chapter

1

The Foundations of Biochemistry

1. The Size of Cells and Their Components

(a) If you were to magnify a cell 10,000-fold (typical of the magnification achieved using an electron microscope), how big would it appear? Assume you are viewing a "typical" eukaryotic cell with a cellular diameter of 50 μm.

(b) If this cell were a muscle cell (myocyte), how many molecules of actin could it hold? (Assume the cell is spherical and no other cellular components are present; actin molecules are spherical, with a diameter of 3.6 nm. The volume of a sphere is $4/3\ \pi r^3$.)

(c) If this were a liver cell (hepatocyte) of the same dimensions, how many mitochondria could it hold? (Assume the cell is spherical; no other cellular components are present; and the mitochondria are spherical, with a diameter of 1.5 μm.)

(d) Glucose is the major energy-yielding nutrient for most cells. Assuming a cellular concentration of 1 mM, calculate how many molecules of glucose would be present in our hypothetical (and spherical) eukaryotic cell. (Avogadro's number, the number of molecules in 1 mol of a nonionized substance, is 6.02×10^{23}.)

(e) Hexokinase is an important enzyme in the metabolism of glucose. If the concentration of hexokinase in our eukaryotic cell is 20 μM, how many glucose molecules are present per hexokinase molecule?

Answer

(a) The magnified cell would have a diameter of $50 \times 10^4\ \mu\text{m} = 500 \times 10^3\ \mu\text{m} = 500$ mm, or 20 inches—about the diameter of a large pizza.

(b) The radius of a globular actin molecule is 3.6 nm/2 = 1.8 nm; the volume of the molecule, in cubic meters, is $(4/3)(3.14)(1.8 \times 10^{-9}\ \text{m})^3 = 2.4 \times 10^{-26}\ \text{m}^3$.*

The number of actin molecules that could fit inside the cell is found by dividing the cell volume (radius = 25 μm) by the actin molecule volume. Cell volume = $(4/3)(3.14)(25 \times 10^{-6}\ \text{m})^3 = 6.5 \times 10^{-14}\ \text{m}^3$. Thus, the number of actin molecules in the hypothetical muscle cell is

$$(6.5 \times 10^{-14}\ \text{m}^3)/(2.4 \times 10^{-26}\ \text{m}^3) = 2.7 \times 10^{12}\ \text{molecules}$$

or 2.7 trillion actin molecules.

**Significant figures:* In multiplication and division, the answer can be expressed with no more significant figures than the least precise value in the calculation. Because some of the data in these problems are derived from measured values, we must round off the calculated answer to reflect this. In this first example, the radius of the actin (1.8 nm) has two significant figures, so the answer (volume of actin = $2.4 \times 10^{-26}\ \text{m}^3$) can be expressed with no more than two significant figures. It will be standard practice in these expanded answers to round off answers to the proper number of significant figures.

(c) The radius of the spherical mitochondrion is 1.5 μm/2 = 0.75 μm, therefore the volume is $(4/3)(3.14)(0.75 \times 10^{-6}\ \text{m})^3 = 1.8 \times 10^{-18}\ \text{m}^3$. The number of mitochondria in the hypothetical liver cell is

$$(6.5 \times 10^{-14}\ \text{m}^3)/(1.8 \times 10^{-18}\ \text{m}^3) = 36 \times 10^3\ \text{mitochondria}$$

(d) The volume of the eukaryotic cell is $6.5 \times 10^{-14}\ \text{m}^3$, which is $6.5 \times 10^{-8}\ \text{cm}^3$ or 6.5×10^{-8} mL. One liter of a 1 mM solution of glucose has (0.001 mol/1000 mL)$(6.02 \times 10^{23}$ molecules/mol) $= 6.02 \times 10^{17}$ molecules/mL. The number of glucose molecules in the cell is the product of the cell volume and glucose concentration:

$$(6.5 \times 10^{-8}\ \text{mL})(6.02 \times 10^{17}\ \text{molecules/mL}) = 3.9 \times 10^{10}\ \text{molecules}$$

or 39 billion glucose molecules.

(e) The concentration ratio of glucose/hexokinase is 0.001 M/0.00002 M, or 50/1, meaning that each enzyme molecule would have about 50 molecules of glucose available as substrate.

2. Components of *E. coli* *E. coli* cells are rod-shaped, about 2 μm long and 0.8 μm in diameter. The volume of a cylinder is $\pi r^2 h$, where h is the height of the cylinder.

(a) If the average density of *E. coli* (mostly water) is 1.1×10^3 g/L, what is the mass of a single cell?

(b) *E. coli* has a protective cell envelope 10 nm thick. What percentage of the total volume of the bacterium does the cell envelope occupy?

(c) *E. coli* is capable of growing and multiplying rapidly because it contains some 15,000 spherical ribosomes (diameter 18 nm), which carry out protein synthesis. What percentage of the cell volume do the ribosomes occupy?

Answer

(a) The volume of a single *E. coli* cell can be calculated from $\pi r^2 h$ (radius = 0.4 μm):

$3.14(4 \times 10^{-5}\ \text{cm})^2(2 \times 10^{-4}\ \text{cm}) = 1.0 \times 10^{-12}\ \text{cm}^3 = 1 \times 10^{-15}\ \text{m}^3 = 1 \times 10^{-15}\ \text{L}$

Density (g/L) multiplied by volume (L) gives the mass of a single cell:

$$(1.1 \times 10^3\ \text{g/L})(1 \times 10^{-15}\ \text{L}) = 1 \times 10^{-12}\ \text{g}$$

or a mass of 1 pg.

(b) First, calculate the proportion of cell volume that does *not* include the cell envelope, that is, the cell volume *without* the envelope—with $r = 0.4\ \mu\text{m} - 0.01\ \mu\text{m}$; and $h = 2\ \mu\text{m} - 2(0.01\ \mu\text{m})$—divided by the total volume.

$$\text{Volume without envelope} = \pi(0.39\ \mu\text{m})^2(1.98\ \mu\text{m})$$

$$\text{Volume with envelope} = \pi(0.4\ \mu\text{m})^2(2\ \mu\text{m})$$

So the percentage of cell that does *not* include the envelope is

$$\frac{\pi(0.39\ \mu\text{m})^2(1.98\ \mu\text{m}) \times 100}{\pi(0.4\ \mu\text{m})^2(2\ \mu\text{m})} = 90\%$$

(Note that we had to calculate to one significant figure, rounding down the 94% to 90%, which here makes a large difference to the answer.) The cell envelope must account for 10% of the total volume of this bacterium.

(c) The volume of all the ribosomes (each ribosome of radius 9 nm) $= 15{,}000 \times (4/3)\pi(9 \times 10^{-3}\ \mu\text{m})^3$

The volume of the cell $= \pi(0.4\ \mu\text{m})^2(2\ \mu\text{m})$

So the percentage of cell volume occupied by the ribosomes is

$$\frac{15{,}000 \times (4/3)\pi(9 \times 10^{-3}\ \mu\text{m})^3 \times 100}{\pi(0.4\ \mu\text{m})^2(2\ \mu\text{m})} = 5\%$$

3. Genetic Information in *E. Coli* DNA The genetic information contained in DNA consists of a linear sequence of coding units, known as codons. Each codon is a specific sequence of three deoxyribonucleotides (three deoxyribonucleotide pairs in double-stranded DNA), and each codon codes for a single amino acid unit in a protein. The molecular weight of an *E. coli* DNA molecule is about 3.1×10^9 g/mol. The average molecular weight of a nucleotide pair is 660 g/mol, and each nucleotide pair contributes 0.34 nm to the length of DNA.

(a) Calculate the length of an *E. coli* DNA molecule. Compare the length of the DNA molecule with the cell dimensions (see Problem 2). How does the DNA molecule fit into the cell?

(b) Assume that the average protein in *E. coli* consists of a chain of 400 amino acids. What is the maximum number of proteins that can be coded by an *E. coli* DNA molecule?

Answer

(a) The number of nucleotide pairs in the DNA molecule is calculated by dividing the molecular weight of DNA by that of a single pair:

$$(3.1 \times 10^9 \text{ g/mol})/(0.66 \times 10^3 \text{ g/mol}) = 4.7 \times 10^6 \text{ pairs}$$

Multiplying the number of pairs by the length per pair gives

$$(4.7 \times 10^6 \text{ pairs})(0.34 \text{ nm/pair}) = 1.6 \times 10^6 \text{ nm} = 1.6 \text{ mm}$$

The length of the cell is 2 μm (from Problem 2), or 0.002 mm, which means the DNA is (1.6 mm)/(0.002 mm) = 800 times longer than the cell. The DNA must be tightly coiled to fit into the cell.

(b) Because the DNA molecule has 4.7×10^6 nucleotide pairs, as calculated in (a), it must have one-third this number of triplet codons:

$$(4.7 \times 10^6)/3 = 1.6 \times 10^6 \text{ codons}$$

If each protein has an average of 400 amino acids, each requiring one codon, the number of proteins that can be coded by *E. coli* DNA is

$$(1.6 \times 10^6 \text{ codons})(1 \text{ amino acid/codon})/(400 \text{ amino acids/protein}) = 4{,}000 \text{ proteins}$$

4. The High Rate of Bacterial Metabolism Bacterial cells have a much higher rate of metabolism than animal cells. Under ideal conditions some bacteria double in size and divide every 20 min, whereas most animal cells under rapid growth conditions require 24 hours. The high rate of bacterial metabolism requires a high ratio of surface area to cell volume.

(a) Why does surface-to-volume ratio affect the maximum rate of metabolism?

(b) Calculate the surface-to-volume ratio for the spherical bacterium *Neisseria gonorrhoeae* (diameter 0.5 μm), responsible for the disease gonorrhea. Compare it with the surface-to-volume ratio for a globular amoeba, a large eukaryotic cell (diameter 150 μm). The surface area of a sphere is $4\pi r^2$.

Answer

(a) Metabolic rate is limited by diffusion of fuels into the cell and waste products out of the cell. This diffusion in turn is limited by the surface area of the cell. As the ratio of surface area to volume decreases, the rate of diffusion cannot keep up with the rate of metabolism within the cell.

(b) For a sphere, surface area = $4\pi r^2$ and volume = 4/3 πr^3. The ratio of the two is the surface-to-volume ratio, *S*/*V*, which is 3/*r* or 6/*D*, where *D* = diameter. Thus, rather than calculating *S* and *V* separately for each cell, we can rapidly calculate and compare *S*/*V* ratios for cells of different diameters.

$$S/V \text{ for } N.\ gonorrhoeae = 6/(0.5\ \mu\text{m}) = 12\ \mu\text{m}^{-1}$$

$$S/V \text{ for amoeba} = 6/(150\ \mu\text{m}) = 0.04\ \mu\text{m}^{-1}$$

$$\frac{S/V \text{ for bacterium}}{S/V \text{ for amoeba}} = \frac{12\mu\text{m}^{-1}}{0.04\ \mu\text{m}^{-1}} = 300$$

Thus, the surface-to-volume ratio is 300 times greater for the bacterium.

5. Fast Axonal Transport Neurons have long thin processes called axons, structures specialized for conducting signals throughout the organism's nervous system. Some axonal processes can be as long as 2 m—for example, the axons that originate in your spinal cord and terminate in the muscles of your toes. Small membrane-enclosed vesicles carrying materials essential to axonal function move along microtubules of the cytoskeleton, from the cell body to the tips of the axons. If the average velocity of a vesicle is 1 μm/s, how long does it take a vesicle to move from a cell body in the spinal cord to the axonal tip in the toes?

Answer Transport time equals distance traveled/velocity, or

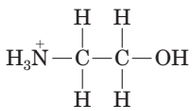
$$(2 \times 10^6\ \mu\text{m})/(1\ \mu\text{m/s}) = 2 \times 10^6\ \text{s}$$

or about 23 days!

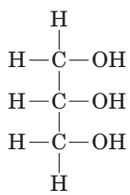
6. Is Synthetic Vitamin C as Good as the Natural Vitamin? A claim put forth by some purveyors of health foods is that vitamins obtained from natural sources are more healthful than those obtained by chemical synthesis. For example, pure L-ascorbic acid (vitamin C) extracted from rose hips is better than pure L-ascorbic acid manufactured in a chemical plant. Are the vitamins from the two sources different? Can the body distinguish a vitamin's source?

Answer The properties of the vitamin—like any other compound—are determined by its chemical structure. Because vitamin molecules from the two sources are structurally identical, their properties are identical, and no organism can distinguish between them. If different vitamin preparations contain different impurities, the biological effects of the *mixtures* may vary with the source. The ascorbic acid in such preparations, however, is identical.

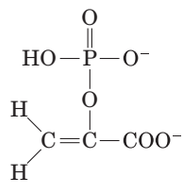
7. Identification of Functional Groups Figures 1–15 and 1–16 show some common functional groups of biomolecules. Because the properties and biological activities of biomolecules are largely determined by their functional groups, it is important to be able to identify them. In each of the compounds below, circle and identify by name each functional group.



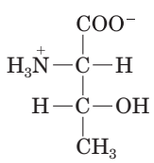
Ethanolamine
(a)



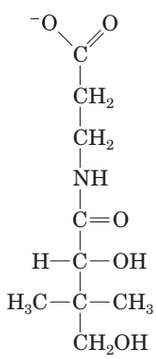
Glycerol
(b)



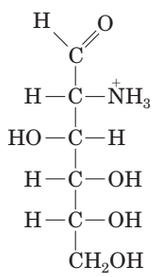
Phosphoenolpyruvate, an intermediate in glucose metabolism
(c)



Threonine, an amino acid
(d)



Pantothenate, a vitamin
(e)



D-Glucosamine
(f)

Answer

(a) $—NH_3^+$ = amino; —OH = hydroxyl

(b) —OH = hydroxyl (three)

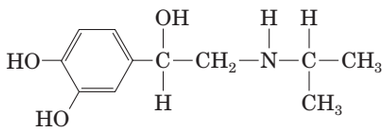
(c) $—P(OH)O_2^-$ = phosphoryl (in its ionized form); $—COO^-$ = carboxyl

(d) $—COO^-$ = carboxyl; $—NH_3^+$ = amino; —OH = hydroxyl; $—CH_3$ = methyl (two)

(e) $—COO^-$ = carboxyl; —CO—NH— = amide; —OH = hydroxyl (two); $—CH_3$ = methyl (two)

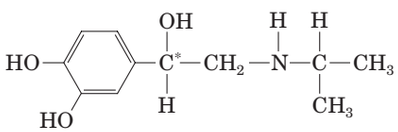
(f) —CHO = aldehyde; $—NH_3^+$ = amino; —OH = hydroxyl (four)

8. Drug Activity and Stereochemistry The quantitative differences in biological activity between the two enantiomers of a compound are sometimes quite large. For example, the D isomer of the drug isoproterenol, used to treat mild asthma, is 50 to 80 times more effective as a bronchodilator than the L isomer. Identify the chiral center in isoproterenol. Why do the two enantiomers have such radically different bioactivity?



Isoproterenol

Answer A chiral center, or chiral carbon, is a carbon atom that is bonded to four different groups. A molecule with a single chiral center has two enantiomers, designated D and L (or in the RS system, *S* and *R*). In isoproterenol, only one carbon (asterisk) has four different groups around it; this is the chiral center:



The bioactivity of a drug is the result of interaction with a biological "receptor," a protein molecule with a binding site that is also chiral and stereospecific. The interaction of the D isomer of a drug with a chiral receptor site will differ from the interaction of the L isomer with that site.

9. Separating Biomolecules In studying a particular biomolecule (a protein, nucleic acid, carbohydrate, or lipid) in the laboratory, the biochemist first needs to separate it from other biomolecules in the sample—that is, to *purify* it. Specific purification techniques are described later in the text. However, by looking at the monomeric subunits of a biomolecule, you should have some ideas about the characteristics of the molecule that would allow you to separate it from other molecules. For example, how would you separate **(a)** amino acids from fatty acids and **(b)** nucleotides from glucose?

Answer

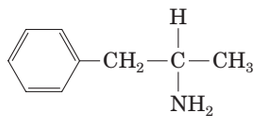
(a) Amino acids and fatty acids have carboxyl groups, whereas only the amino acids have amino groups. Thus, you could use a technique that separates molecules on the basis of the properties (charge or binding affinity) of amino groups. Fatty acids have long hydrocarbon chains and therefore are less soluble in water than amino acids. And finally, the sizes and shapes of these two types of molecules are quite different. Any one or more of these properties may provide ways to separate the two types of compounds.

(b) A nucleotide molecule has three components: a nitrogenous organic base, a five-carbon sugar, and phosphate. Glucose is a six-carbon sugar; it is smaller than a nucleotide. The size difference could be used to separate the molecules. Alternatively, you could use the nitrogenous bases and/or the phosphate groups characteristic of the nucleotides to separate them (based on differences in solubility, charge) from glucose.

10. Silicon-Based Life? Silicon is in the same group of the periodic table as carbon and, like carbon, can form up to four single bonds. Many science fiction stories have been based on the premise of silicon-based life. Is this realistic? What characteristics of silicon make it *less* well adapted than carbon as the central organizing element for life? To answer this question, consider what you have learned about carbon's bonding versatility, and refer to a beginning inorganic chemistry textbook for silicon's bonding properties.

Answer It is improbable that silicon could serve as the central organizing element for life under such conditions as those found on Earth for several reasons. Long chains of silicon atoms are not readily synthesized, and thus the polymeric macromolecules necessary for more complex functions would not readily form. Also, oxygen disrupts bonds between two silicon atoms, so silicon-based life-forms would be unstable in an oxygen-containing atmosphere. Once formed, the bonds between silicon and oxygen are extremely stable and difficult to break, which would prevent the breaking and making (degradation and synthesis) of biomolecules that is essential to the processes of living organisms.

11. Drug Action and Shape of Molecules Several years ago two drug companies marketed a drug under the trade names Dexedrine and Benzedrine. The structure of the drug is shown below.

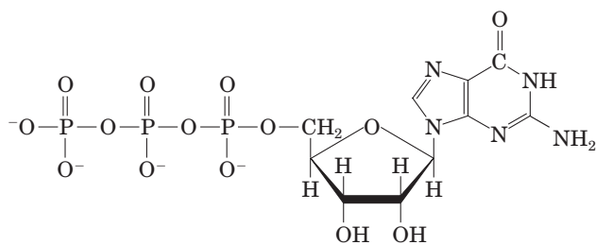


The physical properties (C, H, and N analysis, melting point, solubility, etc.) of Dexedrine and Benzedrine were identical. The recommended oral dosage of Dexedrine (which is still available) was 5 mg/day, but the recommended dosage of Benzedrine (no longer available) was twice that. Apparently, it required considerably more Benzedrine than Dexedrine to yield the same physiological response. Explain this apparent contradiction.

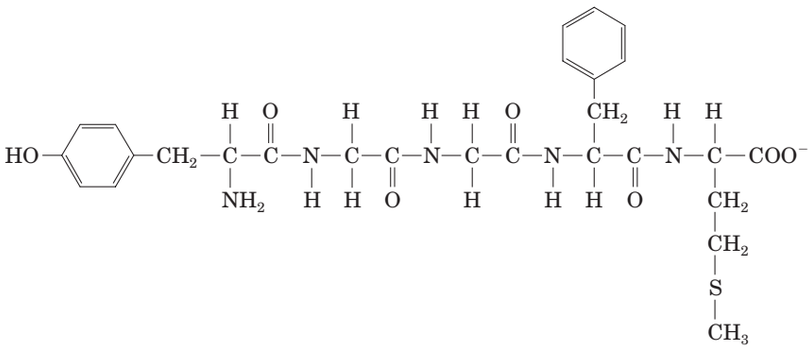
Answer Only one of the two enantiomers of the drug molecule (which has a chiral center) is physiologically active, for reasons described in the answer to Problem 3 (interaction with a stereospecific receptor site). Dexedrine, as manufactured, consists of the single enantiomer (D-amphetamine) recognized by the receptor site. Benzedrine was a racemic mixture (equal amounts of D and L isomers), so a much larger dose was required to obtain the same effect.

12. Components of Complex Biomolecules Figure 1–10 shows the major components of complex biomolecules. For each of the three important biomolecules below (shown in their ionized forms at physiological pH), identify the constituents.

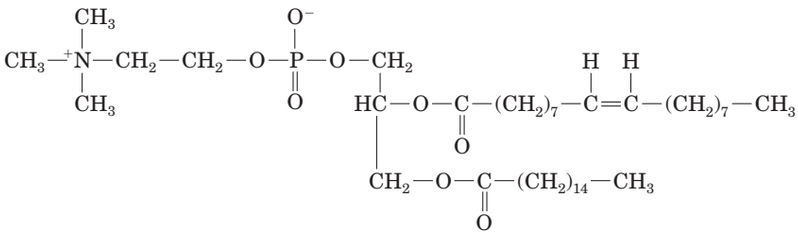
(a) Guanosine triphosphate (GTP), an energy-rich nucleotide that serves as a precursor to RNA:



(b) Methionine enkephalin, the brain's own opiate:



(c) Phosphatidylcholine, a component of many membranes:



Answer

(a) Three phosphoric acid groups (linked by two anhydride bonds), esterified to an α-D-ribose (at the 5′ position), which is attached at C-1 to guanine.

(b) Tyrosine, two glycine, phenylalanine, and methionine residues, all linked by peptide bonds.

(c) Choline esterified to a phosphoric acid group, which is esterified to glycerol, which is esterified to two fatty acids, oleic acid and palmitic acid.

13. Determination of the Structure of a Biomolecule An unknown substance, X, was isolated from rabbit muscle. Its structure was determined from the following observations and experiments. Qualitative analysis showed that X was composed entirely of C, H, and O. A weighed sample of X was completely oxidized, and the H_2O and CO_2 produced were measured; this quantitative analysis revealed that X contained 40.00% C, 6.71% H, and 53.29% O by weight. The molecular mass of X, determined by mass spectrometry, was 90.00 u (atomic mass units; see Box 1–1). Infrared spectroscopy showed that X contained one double bond. X dissolved readily in water to give an acidic solution; the solution demonstrated optical activity when tested in a polarimeter.

(a) Determine the empirical and molecular formula of X.

(b) Draw the possible structures of X that fit the molecular formula and contain one double bond. Consider *only* linear or branched structures and disregard cyclic structures. Note that oxygen makes very poor bonds to itself.

(c) What is the structural significance of the observed optical activity? Which structures in **(b)** are consistent with the observation?

(d) What is the structural significance of the observation that a solution of X was acidic? Which structures in **(b)** are consistent with the observation?

(e) What is the structure of X? Is more than one structure consistent with all the data?

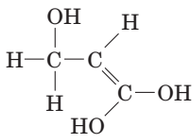
Answer

(a) From the C, H, and O analysis, and knowing the mass of X is 90.00 u, we can calculate the relative atomic proportions by dividing the weight percents by the atomic weights:

Atom	Relative atomic proportion	No. of atoms relative to O
C	(90.00 u)(40.00/100)/(12 u) = 3	3/3 = 1
H	(90.00 u)(6.71/100)/(1.008 u) = 6	6/3 = 2
O	(90.00 u)(53.29/100)/(16.0 u) = 3	3/3 = 1

Thus, the empirical formula is CH_2O, with a formula weight of 12 + 2 + 16 = 30. The molecular formula, based on X having a mass of 90.00 u, must be $C_3H_6O_3$.

(b) Twelve possible structures are shown below. Structures **1** through **5** can be eliminated because they are unstable enol isomers of the corresponding carbonyl derivatives. Structures **9**, **10**, and **12** can also be eliminated on the basis of their instability: they are hydrated carbonyl derivatives (vicinal diols).



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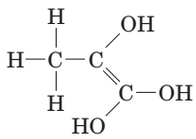

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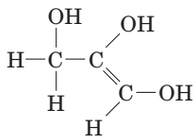

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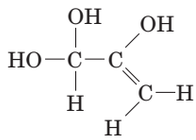

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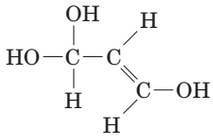
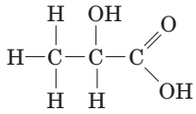


5



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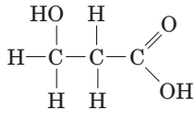

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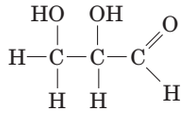

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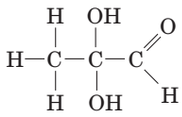

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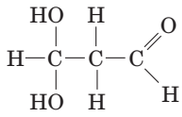

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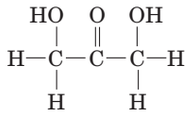

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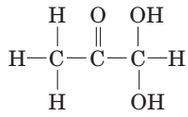


12

(c) Optical activity indicates the presence of a chiral center (a carbon atom surrounded by four different groups). Only structures **6** and **8** have chiral centers.

(d) Of structures **6** and **8**, only **6** contains an acidic group: a carboxyl group.

(e) Structure **6** is substance X. This compound exists in two enantiomeric forms that cannot be distinguished, even by measuring specific rotation. One could determine absolute stereochemistry by x-ray crystallography.

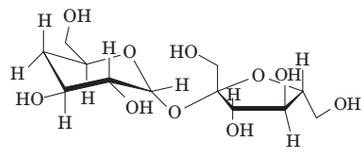
Data Analysis Problem

14. Sweet-Tasting Molecules Many compounds taste sweet to humans. Sweet taste results when a molecule binds to the sweet receptor, one type of taste receptor, on the surface of certain tongue cells. The stronger the binding, the lower the concentration required to saturate the receptor and the sweeter a given concentration of that substance tastes. The standard free-energy change, $\Delta G°$, of the binding reaction between a sweet molecule and a sweet receptor can be measured in kilojoules or kilocalories per mole.

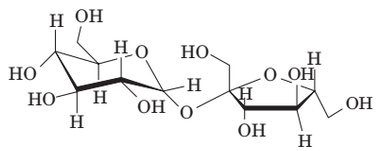
Sweet taste can be quantified in units of "molar relative sweetness" (MRS), a measure that compares the sweetness of a substance to the sweetness of sucrose. For example, saccharin has an MRS of 161; this means that saccharin is 161 times sweeter than sucrose. In practical terms, this is measured by asking human subjects to compare the sweetness of solutions containing different concentrations of each compound. Sucrose and saccharin taste equally sweet when sucrose is at a concentration 161 times higher than that of saccharin.

(a) What is the relationship between MRS and the $\Delta G°$ of the binding reaction? Specifically, would a more negative $\Delta G°$ correspond to a higher or lower MRS? Explain your reasoning.

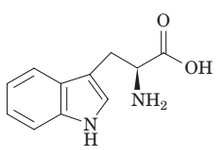
Shown below are the structures of 10 compounds, all of which taste sweet to humans. The MRS and $\Delta G°$ for binding to the sweet receptor are given for each substance.



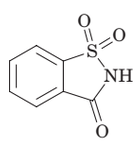
Deoxysucrose
MRS = 0.95
$\Delta G° = -6.67$ kcal/mol



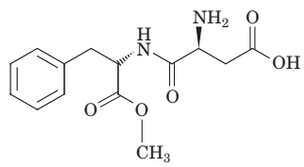
Sucrose
MRS = 1
$\Delta G° = -6.71$ kcal/mol



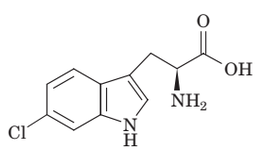
D-Tryptophan
MRS = 21
$\Delta G° = -8.5$ kcal/mol



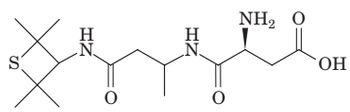
Saccharin
MRS = 161
$\Delta G° = -9.7$ kcal/mol



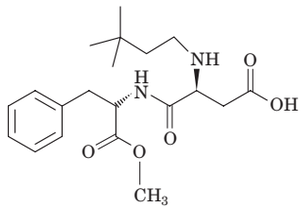
Aspartame
MRS = 172
$\Delta G° = -9.7$ kcal/mol



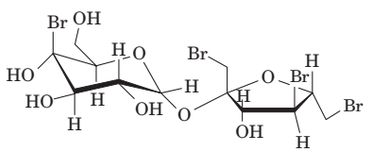
6-Chloro-D-tryptophan
MRS = 906
$\Delta G° = -10.7$ kcal/mol



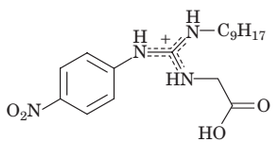
Alitame
MRS = 1,937
$\Delta G° = -11.1$ kcal/mol



Neotame
MRS = 11,057
$\Delta G^\circ = -12.1$ kcal/mol



Tetrabromosucrose
MRS = 13,012
$\Delta G^\circ = -12.2$ kcal/mol



Sucronic acid
MRS = 200,000
$\Delta G^\circ = -13.8$ kcal/mol

Morini, Bassoli, and Temussi (2005) used computer-based methods (often referred to as "in silico" methods) to model the binding of sweet molecules to the sweet receptor.

(b) Why is it useful to have a computer model to predict the sweetness of molecules, instead of a human- or animal-based taste assay?

In earlier work, Schallenberger and Acree (1967) had suggested that all sweet molecules include an "AH-B" structural group, in which "A and B are electronegative atoms separated by a distance of greater than 2.5 Å [0.25 nm] but less than 4 Å [0.4 nm]. H is a hydrogen atom attached to one of the electronegative atoms by a covalent bond" (p. 481).

(c) Given that the length of a "typical" single bond is about 0.15 nm, identify the AH-B group(s) in each of the molecules shown above.

(d) Based on your findings from **(c),** give two objections to the statement that "molecules containing an AH-B structure will taste sweet."

(e) For two of the molecules shown above, the AH-B model *can* be used to explain the difference in MRS and ΔG°. Which two molecules are these, and how would you use them to support the AH-B model?

(f) Several of the molecules have closely related structures but very different MRS and ΔG° values. Give two such examples, and use these to argue that the AH-B model is unable to explain the observed differences in sweetness.

In their computer-modeling study, Morini and coauthors used the three-dimensional structure of the sweet receptor and a molecular dynamics modeling program called GRAMM to predict the ΔG° of binding of sweet molecules to the sweet receptor. First, they "trained" their model—that is, they refined the parameters so that the ΔG° values predicted by the model matched the known ΔG° values for one set of sweet molecules (the "training set"). They then "tested" the model by asking it to predict the ΔG° values for a new set of molecules (the "test set").

(g) Why did Morini and colleagues need to test their model against a different set of molecules from the set it was trained on?

(h) The researchers found that the predicted ΔG° values for the test set differed from the actual values by, on average, 1.3 kcal/mol. Using the values given with the structures above, estimate the resulting error in MRS values.

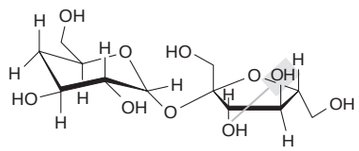
Answer

(a) A more negative $\Delta G°$ corresponds to a larger K_{eq} for the binding reaction, so the equilibrium is shifted more toward products and tighter binding—and thus greater sweetness and higher MRS.

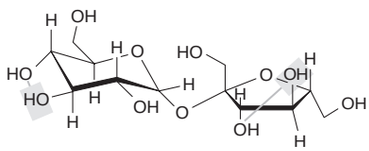
(b) Animal-based sweetness assays are time-consuming. A computer program to predict sweetness, even if not always completely accurate, would allow chemists to design effective sweeteners much faster. Candidate molecules could then be tested in the conventional assay.

(c) The range 0.25 to 0.4 nm corresponds to about 1.5 to 2.5 single-bond lengths. The figure below can be used to construct an approximate ruler; any atoms in the gray rectangle are between 0.25 and 0.4 nm from the origin of the ruler.

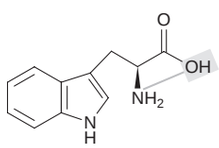
There are many possible AH-B groups in the molecules; a few are shown here.



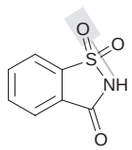
Deoxysucrose



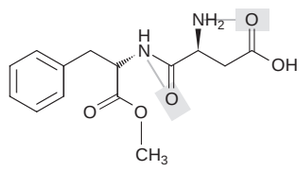
Sucrose



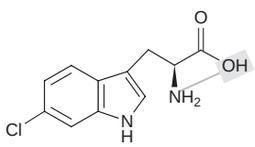
D-Tryptophan



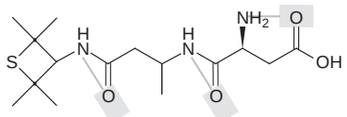
Saccharin



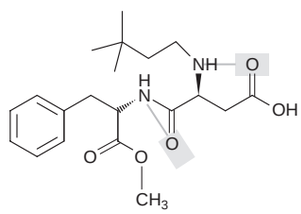
Aspartame



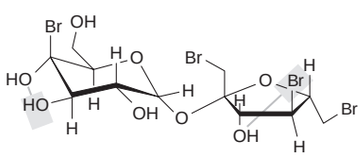
6-Chloro-D-tryptophan



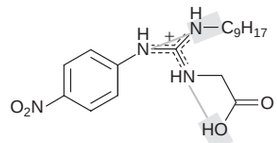
Alitame



Neotame



Tetrabromosucrose



Sucronic acid

(d) First, each molecule has multiple AH-B groups, so it is difficult to know which is the important one. Second, because the AH-B motif is very simple, many nonsweet molecules will have this group.

(e) Sucrose and deoxysucrose. Deoxysucrose lacks one of the AH-B groups present in sucrose and has a slightly lower MRS than sucrose—as is expected if the AH-B groups are important for sweetness.

(f) There are many such examples; here are a few: (1) D-Tryptophan and 6-chloro-D-tryptophan have the same AH-B group but very different MRS values. (2) Aspartame and neotame have the same AH-B groups but very different MRS values. (3) Neotame has two AH-B groups and alitame has three, yet neotame is more than five times sweeter than alitame. (4) Bromine is less electronegative than oxygen and thus is expected to weaken an AH-B group, yet tetrabromosucrose is much sweeter than sucrose.

(g) Given enough "tweaking" of parameters, any model can be made to fit a defined dataset. Because the objective was to create a model to predict $\Delta G°$ for molecules not tested in vivo, the researchers needed to show that the model worked well for molecules it had not been trained on. The degree of inaccuracy with the test set could give researchers an idea of how the model would behave for novel molecules.

(h) MRS is related to K_{eq}, which is related exponentially to $\Delta G°$, so adding a constant amount to $\Delta G°$ multiplies the MRS by a constant amount. Based on the values given with the structures, a change in $\Delta G°$ of 1.3 kcal/mol corresponds to a 10-fold change in MRS.

References

Morini, G., Bassoli, A., & Temussi, P.A. (2005) From small sweeteners to sweet proteins: anatomy of the binding sites of the human T1R2_T1R3 receptor. *J. Med. Chem.* **48,** 5520–5529.

Schallenberger, R.S. & Acree, T.E. (1967) Molecular theory of sweet taste. *Nature* **216,** 480–482.

chapter

2

Water

1. Solubility of Ethanol in Water Explain why ethanol (CH_3CH_2OH) is more soluble in water than is ethane (CH_3CH_3).

Answer Ethanol is polar; ethane is not. The ethanol —OH group can hydrogen-bond with water.

2. Calculation of pH from Hydrogen Ion Concentration What is the pH of a solution that has an H^+ concentration of **(a)** 1.75×10^{-5} mol/L; **(b)** 6.50×10^{-10} mol/L; **(c)** 1.0×10^{-4} mol/L; **(d)** 1.50×10^{-5} mol/L?

Answer Using $pH = -\log [H^+]$:
(a) $-\log (1.75 \times 10^{-5}) = 4.76$; **(b)** $-\log (6.50 \times 10^{-10}) = 9.19$; **(c)** $-\log (1.0 \times 10^{-4}) = 4.0$; **(d)** $-\log (1.50 \times 10^{-5}) = 4.82$.

3. Calculation of Hydrogen Ion Concentration from pH What is the H^+ concentration of a solution with pH of **(a)** 3.82; **(b)** 6.52; **(c)** 11.11?

Answer Using $[H^+] = 10^{-pH}$:
(a) $[H^+] = 10^{-3.82} = 1.51 \times 10^{-4}$ M; **(b)** $[H^+] = 10^{-6.52} = 3.02 \times 10^{-7}$ M; **(c)** $[H^+] = 10^{-11.11} = 7.76 \times 10^{-12}$ M.

4. Acidity of Gastric HCl In a hospital laboratory, a 10.0 mL sample of gastric juice, obtained several hours after a meal, was titrated with 0.1 M NaOH to neutrality; 7.2 mL of NaOH was required. The patient's stomach contained no ingested food or drink, thus assume that no buffers were present. What was the pH of the gastric juice?

Answer Multiplying volume (L) by molar concentration (mol/L) gives the number of moles in that volume of solution. If x is the concentration of gastric HCl (mol/L),

$$(0.010 \text{ L})x = (0.0072 \text{ L})(0.1 \text{ mol/L})$$

$$x = 0.072 \text{ M gastric HCl}$$

Given that $pH = -\log [H^+]$ and that HCl is a strong acid,

$$pH = -\log (7.2 \times 10^{-2}) = 1.1$$

5. Calculation of the pH of a Strong Acid or Base **(a)** Write out the acid dissociation reaction for hydrochloric acid. **(b)** Calculate the pH of a solution of 5.0×10^{-4} M HCl. **(c)** Write out the acid dissociation reaction for sodium hydroxide. **(d)** Calculate the pH of a solution of 7.0×10^{-5} M NaOH.

Answer

(a) $HCl \rightleftharpoons H^+ + Cl^-$

(b) HCl is a strong acid and fully dissociates into H^+ and Cl^-. Thus, $[H^+] = [Cl^-] = [HCl]$.
$pH = -\log [H^+] = -\log (5.0 \times 10^{-4}\ \text{M}) = 3.3$ (two significant figures)

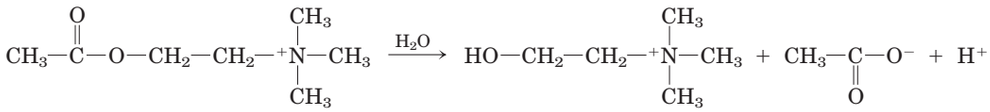
(c) $NaOH \rightleftharpoons Na^+ + OH^-$

(d) NaOH is a strong base; dissociation in aqueous solution is essentially complete, so $[Na^+] = [OH^-] = [NaOH]$.
$pH + pOH = 14$
$pOH = -\log [OH^-]$
$pH = 14 + \log [OH^-]$
$= 14 + \log (7.0 \times 10^{-5}) = 9.8$ (two significant figures)

6. Calculation of pH from Concentration of Strong Acid Calculate the pH of a solution prepared by diluting 3.0 mL of 2.5 M HCl to a final volume of 100 mL with H_2O.

Answer Because HCl is a strong acid, it dissociates completely to $H^+ + Cl^-$. Therefore, 3.0 mL × 2.5 M HCl = 7.5 meq of H^+. In 100 mL of solution, this is 0.075 M H^+.
$pH = -\log [H^+] = -\log (0.075) = -(-1.1) = 1.1$ (two significant figures)

7. Measurement of Acetylcholine Levels by pH Changes The concentration of acetylcholine (a neurotransmitter) in a sample can be determined from the pH changes that accompany its hydrolysis. When the sample is incubated with the enzyme acetylcholinesterase, acetylcholine is quantitatively converted into choline and acetic acid, which dissociates to yield acetate and a hydrogen ion:



In a typical analysis, 15 mL of an aqueous solution containing an unknown amount of acetylcholine had a pH of 7.65. When incubated with acetylcholinesterase, the pH of the solution decreased to 6.87. Assuming that there was no buffer in the assay mixture, determine the number of moles of acetylcholine in the 15 mL sample.

Answer Given that $pH = -\log [H^+]$, we can calculate $[H^+]$ at the beginning and at the end of the reaction:

At pH 7.65, $\log [H^+] = -7.65$ $\quad [H^+] = 10^{-7.65} = 2.24 \times 10^{-8}$ M

At pH 6.87, $\log [H^+] = -6.87$ $\quad [H^+] = 10^{-6.87} = 1.35 \times 10^{-7}$ M

The difference in $[H^+]$ is

$$(1.35 - 0.22) \times 10^{-7}\ \text{M} = 1.13 \times 10^{-7}\ \text{M}$$

For a volume of 15 mL, or 0.015 L, multiplying volume by molarity gives

$$(0.015\ \text{L})(1.13 \times 10^{-7}\ \text{mol/L}) = 1.7 \times 10^{-9}\ \text{mol of acetylcholine}$$

8. Physical Meaning of pK_a Which of the following aqueous solutions has the lowest pH: 0.1 M HCl; 0.1 M acetic acid ($pK_a = 4.86$); 0.1 M formic acid ($pK_a = 3.75$)?

Answer A 0.1 M HCl solution has the lowest pH because HCl is a strong acid and dissociates completely to $H^+ + Cl^-$, yielding the highest $[H^+]$.

9. Simulated Vinegar One way to make vinegar (*not* the preferred way) is to prepare a solution of acetic acid, the sole acid component of vinegar, at the proper pH (see Fig. 2–14) and add appropriate flavoring agents. Acetic acid (M_r 60) is a liquid at 25 °C, with a density of 1.049 g/mL. Calculate the volume that must be added to distilled water to make 1 L of simulated vinegar (see Fig. 2–15).

Answer From Figure 2–15, the pK_a of acetic acid is 4.76. From Figure 2–14, the pH of vinegar is ~3; we will calculate for a solution of pH 3.0. Using the Henderson-Hasselbalch equation

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

and the fact that dissociation of HA gives equimolar $[H^+]$ and $[A^-]$ (where HA is CH_3COOH, and A^- is CH_3COO^-), we can write

$$3.0 = 4.76 + \log ([\text{A}^-]/[\text{HA}])$$

$$-1.76 = \log ([\text{A}^-]/[\text{HA}]) = -\log ([\text{HA}]/[\text{A}^-])$$

$$[\text{HA}]/[\text{A}^-] = 10^{1.76} = 58$$

Thus, $[HA] = 58[A^-]$. At pH 3.0, $[H^+] = [A^-] = 10^{-3}$, so

$$[\text{HA}] = 58 \times 10^{-3}\ \text{M} = 0.058\ \text{mol/L}$$

Dividing density (g/mL) by molecular weight (g/mol) for acetic acid gives

$$\frac{1.049\ \text{g/mL}}{60\ \text{g/mol}} = 0.017\ \text{mol/mL}$$

Dividing this answer into 0.058 mol/L gives the volume of acetic acid needed to prepare 1.0 L of a 0.058 M solution:

$$\frac{0.058\ \text{mol/L}}{0.017\ \text{mol/mL}} = 3.3\ \text{mL/L}$$

10. Identifying the Conjugate Base Which is the conjugate base in each of the pairs below?

(a) $RCOOH$, $RCOO^-$

(b) RNH_2, RNH_3^+

(c) $H_2PO_4^-$, H_3PO_4

(d) H_2CO_3, HCO_3^-

Answer In each pair, the acid is the species that gives up a proton; the conjugate base is the deprotonated species. By inspection, the conjugate base is the species with fewer hydrogen atoms. **(a)** $RCOO^-$ **(b)** RNH_2 **(c)** $H_2PO_4^-$ **(d)** HCO_3^-

11. Calculation of the pH of a Mixture of a Weak Acid and Its Conjugate Base Calculate the pH of a dilute solution that contains a molar ratio of potassium acetate to acetic acid (pK_a = 4.76) of **(a)** 2:1; **(b)** 1:3; **(c)** 5:1; **(d)** 1:1; **(e)** 1:10.

Answer Using the Henderson-Hasselbalch equation,

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

pH = 4.76 + log ([acetate]/[acetic acid]), where [acetate]/[acetic acid] is the ratio given for each part of the question.

(a) log (2/1) = 0.30; pH = 4.76 + 0.30 = 5.06

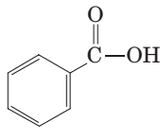
(b) log (1/3) = −0.48; pH = 4.76 + (−0.48) = 4.28

(c) log (5/1) = 0.70; pH = 4.76 + 0.70 = 5.46

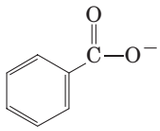
(d) log (1/1) = 0; pH = 4.76

(e) log (1/10) = −1.00; pH = 4.76 + (−1.00) = 3.76

12. Effect of pH on Solubility The strongly polar hydrogen-bonding properties of water make it an excellent solvent for ionic (charged) species. By contrast, nonionized, nonpolar organic molecules, such as benzene, are relatively insoluble in water. In principle, the aqueous solubility of an organic acid or base can be increased by converting the molecules to charged species. For example, the solubility of benzoic acid in water is low. The addition of sodium bicarbonate to a mixture of water and benzoic acid raises the pH and deprotonates the benzoic acid to form benzoate ion, which is quite soluble in water.

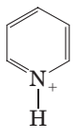


Benzoic acid
$pK_a \approx 5$



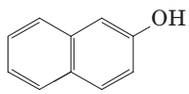
Benzoate ion

Are the following compounds more soluble in an aqueous solution of 0.1 M NaOH or 0.1 M HCl? (The dissociable proton in **(c)** is that of the —OH group.)



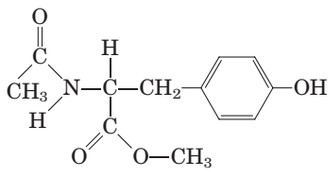
Pyridine ion
$pK_a \approx 5$

(a)



β-Naphthol
$pK_a \approx 10$

(b)



N-Acetyltyrosine methyl ester
$pK_a \approx 10$

(c)

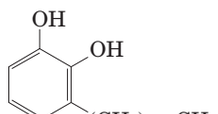
Answer

(a) Pyridine is ionic in its protonated form and therefore more soluble at the lower pH, in 0.1 M HCl.

(b) β-Naphthol is ionic when *de*protonated and thus more soluble at the higher pH, in 0.1 M NaOH.

(c) *N*-Acetyltyrosine methyl ester is ionic when *de*protonated and thus more soluble in 0.1 M NaOH.

13. Treatment of Poison Ivy Rash The components of poison ivy and poison oak that produce the characteristic itchy rash are catechols substituted with long-chain alkyl groups.



$pK_a \approx 8$

If you were exposed to poison ivy, which of the treatments below would you apply to the affected area? Justify your choice.

(a) Wash the area with cold water.

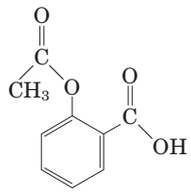
(b) Wash the area with dilute vinegar or lemon juice.

(c) Wash the area with soap and water.

(d) Wash the area with soap, water, and baking soda (sodium bicarbonate).

Answer The best choice is **(d).** Soap helps to emulsify and dissolve the hydrophobic alkyl group of an alkylcatechol. Given that the pK_a of an alkylcatechol is about 8, in a mildly alkaline solution of bicarbonate ($NaHCO_3$) its —OH group ionizes, making the compound much more water-soluble. A neutral or acidic solution, as in **(a)** or **(b),** would not be effective.

14. pH and Drug Absorption Aspirin is a weak acid with a pK_a of 3.5.



It is absorbed into the blood through the cells lining the stomach and the small intestine. Absorption requires passage through the plasma membrane, the rate of which is determined by the polarity of the molecule: charged and highly polar molecules pass slowly, whereas neutral hydrophobic ones pass rapidly. The pH of the stomach contents is about 1.5, and the pH of the contents of the small intestine is about 6. Is more aspirin absorbed into the bloodstream from the stomach or from the small intestine? Clearly justify your choice.

Answer With a pK_a of 3.5, aspirin is in its protonated (neutral) form at pH below 2.5. At higher pH, it becomes increasingly deprotonated (anionic). Thus, aspirin is better absorbed in the more acidic environment of the stomach.

15. Calculation of pH from Molar Concentrations What is the pH of a solution containing 0.12 mol/L of NH_4Cl and 0.03 mol/L of NaOH (pK_a of NH_4^+/NH_3 is 9.25)?

Answer For the equilibrium

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$

$$pH = pK_a + \log ([NH_3]/[NH_4^+])$$

we know that $[NH_4^+] + [NH_3] = 0.12$ mol/L, and that NaOH completely dissociates to give $[OH^-] = 0.03$ mol/L. Thus, $[NH_3] = 0.03$ mol/L and $[NH_4^+] = 0.09$ mol/L, and

$$pH = 9.25 + \log (0.03/0.09) = 9.25 - 0.48 = 8.77, \text{ which rounds to } 9.$$

16. Calculation of pH after Titration of Weak Acid A compound has a pK_a of 7.4. To 100 mL of a 1.0 M solution of this compound at pH 8.0 is added 30 mL of 1.0 M hydrochloric acid. What is the pH of the resulting solution?

Answer Begin by calculating the ratio of conjugate base to acid in the starting solution, using the Henderson-Hasselbalch equation:

$$pH = pK_a + \log ([A^-]/[HA])$$

$$8.0 = 7.4 + \log ([A^-]/[HA])$$

$$\log ([A^-]/[HA]) = 0.6$$

$$[A^-]/[HA] = 10^{0.6} = 4$$

The solution contains 100 meq of the compound (conjugate base plus acid), so 80 meq are in the conjugate base form and 20 meq are in the acid form, for a [base]/[acid] ratio of 4.

Because HCl is a strong acid and dissociates completely, adding 30 mL of 1.0 M HCl adds 30 meq of H^+ to the solution. These 30 meq titrate 30 meq of the conjugate base, so the [base]/[acid] ratio is 1. Solving the Henderson-Hasselbalch equation for pH:

$$\text{pH} = \text{p}K_\text{a} + \log ([\text{A}^-]/[\text{HA}])$$
$$= 7.4 + \log 1 = 7.4$$

17. Properties of a Buffer The amino acid glycine is often used as the main ingredient of a buffer in biochemical experiments. The amino group of glycine, which has a pK_a of 9.6, can exist either in the protonated form ($—NH_3^+$) or as the free base ($—NH_2$), because of the reversible equilibrium

$$R—NH_3^+ \rightleftharpoons R—NH_2 + H^+$$

(a) In what pH range can glycine be used as an effective buffer due to its amino group?

(b) In a 0.1 M solution of glycine at pH 9.0, what fraction of glycine has its amino group in the $—NH_3^+$ form?

(c) How much 5 M KOH must be added to 1.0 L of 0.1 M glycine at pH 9.0 to bring its pH to exactly 10.0?

(d) When 99% of the glycine is in its $—NH_3^+$ form, what is the numerical relation between the pH of the solution and the pK_a of the amino group?

Answer

(a) In general, a buffer functions best in the zone from about one pH unit below to one pH unit above its pK_a. Thus, glycine is a good buffer (through ionization of its amino group) between pH 8.6 and pH 10.6.

(b) Using the Henderson-Hasselbalch equation

$$\text{pH} = \text{p}K_\text{a} + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

we can write

$$9.0 = 9.6 + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

$$\frac{[\text{A}^-]}{[\text{HA}]} = 10^{-0.6} = 0.25$$

which corresponds to a ratio of 1/4. This indicates that the amino group of glycine is about 1/5 deprotonated and 4/5 protonated at pH 9.0.

(c) From **(b)** we know that the amino group is about 1/5, or 20%, deprotonated at pH 9.0. Thus, in moving from pH 9.0 to pH 9.6 (at which, by definition, the amino group is 50% deprotonated), 30%, or 0.3, of the glycine is titrated. We can now calculate from the Henderson-Hasselbalch equation the percentage protonation at pH 10.0:

$$10.0 = 9.6 + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

$$\frac{[\text{A}^-]}{[\text{HA}]} = 10^{0.4} = 2.5 = 5/2$$

This ratio indicates that glycine is 5/7, or 71%, deprotonated at pH 10.0, an additional 21%, or 0.21, deprotonation above that (50%, or 0.5) at the pK_a. Thus, the total fractional deprotonation in moving from pH 9.0 to 10.0 is 0.30 + 0.21 = 0.51, which corresponds to

$$0.51 \times 0.1 \text{ mol} = 0.05 \text{ mol of KOH}$$

Thus, the volume of 5 M KOH solution required is (0.5 mol)/(5 mol/L) = 0.01 L, or 10 mL.

(d) From the Henderson-Hasselbalch equation,

$$\begin{aligned} \text{pH} &= \text{p}K_a + \log ([\text{—NH}_2]/[\text{—NH}_3^+]) \\ &= \text{p}K_a + \log (0.01/0.99) \\ &= \text{p}K_a + (-2) = \text{p}K_a - 2 \end{aligned}$$

In general, any group with an ionizable hydrogen is almost completely protonated at a pH at least two pH units below its pK_a value.

18. Preparation of a Phosphate Buffer What molar ratio of HPO_4^{2-} to $H_2PO_4^-$ in solution would produce a pH of 7.0? Phosphoric acid (H_3PO_4), a triprotic acid, has 3 pK_a values: 2.14, 6.86, and 12.4. Hint: Only one of the pK_a values is relevant here.

Answer Only the pK_a close to the pH is relevant here, because the concentrations of the other species (H_3PO_4 and PO_4^{3-}) are insignificant compared with the concentrations of HPO_4^{2-} and $H_2PO_4^-$. Begin with the Henderson-Hasselbalch equation:

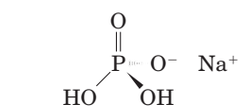
$$\text{pH} = \text{p}K_a + \log ([\text{conjugate base}]/[\text{acid}])$$

$$\log ([HPO_4^{2-}]/[H_2PO_4^-]) = \text{pH} - \text{p}K_a = 7.0 - 6.86 = 0.14$$

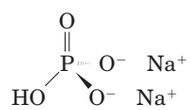
$$[HPO_4^{2-}]/[H_2PO_4^-] = 10^{0.14} = 1.38 = 1.4 \text{ (two significant figures)}$$

19. Preparation of Standard Buffer for Calibration of a pH Meter The glass electrode used in commercial pH meters gives an electrical response proportional to the concentration of hydrogen ion. To convert these responses to a pH reading, the electrode must be calibrated against standard solutions of known H^+ concentration. Determine the weight in grams of sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$; FW 138) and disodium hydrogen phosphate (Na_2HPO_4; FW 142) needed to prepare 1 L of a standard buffer at pH 7.00 with a total phosphate concentration of 0.100 M (see Fig. 2–15). See Problem 18 for the pK_a values of phosphoric acid.

Answer In solution, the two salts ionize as indicated below.



Sodium dihydrogen phosphate
(sodium phosphate, monobasic)
$NaH_2PO_4 \cdot H_2O$



Disodium hydrogen phosphate
(sodium phosphate, dibasic)
Na_2HPO_4

The buffering capacity of the solution is determined by the concentration ratio of proton acceptor (A^-) to proton donor (HA), or $[HPO_4^{2-}]/[H_2PO_4^-]$. From Figure 2–15, the pK_a for the dissociation of the ionizable hydrogen of dihydrogen phosphate

$$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$$

is 6.86. Using the Henderson-Hasselbalch equation,

$$\text{pH} = \text{p}K_a + \log \frac{[A^-]}{[HA]}$$

we calculate:

$$7.00 - 6.86 = \log \frac{[A^-]}{[HA]}$$

$$\frac{[A^-]}{[HA]} = 10^{0.14} = 1.38$$

This ratio is approximately 7/5; that is, 7 parts Na_2HPO_4 to 5 parts $NaH_2PO_4 \cdot H_2O$. Because $[HPO_4^{2-}] + [H_2PO_4^-] = 0.100$ M, $[H_2PO_4^-] = 0.100$ M $- [HPO_4^{2-}]$, and we can now calculate the amount of each salt required for a 0.100 M solution:

$$\frac{[HPO_4^{2-}]}{0.100 \text{ M} - [HPO_4^{2-}]} = 1.38$$

Solving for $[HPO_4^{2-}]$,

$$[HPO_4^{2-}] = \frac{0.138}{2.38} \text{ M} = 0.058 \text{ M} = 0.058 \text{ mol/L}$$

and $[H_2PO_4^-] = 0.100$ M $- 0.058$ M $= 0.042$ M $= 0.042$ mol/L.

The amount needed for 1 L of solution = FW (mol/L).

For $NaH_2PO_4 \cdot H_2O$: (138 g/mol)(0.042 mol/L) = 5.8 g/L

For Na_2HPO_4: (142 g/mol)(0.058 mol/L) = 8.2 g/L

20. Calculation of Molar Ratios of Conjugate Base to Weak Acid from pH For a weak acid with a pK_a of 6.0, calculate the ratio of conjugate base to acid at a pH of 5.0.

Answer Using the Henderson-Hasselbalch equation,

$$\text{pH} = \text{p}K_a + \log ([A^-]/[HA])$$

$$5.0 = 6.0 + \log ([A^-]/[HA])$$

$$\log ([A^-]/[HA]) = -1.0$$

$$[A^-]/[HA] = 10^{-1.0} = 0.10$$

21. Preparation of Buffer of Known pH and Strength Given 0.10 M solutions of acetic acid (pK_a = 4.76) and sodium acetate, describe how you would go about preparing 1.0 L of 0.10 M acetate buffer of pH 4.00.

Answer Use the Henderson-Hasselbalch equation to calculate the ratio $[Ac^-]/[HAc]$ in the final buffer.

$$\text{pH} = \text{p}K_a + \log ([Ac^-]/[HAc])$$

$$\log ([Ac^-]/[HAc]) = \text{pH} - \text{p}K_a = 4.00 - 4.76 = -0.76$$

$$[Ac^-]/[HAc] = 10^{-0.76}$$

The fraction of the solution that is $Ac^- = [Ac^-]/[HAc + Ac^-] = 10^{-0.76}/(1 + 10^{-0.76}) = 0.148$, which must be rounded to 0.15 (two significant figures). Therefore, to make 1.0 L of acetate buffer, use 150 mL of sodium acetate and 850 mL of acetic acid.

22. Choice of Weak Acid for a Buffer Which of these compounds would be the best buffer at pH 5.0: formic acid (pK_a = 3.8), acetic acid (pK_a = 4.76), or ethylamine (pK_a = 9.0)? Briefly justify your answer.

Answer Acetic acid; its pK_a is closest to the desired pH.

23. Working with Buffers A buffer contains 0.010 mol of lactic acid ($pK_a = 3.86$) and 0.050 mol of sodium lactate per liter. **(a)** Calculate the pH of the buffer. **(b)** Calculate the change in pH when 5 mL of 0.5 M HCl is added to 1 L of the buffer. **(c)** What pH change would you expect if you added the same quantity of HCl to 1 L of pure water?

Answer Using the Henderson-Hasselbalch equation,

$$\text{pH} = pK_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

(a) pH = pK_a + log ([lactate]/[lactic acid]) = 3.86 + log (0.050 M/0.010 M) = 3.86 + 0.70 = 4.56. Thus, the pH is 4.6.

(b) Strong acids ionize completely, so 0.005 L × 0.5 mol/L = 0.002 mol of H^+ is added. The added acid will convert some of the salt form to the acid form. Thus, the final pH is

$$\text{pH} = 3.86 + \log [(0.050 - 0.0025)/(0.010 - 0.0025)]$$

$$= 3.86 + 0.58 = 4.44$$

The change in pH = 4.56 − 4.44 = 0.12, which rounds to 0.1 pH unit.

(c) HCl completely dissociates. So, when 5 mL of 0.5 M HCl is added to 1 L of water,

$$[\text{H}^+] = (0.002 \text{ mol})/(1 \text{ L}) = 0.002 \text{ mol/L} = 0.002 \text{ M}$$

$$\text{pH} = -\log 0.002 = 2.7$$

The pH of pure water is 7.0, so the change in pH = 7.0 – 2.7 = 4.3, which rounds to 4 pH units.

24. Use of Molar Concentrations to Calculate pH What is the pH of a solution that contains 0.20 M sodium acetate and 0.60 M acetic acid ($pK_a = 4.76$)?

Answer pH = pK_a + log ([base]/[acid])

= pK_a + log ([acetate]/[acetic acid])

= 4.76 + log (0.20/0.60)

= 4.76 + (−0.48) = 4.3 (two significant figures, based on precision of concentrations)

25. Preparation of an Acetate Buffer Calculate the concentrations of acetic acid ($pK_a = 4.76$) and sodium acetate necessary to prepare a 0.2 M buffer solution at pH 5.0.

Answer First, calculate the required ratio of conjugate base to acid.

$$\text{pH} = pK_a + \log ([\text{acetate}]/[\text{acetic acid}])$$

$$\log ([\text{acetate}]/[\text{acetic acid}]) = \text{pH} - pK_a = 5.0 - 4.76 = 0.24$$

$$[\text{acetate}]/[\text{acetic acid}] = 10^{0.24} = 1.7$$

$$[\text{acetate}]/[\text{acetate + acetic acid}] = 1.7/2.7 = 0.63 \text{ (two significant figures)}$$

Thus, 63% of the 0.2 M buffer is acetate and 27% is acetic acid. So at pH 5.0 the buffer has 0.13 M acetate and 0.07 M acetic acid.

26. pH of Insect Defensive Secretion You have been observing an insect that defends itself from enemies by secreting a caustic liquid. Analysis of the liquid shows it to have a total concentration of formate plus formic acid ($K_a = 1.8 \times 10^{-4}$) of 1.45 M; the concentration of formate ion is 0.015 M. What is the pH of the secretion?

Answer Solve the Henderson-Hasselbalch equation for pH.

$$\text{pH} = \text{p}K_a + \log\,([\text{conjugate base}]/[\text{acid}])$$

Given the K_a of formic acid ($K_a = 1.8 \times 10^{-4}$), you can calculate pK_a as $-\log K_a = 3.7$. If the concentration of formate + formic acid = 1.45 M and the concentration of formate is 0.015 M, then the concentration of formic acid is 1.45 M − 0.015 M + 1.435 M.

$$\log\,([\text{formate}]/[\text{formic acid}]) = \log\,(0.015/1.435) = -2.0$$

$$\text{pH} = 3.7 - 2.0 = 1.7 \text{ (two significant figures)}$$

27. Calculation of pK_a An unknown compound, X, is thought to have a carboxyl group with a pK_a of 2.0 and another ionizable group with a pK_a between 5 and 8. When 75 mL of 0.1 M NaOH is added to 100 mL of a 0.1 M solution of X at pH 2.0, the pH increases to 6.72. Calculate the pK_a of the second ionizable group of X.

Answer At the first pH (pH = 2), 50% of the carboxyl group is dissociated (pK_a = pH). Then

$$\text{Amount of NaOH added} = 0.075 \text{ L} \times 0.1 \text{ mol/L} = 0.0075 \text{ mol}$$

$$\text{Amount of X present} = 0.1 \text{ L} \times 0.1 \text{ mol/L} = 0.01 \text{ mol}$$

At the new pH of 6.72, the carboxyl group is completely dissociated (because pH is much greater than the pK_a). The amount of NaOH required to titrate this remaining 50% of the carboxyl group is 0.5 × 0.01 mol = 0.005 mol.

Thus, 0.0075 mol − 0.005 mol = 0.0025 mol of NaOH is available to titrate the other group, and, using the Henderson-Hasselbalch equation,

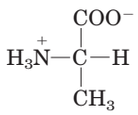
$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

we can find the pK_a of the second ionizable group of X:

$$6.72 = \text{p}K_a + \log\,[0.0025/(0.01 - 0.0025)]$$

$$\text{p}K_a = 6.72 - (-0.48) = 7.20, \text{ which rounds to } 7.$$

28. Ionic Forms of Alanine Alanine is a diprotic acid that can undergo two dissociation reactions (see Table 3–1 for pK_a values). **(a)** Given the structure of the partially protonated form (or zwitterion; see Fig. 3–9) below, draw the chemical structures of the other two forms of alanine that predominate in aqueous solution: the fully protonated form and the fully deprotonated form.

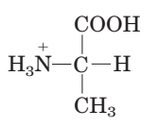


Alanine

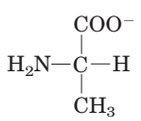
Of the three possible forms of alanine, which would be present at the highest concentration in solutions of the following pH: **(b)** 1.0; **(c)** 6.2; **(d)** 8.02; **(e)** 11.9. Explain your answers in terms of pH relative to the two pK_a values.

Answer

(a)



Fully protonated



Fully deprotonated

(b) At pH 1.0, 1.3 pH units below the pK_a of the carboxyl group, more than 90% of the carboxyl groups are protonated, and protonated amino groups predominate by a factor of more than 107.

(c) At pH 6.2 the zwitterion predominates. This is 4 pH units above the pK_a of the carboxyl group, so the vast majority of carboxyl groups are deprotonated. It is 3.5 pH units below the pK_a of the amino group, so the vast majority of amino groups are protonated.

(d) At pH 8.02 the zwitterion still predominates. The carboxyl groups are deprotonated and, with the pH still 1.6 units below the pK_a of the amino group, the vast majority of amino groups are protonated.

(e) At pH 11.9, 2.2 pH units above the pK_a of the amino group, the vast majority of amino groups are deprotonated; and the carboxyl groups, at 9.6 pH units above their pK_a, remain deprotonated.

29. Control of Blood pH by Respiration Rate

(a) The partial pressure of CO_2 in the lungs can be varied rapidly by the rate and depth of breathing. For example, a common remedy to alleviate hiccups is to increase the concentration of CO_2 in the lungs. This can be achieved by holding one's breath, by very slow and shallow breathing (hypoventilation), or by breathing in and out of a paper bag. Under such conditions, pCO_2 in the air space of the lungs rises above normal. Qualitatively explain the effect of these procedures on the blood pH.

(b) A common practice of competitive short-distance runners is to breathe rapidly and deeply (hyperventilate) for about half a minute to remove CO_2 from their lungs just before the race begins. Blood pH may rise to 7.60. Explain why the blood pH increases.

(c) During a short-distance run, the muscles produce a large amount of lactic acid ($CH_3CH(OH)COOH$, $K_a = 1.38 \times 10^{-4}$) from their glucose stores. In view of this fact, why might hyperventilation before a dash be useful?

Answer

(a) Blood pH is controlled by the carbon dioxide–bicarbonate buffer system, as shown by the net equation

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

During *hypoventilation,* the concentration of CO_2 in the lungs and arterial blood increases, driving the equilibrium to the right and raising the $[H^+]$; that is, the pH is lowered.

(b) During *hyperventilation,* the concentration of CO_2 in the lungs and arterial blood falls. This drives the equilibrium to the left, which requires the consumption of hydrogen ions, reducing $[H^+]$ and increasing pH.

(c) Lactate is a moderately strong acid (pK_a = 3.86) that completely dissociates under physiological conditions:

$$CH_3CH(OH)COOH \rightleftharpoons CH_3CH(OH)COO^- + H^+$$

This lowers the pH of the blood and muscle tissue. Hyperventilation is useful because it removes hydrogen ions, raising the pH of the blood and tissues in anticipation of the acid buildup.

30. Calculation of Blood pH from CO_2 and Bicarbonate Levels Calculate the pH of a blood plasma sample with a total CO_2 concentration of 26.9 mM and bicarbonate concentration of 25.6 mM. Recall from page 63 that the relevant pK_a of carbonic acid is 6.1.

Answer Use the Henderson-Hasselbalch equation:

$$\text{pH} = \text{p}K_a + \log\,([\text{bicarbonate}]/[\text{carbonic acid}])$$

If total $[CO_2] = 26.9$ M and [bicarbonate] = 25.6 M, then the concentration of carbonic acid is 26.9 M − 25.6 M = 1.3 M.

$$\text{pH} = 6.1 + \log\,(25.6/1.3) = 7.4 \text{ (two significant figures)}$$

31. Effect of Holding One's Breath on Blood pH The pH of the extracellular fluid is buffered by the bicarbonate/carbonic acid system. Holding your breath can increase the concentration of $CO_2(g)$ in the blood. What effect might this have on the pH of the extracellular fluid? Explain by showing the relevant equilibrium equation(s) for this buffer system.

Answer Dissolving more CO_2 in the blood increases $[H^+]$ in blood and extracellular fluids, lowering pH: $CO_2(d) + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$

Data Analysis Problem

32. "Switchable" Surfactants Hydrophobic molecules do not dissolve well in water. Given that water is a very commonly used solvent, this makes certain processes very difficult: washing oily food residue off dishes, cleaning up spilled oil, keeping the oil and water phases of salad dressings well mixed, and carrying out chemical reactions that involve both hydrophobic and hydrophilic components.

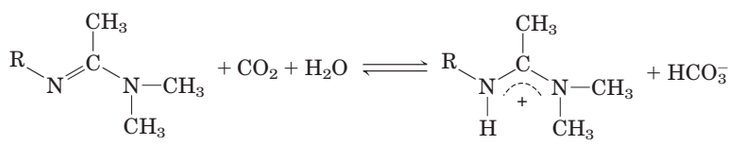
Surfactants are a class of amphipathic compounds that includes soaps, detergents, and emulsifiers. With the use of surfactants, hydrophobic compounds can be suspended in aqueous solution by forming micelles (see Fig. 2–7). A micelle has a hydrophobic core consisting of the hydrophobic compound and the hydrophobic "tails" of the surfactant; the hydrophilic "heads" of the surfactant cover the surface of the micelle. A suspension of micelles is called an emulsion. The more hydrophilic the head group of the surfactant, the more powerful it is—that is, the greater its capacity to emulsify hydrophobic material.

When you use soap to remove grease from dirty dishes, the soap forms an emulsion with the grease that is easily removed by water through interaction with the hydrophilic head of the soap molecules. Likewise, a detergent can be used to emulsify spilled oil for removal by water. And emulsifiers in commercial salad dressings keep the oil suspended evenly throughout the water-based mixture.

There are some situations in which it would be very useful to have a "switchable" surfactant: a molecule that could be reversibly converted between a surfactant and a nonsurfactant.

(a) Imagine such a "switchable" surfactant existed. How would you use it to clean up and then recover the oil from an oil spill?

Liu et al. describe a prototypical switchable surfactant in their 2006 article "Switchable Surfactants." The switching is based on the following reaction:



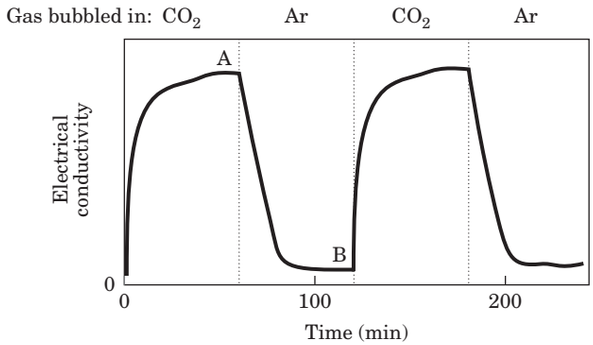
Amidine form Amidinium form

(b) Given that the pK_a of a typical amidinium ion is 12.4, in which direction (left or right) would you expect the equilibrium of the above reaction to lie? (See Fig. 2–16 for relevant pK_a values.) Justify your answer. Hint: Remember the reaction $H_2O + CO_2 \rightleftharpoons H_2CO_3$.

Liu and colleagues produced a switchable surfactant for which $R = C_{16}H_{33}$. They do not name the molecule in their article; for brevity, we'll call it s-surf.

(c) The amidinium form of s-surf is a powerful surfactant; the amidine form is not. Explain this observation.

Liu and colleagues found that they could switch between the two forms of s-surf by changing the gas that they bubbled through a solution of the surfactant. They demonstrated this switch by measuring the electrical conductivity of the s-surf solution; aqueous solutions of ionic compounds have higher conductivity than solutions of nonionic compounds. They started with a solution of the amidine form of s-surf in water. Their results are shown below; dotted lines indicate the switch from one gas to another.



(d) In which form is the majority of s-surf at point A? At point B?

(e) Why does the electrical conductivity rise from time 0 to point A?

(f) Why does the electrical conductivity fall from point A to point B?

(g) Explain how you would use s-surf to clean up and recover the oil from an oil spill.

Answer

(a) Use the substance in its surfactant form to emulsify the spilled oil, collect the emulsified oil, then switch to the nonsurfactant form. The oil and water will separate and the oil can be collected for further use.

(b) The equilibrium lies strongly to the right. The stronger acid (lower pK_a), H_2CO_3, donates a proton to the conjugate base of the weaker acid (higher pK_a), amidine.

(c) The strength of a surfactant depends on the hydrophilicity of its head groups: the more hydrophilic, the more powerful the surfactant. The amidinium form of s-surf is much more hydrophilic than the amidine form, so it is a more powerful surfactant.

(d) *Point A:* amidinium; the CO_2 has had plenty of time to react with the amidine to produce the amidinium form. *Point B:* amidine; Ar has removed CO_2 from the solution, leaving the amidine form.

(e) The conductivity rises as uncharged amidine reacts with CO_2 to produce the charged amidinium form.

(f) The conductivity falls as Ar removes CO_2, shifting the equilibrium to the uncharged amidine form.

(g) Treat s-surf with CO_2 to produce the surfactant amidinium form and use this to emulsify the spill. Treat the emulsion with Ar to remove the CO_2 and produce the nonsurfactant amidine from. The oil will separate from the water and can be recovered.

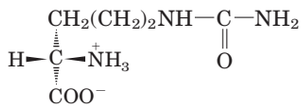
Reference

Liu, Y., Jessop, P.G., Cunningham, M., Eckert, C.A., & Liotta, C.L. (2006) *Science* **313,** 958–960.

chapter

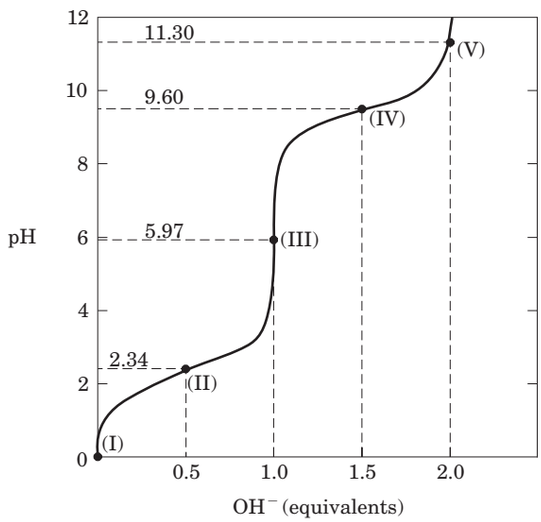
3 Amino Acids, Peptides, and Proteins

1. Absolute Configuration of Citrulline The citrulline isolated from watermelons has the structure shown below. Is it a D- or L-amino acid? Explain.



Answer Rotating the structural representation 180° in the plane of the page puts the most highly oxidized group—the carboxyl (—COO^-) group—at the top, in the same position as the —CHO group of glyceraldehyde in Figure 3–4. In this orientation, the α-amino group is on the left, and thus the absolute configuration of the citrulline is L.

2. Relationship between the Titration Curve and the Acid-Base Properties of Glycine A 100 mL solution of 0.1 M glycine at pH 1.72 was titrated with 2 M NaOH solution. The pH was monitored and the results were plotted as shown in the following graph. The key points in the titration are designated I to V. For each of the statements **(a)** to **(o),** *identify* the appropriate key point in the titration and *justify* your choice.



Note: before considering statements **(a)** through **(o),** refer to Figure 3–10. The three species involved in the titration of glycine can be considered in terms of a useful physical analogy. Each ionic species can be viewed as a different floor of a building, each with a different net charge:

$^{+}H_3N—CH_2—COOH$	+1
$^{+}H_3N—CH_2—COO^{-}$	0 (zwitterion)
$H_2N—CH_2—COO^{-}$	−1

The floors are connected by steep stairways, and each stairway has a landing halfway between the floors. A titration curve traces the path one would follow between the different floors as the pH changes in response to added OH^{-}. Recall that the pK_a of an acid (on a halfway landing) represents the pH at which half of the acid is deprotonated. The isoelectric point (pI) is the pH at which the average net charge is zero. Now you are ready to consider statements **(a)** through **(o).**

(a) Glycine is present predominantly as the species $^{+}H_3N—CH_2—COOH$.

(b) The *average* net charge of glycine is $+\frac{1}{2}$.

(c) Half of the amino groups are ionized.

(d) The pH is equal to the pK_a of the carboxyl group.

(e) The pH is equal to the pK_a of the protonated amino group.

(f) Glycine has its maximum buffering capacity.

(g) The *average* net charge of glycine is zero.

(h) The carboxyl group has been completely titrated (first equivalence point).

(i) Glycine is completely titrated (second equivalence point).

(j) The predominant species is $^{+}H_3N—CH_2—COO^{-}$.

(k) The *average* net charge of glycine is −1.

(l) Glycine is present predominantly as a 50:50 mixture of $^{+}H_3N—CH_2—COOH$ and $^{+}H_3N—CH_2—COO^{-}$.

(m) This is the isoelectric point.

(n) This is the end of the titration.

(o) These are the *worst* pH regions for buffering power.

Answer

(a) I; maximum protonation occurs at the lowest pH (the highest $[H^{+}]$).

(b) II; at the first pK_a, or pK_1 (2.34), half of the protons are removed from the α-carboxyl group (i.e., it is half deprotonated), changing its charge from 0 to $-\frac{1}{2}$. The average net charge of glycine is $(-\frac{1}{2}) + 1 = \frac{1}{2}$.

(c) IV; the α-amino group is half-deprotonated at its pK_a, or pK_2 (9.60).

(d) II; from the Henderson-Hasselbalch equation, pH = pK_a + log ($[A^{-}]/[HA]$). If $[A^{-}]/[HA] = 1$, or $[A^{-}] = [HA]$, then pH = pK_a. (Recall that log 1 = 0.)

(e) IV; see answers **(c)** and **(d).**

(f) II and IV; in the pK_a regions, acid donates protons to or base abstracts protons from glycine, with minimal pH changes.

(g) III; this occurs at the isoelectric point; pI = (pK_1 + pK_2)/2 = (2.34 + 9.60)/2 = 5.97.

(h) III; the pH at which 1.0 equivalent of OH^{-} has been added, pH 5.97 (3.6 pH units away from either pK_a).

(i) V; pH 11.3 (1.7 pH units above pK_2).

(j) III; at pI (5.97) the carboxyl group is fully negatively charged (deprotonated) and the amino group is fully positively charged (protonated).

(k) V; both groups are fully deprotonated, with a neutral amino group and a negatively charged carboxyl group (net charge = −1).

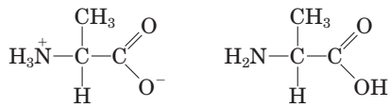
(l) II; the carboxyl group is half ionized at pH = pK_1.

(m) III; see answers **(g)** and **(j).**

(n) V; glycine is fully titrated after 2.0 equivalents of OH^- have been added.

(o) I, III, and V; each is several pH units removed from either pK_a, where the best pH buffering action occurs.

3. How Much Alanine Is Present as the Completely Uncharged Species? At a pH equal to the isoelectric point of alanine, the *net* charge on alanine is zero. Two structures can be drawn that have a net charge of zero, but the predominant form of alanine at its pI is zwitterionic.



Zwitterionic Uncharged

(a) Why is alanine predominantly zwitterionic rather than completely uncharged at its pI?

(b) What fraction of alanine is in the completely uncharged form at its pI? Justify your assumptions.

Answer

(a) The pI of alanine is well above the pK_a of the α-carboxyl group and well below the pK_a of the α-amino group. Hence, at pH = pI, both groups are present predominantly in their charged (ionized) forms.

(b) From Table 3–1, the pI of alanine is 6.01, midway between the two pK_a values 2.34 and 9.69. From the Henderson-Hasselbalch equation, $\text{pH} - \text{p}K_a = \log([A^-]/[HA])$. For the carboxyl group:

$$\log \frac{[A^-]}{[HA]} = 6.01 - 2.34 = 3.67$$

$$\frac{[HA]}{[A^-]} = 10^{-3.67} = \frac{1}{4.68 \times 10^3}$$

That is, one molecule in 4,680 is still in the form —COOH. Similarly, at pH = pI, one molecule in 4,680 is in the form $—NH_2$. Thus, the fraction of molecules with both groups uncharged (—COOH *and* $—NH_2$) is 1 in 4,680 × 4,680, or 1 in 2.19×10^7.

4. Ionization State of Histidine Each ionizable group of an amino acid can exist in one of two states, charged or neutral. The electric charge on the functional group is determined by the relationship between its pK_a and the pH of the solution. This relationship is described by the Henderson-Hasselbalch equation.

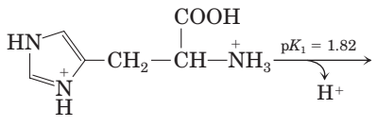
(a) Histidine has three ionizable functional groups. Write the equilibrium equations for its three ionizations and assign the proper pK_a for each ionization. Draw the structure of histidine in each ionization state. What is the net charge on the histidine molecule in each ionization state?

(b) Draw the structures of the predominant ionization state of histidine at pH 1, 4, 8, and 12. Note that the ionization state can be approximated by treating each ionizable group independently.

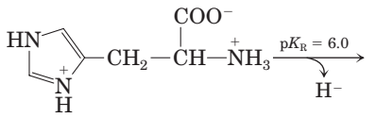
(c) What is the net charge of histidine at pH 1, 4, 8, and 12? For each pH, will histidine migrate toward the anode (+) or cathode (−) when placed in an electric field?

Answer

(a)



1



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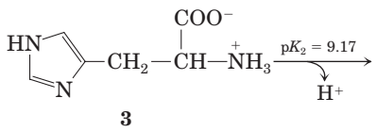

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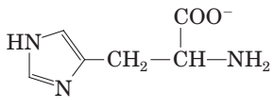


4

We start with the most highly protonated species of histidine (structure **1,** found at the most acidic pH). The pK_a values are from Table 3–1. As base is added, the group with the lowest pK_a loses its proton first, followed by the group with the next lowest pK_a, then the group with the highest pK_a. (In the following table, R = —CH_2–imidazole.)

Structure	Net charge
1 $^{+}H_3N$—CH(RH^{+})—COOH	+2
2 $^{+}H_3N$—CH(RH^{+})—COO^{-}	+1
3 $^{+}H_3N$—CH(R)—COO^{-}	0
4 H_2N—CH(R)—COO^{-}	−1

(b) and **(c)** See the structures in **(a).**

pH	Structure	Net charge	Migrates toward:
1	**1**	+2	Cathode
4	**2**	+1	Cathode
8	**3**	0	Does not migrate
12	**4**	−1	Anode

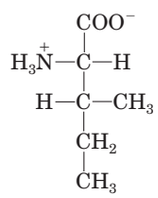
5. Separation of Amino Acids by Ion-Exchange Chromatography Mixtures of amino acids can be analyzed by first separating the mixture into its components through ion-exchange chromatography. Amino acids placed on a cation-exchange resin (see Fig. 3–17a) containing sulfonate ($—SO_3^-$) groups flow down the column at different rates because of two factors that influence their movement: (1) ionic attraction between the sulfonate residues on the column and positively charged functional groups on the amino acids, and (2) hydrophobic interactions between amino acid side chains and the strongly hydrophobic backbone of the polystyrene resin. For each pair of amino acids listed, determine which will be eluted first from an ion-exchange column by a pH 7.0 buffer.

(a) Asp and Lys
(b) Arg and Met
(c) Glu and Val
(d) Gly and Leu
(e) Ser and Ala

Answer See Table 3–1 for pK_a values for the amino acid side chains. At pH < pI, an amino acid has a net positive charge; at pH > pI, it has a net negative charge. For any pair of amino acids, the more negatively charged one passes through the sulfonated resin faster. For two neutral amino acids, the less polar one passes through more slowly because of its stronger hydrophobic interactions with the polystyrene.

	pI values	Net charge (pH 7)	Elution order	Basis for separation
(a) Asp, Lys	2.77, 9.74	−1, +1	Asp, Lys	Charge
(b) Arg, Met	10.76, 5.74	+1, 0	Met, Arg	Charge
(c) Glu, Val	3.22, 5.97	−1, 0	Glu, Val	Charge
(d) Gly, Leu	5.97, 5.98	0, 0	Gly, Leu	Polarity
(e) Ser, Ala	5.68, 6.01	0, 0	Ser, Ala	Polarity

6. Naming the Stereoisomers of Isoleucine The structure of the amino acid isoleucine is

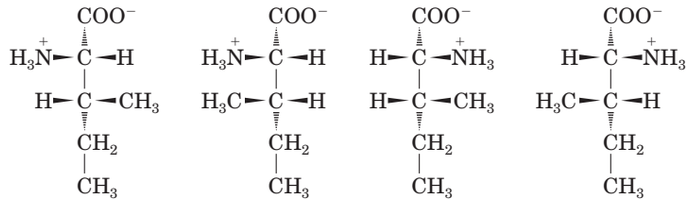


(a) How many chiral centers does it have?
(b) How many optical isomers?
(c) Draw perspective formulas for all the optical isomers of isoleucine.

Answer

(a) Two; at C-2 and C-3 (the α and β carbons).
(b) Four; the two chiral centers permit four possible diastereoisomers: (*S,S*), (*S,R*), (*R,R*), and (*R,S*).

(c)



7. Comparing the pK_a Values of Alanine and Polyalanine The titration curve of alanine shows the ionization of two functional groups with pK_a values of 2.34 and 9.69, corresponding to the ionization of the carboxyl and the protonated amino groups, respectively. The titration of di-, tri-, and larger oligopeptides of alanine also shows the ionization of only two functional groups, although the experimental pK_a values are different. The trend in pK_a values is summarized in the table.

Amino acid or peptide	pK_1	pK_2
Ala	2.34	9.69
Ala–Ala	3.12	8.30
Ala–Ala–Ala	3.39	8.03
Ala–(Ala)$_n$–Ala, $n \geq 4$	3.42	7.94

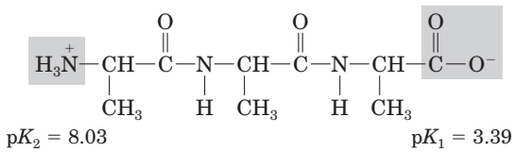
(a) Draw the structure of Ala–Ala–Ala. Identify the functional groups associated with pK_1 and pK_2.

(b) Why does the value of pK_1 *increase* with each additional Ala residue in the Ala oligopeptide?

(c) Why does the value of pK_2 *decrease* with each additional Ala residue in the Ala oligopeptide?

Answer

(a) The structure at pH 7 is:



Note that only the amino- and carboxyl-terminal groups ionize.

(b) As the length of poly(Ala) increases, the two terminal groups move farther apart, separated by an intervening sequence with an "insulating" nonpolar structure. The carboxyl group becomes a weaker acid, as reflected in its higher pK_a, because the electrostatic repulsion between the carboxyl proton and the positive charge on the NH_3^+ group diminishes as the groups become more distant.

(c) The negative charge on the terminal carboxyl group has a stabilizing effect on the positively charged (protonated) terminal amino group. With increasing numbers of intervening Ala residues, this stabilizing effect is diminished and the NH_3^+ group loses its

proton more easily. The lower pK_2 indicates that the terminal amino group has become a weaker base (stronger acid). The intramolecular effects of the amide (peptide bond) linkages keep pK_a values lower than they would be for an alkyl-substituted amine.

8. The Size of Proteins What is the approximate molecular weight of a protein with 682 amino acid residues in a single polypeptide chain?

Answer Assuming that the average M_r per residue is 110 (corrected for loss of water in formation of the peptide bond), a protein containing 682 residues has an M_r of approximately $682 \times 110 = 75{,}000$.

9. The Number of Tryptophan Residues in Bovine Serum Albumin A quantitative amino acid analysis reveals that bovine serum albumin (BSA) contains 0.58% tryptophan (M_r 204) by weight.

(a) Calculate the *minimum* molecular weight of BSA (i.e., assuming there is only one tryptophan residue per protein molecule).

(b) Gel filtration of BSA gives a molecular weight estimate of 70,000. How many tryptophan residues are present in a molecule of serum albumin?

Answer

(a) The M_r of a Trp residue must be adjusted to account for the removal of water during peptide bond formation: $M_r = 204 - 18 = 186$. The molecular weight of BSA can be calculated using the following proportionality, where n is the number of Trp residues in the protein:

$$\frac{\text{wt Trp}}{\text{wt BSA}} = \frac{n(M_r\ \text{Trp})}{M_r\ \text{BSA}}$$

$$\frac{0.58\ \text{g}}{100\ \text{g}} = \frac{n(186)}{M_r\ \text{BSA}}$$

A *minimum* molecular weight can be found by assuming only one Trp residue per BSA molecule ($n = 1$).

$$\frac{(100\ \text{g})(186)(1)}{0.58\text{g}} = 32{,}000$$

(b) Given that the M_r of BSA is approximately 70,000, BSA has ~ 70,000/32,000 = 2.2, or 2 Trp residues per molecule. (The remainder from this division suggests that the estimate of M_r 70,000 for BSA is somewhat high.)

10. Subunit Composition of a Protein A protein has a molecular mass of 400 kDa when measured by gel filtration. When subjected to gel electrophoresis in the presence of sodium dodecyl sulfate (SDS), the protein gives three bands with molecular masses of 180, 160, and 60 kDa. When electrophoresis is carried out in the presence of SDS and dithiothreitol, three bands are again formed, this time with molecular masses of 160, 90, and 60 kDa. Determine the subunit composition of the protein.

Answer The protein has four subunits, with molecular masses of 160, 90, 90, and 60 kDa. The two 90 kDa subunits (possibly identical) are linked by one or more disulfide bonds.

11. Net Electric Charge of Peptides A peptide has the sequence

Glu–His–Trp–Ser–Gly–Leu–Arg–Pro–Gly

(a) What is the net charge of the molecule at pH 3, 8, and 11? (Use pK_a values for side chains and terminal amino and carboxyl groups as given in Table 3–1.)

(b) Estimate the pI for this peptide.

Answer

(a) When pH > pK_a, ionizing groups lose their protons. The pK_a values of importance here are those of the amino-terminal (9.67) and carboxyl-terminal (2.34) groups and those of the R groups of the Glu (4.25), His (6.00), and Arg (12.48) residues. **Note:** here we are using the available pK_a values: those for the *free* amino acids as given in Table 3–1. As demonstrated in Problem 7, however, the pK_a values of the α-amino and α-carboxyl groups shift when the amino acid is at the amino or carboxyl terminus, respectively, of a peptide, and this would affect the net charge and the pI of the peptide.

pH	^+H_3N	Glu	His	Arg	COO^-	Net charge
3	+1	0	+1	+1	−1	+2
8	+1	−1	0	+1	−1	0
11	0	−1	0	+1	−1	−1

(b) Two different methods can be used to estimate pI. Find the two ionizable groups with pK_a values that "straddle" the point at which net peptide charge = 0 (here, two groups that ionize near pH 8): the amino-terminal α-amino group of Glu and the His imidazole group. Thus, we can estimate

$$\text{pI} = \frac{9.67 + 6.00}{2} = 7.8$$

Alternatively, plot the calculated net charges as a function of pH, and determine graphically the pH at which the net charge is zero on the vertical axis. More data points are needed to use this method accurately.

Note: although at any instant an individual amino acid molecule will have an integral charge, it is possible for a population of amino acid molecules in solution to have a fractional charge. For example, at pH 1.0 glycine exists entirely as the form $^+H_3N—CH_2—COOH$ with a net positive charge of 1.0. However, at pH 2.34, where there is an equal mixture of $^+H_3N—CH_2—COOH$ and $^+H_3N—CH_2—COO^-$, the average or net charge on the population of glycine molecules is 0.5 (see the discussion on pp. 80–81). You can use the Henderson-Hasselbalch equation to calculate the exact ratio of charged and uncharged species at equilibrium at various pH values.

12. Isoelectric Point of Pepsin Pepsin is the name given to a mix of several digestive enzymes secreted (as larger precursor proteins) by glands that line the stomach. These glands also secrete hydrochloric acid, which dissolves the particulate matter in food, allowing pepsin to enzymatically cleave individual protein molecules. The resulting mixture of food, HCl, and digestive enzymes is known as chyme and has a pH near 1.5. What pI would you predict for the pepsin proteins? What functional groups must be present to confer this pI on pepsin? Which amino acids in the proteins would contribute such groups?

Answer Pepsin proteins have a relatively low pI (near the pH of gastric juice) in order to remain soluble and thus functional in the stomach. (Pepsin—the mixture of enzymes—has a pI of ~1.) As pH increases, pepsins acquire a net charge and undergo ionic interactions with oppositely charged molecules (such as dissolved salts), causing the pepsin proteins to precipitate. Pepsin is active only in the stomach. In the relatively high pH of the intestine, pepsin proteins precipitate and become inactive.

A low pI requires large numbers of negatively charged (low pK_a) groups. These are contributed by the carboxylate groups of Asp and Glu residues.

13. The Isoelectric Point of Histones Histones are proteins found in eukaryotic cell nuclei, tightly bound to DNA, which has many phosphate groups. The pI of histones is very high, about 10.8. What amino acid residues must be present in relatively large numbers in histones? In what way do these residues contribute to the strong binding of histones to DNA?

Answer Large numbers of positively charged (high pK_a) groups in a protein give it a high pI. In histones, the positively charged R groups of Lys, Arg, and His residues interact strongly with the negatively charged phosphate groups of DNA through ionic interactions.

14. Solubility of Polypeptides One method for separating polypeptides makes use of their different solubilities. The solubility of large polypeptides in water depends upon the relative polarity of their R groups, particularly on the number of ionized groups: the more ionized groups there are, the more soluble the polypeptide. Which of each pair of the polypeptides that follow is more soluble at the indicated pH?

(a) $(Gly)_{20}$ or $(Glu)_{20}$ at pH 7.0

(b) $(Lys–Ala)_3$ or $(Phe–Met)_3$ at pH 7.0

(c) $(Ala–Ser–Gly)_5$ or $(Asn–Ser–His)_5$ at pH 6.0

(d) $(Ala–Asp–Gly)_5$ or $(Asn–Ser–His)_5$ at pH 3.0

Answer

(a) $(Glu)_{20}$; it is highly negatively charged (polar) at pH 7. $(Gly)_{20}$ is uncharged except for the amino- and carboxyl-terminal groups.

(b) $(Lys–Ala)_3$; this is highly positively charged (polar) at pH 7. $(Phe–Met)_3$ is much less polar and hence less soluble.

(c) $(Asn–Ser–His)_5$; both polymers have polar Ser side chains, but $(Asn–Ser–His)_5$ also has the polar Asn side chains and partially protonated His side chains.

(d) $(Asn–Ser–His)_5$; at pH 3, the carboxylate groups of Asp residues are partially protonated and neutral, whereas the imidazole groups of His residues are fully protonated and positively charged.

15. Purification of an Enzyme A biochemist discovers and purifies a new enzyme, generating the purification table below.

Procedure	Total protein (mg)	Activity (units)
1. Crude extract	20,000	4,000,000
2. Precipitation (salt)	5,000	3,000,000
3. Precipitation (pH)	4,000	1,000,000
4. Ion-exchange chromatography	200	800,000
5. Affinity chromatography	50	750,000
6. Size-exclusion chromatography	45	675,000

(a) From the information given in the table, calculate the specific activity of the enzyme after each purification procedure.

(b) Which of the purification procedures used for this enzyme is most effective (i.e., gives the greatest relative increase in purity)?

(c) Which of the purification procedures is least effective?

(d) Is there any indication based on the results shown in the table that the enzyme after step 6 is now pure? What else could be done to estimate the purity of the enzyme preparation?

Answer

(a) From the percentage recovery of activity (units), we calculate percentage yield and specific activity (units/mg).

Procedure	Protein (mg)	Activity (units)	% Yield	Specific activity (units/mg)	Purification factor (overall)
1	20,000	4,000,000	(100)	200	(1.0)
2	5,000	3,000,000	75	600	× 3.0
3	4,000	1,000,000	25	250	× 1.25
4	200	800,000	20	4,000	× 20
5	50	750,000	19	15,000	× 75
6	45	675,000	17	15,000	× 75

(b) Step 4, ion-exchange chromatography; this gives the greatest increase in specific activity (an index of purity and degree of increase in purification).

(c) Step 3, pH precipitation; two-thirds of the total activity from the previous step was lost here.

(d) Yes. The specific activity did not increase further after step 5. SDS polyacrylamide gel electrophoresis is an excellent, standard way of checking homogeneity and purity.

16. Dialysis A purified protein is in a Hepes (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)) buffer at pH 7 with 500 mM NaCl. A sample (1 mL) of the protein solution is placed in a tube made of dialysis membrane and dialyzed against 1 L of the same Hepes buffer with 0 mM NaCl. Small molecules and ions (such as Na^+, Cl^-, and Hepes) can diffuse across the dialysis membrane, but the protein cannot.

(a) Once the dialysis has come to equilibrium, what is the concentration of NaCl in the protein sample? Assume no volume changes occur in the sample during the dialysis.

(b) If the original 1 mL sample were dialyzed twice, successively, against 100 mL of the same Hepes buffer with 0 mM NaCl, what would be the final NaCl concentration in the sample?

Answer

(a) [NaCl] = 0.5 mM

(b) [NaCl] = 0.05 mM.

17. Peptide Purification At pH 7.0, in what order would the following three peptides be eluted from a column filled with a cation-exchange polymer? Their amino acid compositions are:

Protein A: Ala 10%, Glu 5%, Ser 5%, Leu 10%, Arg 10%, His 5%, Ile 10%, Phe 5%, Tyr 5%, Lys 10%, Gly 10%, Pro 5%, and Trp 10%.

Protein B: Ala 5%, Val 5%, Gly 10%, Asp 5%, Leu 5%, Arg 5%, Ile 5%, Phe 5%, Tyr 5%, Lys 5%, Trp 5%, Ser 5%, Thr 5%, Glu 5%, Asn 5%, Pro 10%, Met 5%, and Cys 5%.

Protein C: Ala 10%, Glu 10%, Gly 5%, Leu 5%, Asp 10%, Arg 5%, Met 5%, Cys 5%, Tyr 5%, Phe 5%, His 5%, Val 5%, Pro 5%, Thr 5%, Ser 5%, Asn 5%, and Gln 5%.

Answer Protein C has a net negative charge because there are more Glu and Asp residues than Lys, Arg, and His residues. Protein A has a net positive charge. Protein B has no net charge at neutral pH. A cation-exchange column has a negatively charged polymer, so protein C interacts most weakly with the column and is eluted first, followed by B, then A.

18. Sequence Determination of the Brain Peptide Leucine Enkephalin A group of peptides that influence nerve transmission in certain parts of the brain has been isolated from normal brain tissue. These peptides are known as opioids because they bind to specific receptors that also bind opiate drugs, such as morphine and naloxone. Opioids thus mimic some of the properties of opiates. Some researchers consider these peptides to be the brain's own painkillers. Using the information below, determine the amino acid sequence of the opioid leucine enkephalin. Explain how your structure is consistent with each piece of information.

(a) Complete hydrolysis by 6 M HCl at 110 °C followed by amino acid analysis indicated the presence of Gly, Leu, Phe, and Tyr in a 2:1:1:1 molar ratio.

(b) Treatment of the peptide with 1-fluoro-2,4-dinitrobenzene followed by complete hydrolysis and chromatography indicated the presence of the 2,4-dinitrophenyl derivative of tyrosine. No free tyrosine could be found.

(c) Complete digestion of the peptide with chymotrypsin followed by chromatography yielded free tyrosine and leucine, plus a tripeptide containing Phe and Gly in a 1:2 ratio.

Answer

(a) The empirical composition is (2 Gly, Leu, Phe, Tyr)$_n$.

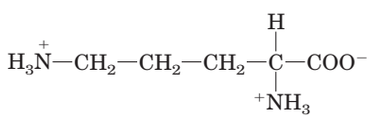
(b) Tyr is the amino-terminal residue, and there are no other Tyr residues, so $n = 1$ and the sequence is Tyr–(2 Gly, Leu, Phe).

(c) As shown in Table 3–7, chymotrypsin cleaves on the carboxyl side of aromatic residues (Phe, Trp, and Tyr). The peptide has only two aromatic residues, Tyr at the amino terminus and a Phe. Because there are three cleavage products, the Phe residue cannot be at the carboxyl terminus. Rather, release of free leucine means that Leu must be at the carboxyl terminus and must be on the carboxyl side of Phe in the peptide. Thus the sequence must be

$$\text{Tyr–(2 Gly)–Phe–Leu} = \text{Tyr–Gly–Gly–Phe–Leu}$$

19. Structure of a Peptide Antibiotic from *Bacillus brevis* Extracts from the bacterium *Bacillus brevis* contain a peptide with antibiotic properties. This peptide forms complexes with metal ions and seems to disrupt ion transport across the cell membranes of other bacterial species, killing them. The structure of the peptide has been determined from the following observations.

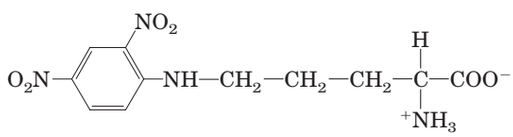
(a) Complete acid hydrolysis of the peptide followed by amino acid analysis yielded equimolar amounts of Leu, Orn, Phe, Pro, and Val. Orn is ornithine, an amino acid not present in proteins but present in some peptides. It has the structure



(b) The molecular weight of the peptide was estimated as about 1,200.

(c) The peptide failed to undergo hydrolysis when treated with the enzyme carboxypeptidase. This enzyme catalyzes the hydrolysis of the carboxyl-terminal residue of a polypeptide unless the residue is Pro or, for some reason, does not contain a free carboxyl group.

(d) Treatment of the intact peptide with 1-fluoro-2,4-dinitrobenzene, followed by complete hydrolysis and chromatography, yielded only free amino acids and the following derivative:



(Hint: note that the 2,4-dinitrophenyl derivative involves the amino group of a side chain rather than the α-amino group.)

(e) Partial hydrolysis of the peptide followed by chromatographic separation and sequence analysis yielded the following di- and tripeptides (the amino-terminal amino acid is always at the left):

Leu–Phe Phe–Pro Orn–Leu Val–Orn

Val–Orn–Leu Phe–Pro–Val Pro–Val–Orn

Given the above information, deduce the amino acid sequence of the peptide antibiotic. Show your reasoning. When you have arrived at a structure, demonstrate that it is consistent with *each* experimental observation.

Answer The information obtained from each experiment is as follows.

(a) The simplest empirical formula for the peptide is $(\text{Leu, Orn, Phe, Pro, Val})_n$.

(b) Assuming an average residue M_r of 110, the minimum molecular weight for the peptide is 550. Because $1{,}200/550 \approx 2$, the empirical formula is $(\text{Leu, Orn, Phe, Pro, Val})_2$.

(c) Failure of carboxypeptidase to cleave the peptide could result from Pro at the carboxyl terminus *or* the absence of a carboxyl-terminal residue—as in a cyclic peptide.

(d) Failure of FDNB to derivatize an α-amino group indicates either the absence of a free amino-terminal group or that Pro (an imino acid) is at the amino-terminal position. (The derivative formed is 2,4 dinitrophenyl-ε-ornithine.)

(e) The presence of Pro at an internal position in the peptide Phe–Pro–Val indicates that it is *not* at the amino or carboxyl terminus. The information from these experiments suggests that the peptide is cyclic. The alignment of overlapping sequences is

Leu–Phe

Phe–Pro

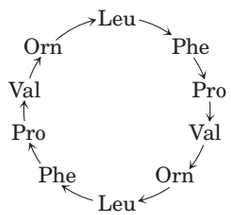
Phe–Pro–Val

Pro–Val–Orn

Val–Orn

Orn–(Leu)

Thus, the peptide is a cyclic dimer of Leu–Phe–Pro–Val–Orn:



where the arrows indicate the –CO → NH–, or C → N, direction of the peptide bonds. This structure is consistent with all the data.

20. Efficiency in Peptide Sequencing A peptide with the primary structure Lys–Arg–Pro–Leu–Ile–Asp–Gly–Ala is sequenced by the Edman procedure. If each Edman cycle is 96% efficient, what percentage of the amino acids liberated in the fourth cycle will be leucine? Do the calculation a second time, but assume a 99% efficiency for each cycle.

Answer 88%, 97%. The formula for calculating the percentage of correct amino acid liberated after sequencing cycle n, given an efficiency x, is x^n/x, or x^{n-1}. If the efficiency is 0.96, the fraction of correct amino acid liberated in the fourth cycle is $(0.96)^3 = 0.88$. If the efficiency is 0.99, the fraction is $(0.99)^3 = 0.97$.

The $n - 1$ term can be explained by considering what happens in each cycle. For example, in the first cycle, a Lys residue is liberated from 96% of the ends, so that 96% of the termini now have Arg and the remaining 4% still have Lys. However, 100% of the residues actually removed in this cycle are Lys. In the second cycle, Arg is removed from 96% of the ends that contain it, or from $0.96 \times 0.96 = 0.92 = 92\%$ of the ends. Lys is removed from $0.96 \times 0.04 = 0.04$ of the ends. However, the fraction of liberated residues that are Arg is greater than 92% because only 96% of the ends had residues removed. Hence, the fraction of residues liberated as Arg in the second cycle is $0.92/0.96 = 0.96 = 96\%$—and so forth.

21. Sequence Comparisons Proteins called molecular chaperones (described in Chapter 4) assist in the process of protein folding. One class of chaperone found in organisms from bacteria to mammals is heat shock protein 90 (Hsp90). All Hsp90 chaperones contain a 10 amino acid "signature sequence," which allows for ready identification of these proteins in sequence databases. Two representations of this signature sequence are shown below.

Y-x-[NQHD]-[KHR]-[DE]-[IVA]-F-[LM]-R-[ED].

(a) In this sequence, which amino acid residues are invariant (conserved across all species)?

(b) At which position(s) are amino acids limited to those with positively charged side chains? For each position, which amino acid is more commonly found?

(c) At which positions are substitutions restricted to amino acids with negatively charged side chains? For each position, which amino acid predominates?

(d) There is one position that can be any amino acid, although one amino acid appears much more often than any other. What position is this, and which amino acid appears most often?

Answer

(a) Y (1), F (7), and R (9)

(b) Positions 4 and 9; K (Lys) is more common at 4, R (Arg) is invariant at 9

(c) Positions 5 and 10; E (Glu) is more common at both positions

(d) Position 2; S (Ser)

22. Biochemistry Protocols: Your First Protein Purification As the newest and least experienced student in a biochemistry research lab, your first few weeks are spent washing glassware and labeling test tubes. You then graduate to making buffers and stock solutions for use in various laboratory procedures. Finally, you are given responsibility for purifying a protein. It is citrate synthase (an enzyme of the citric acid cycle, to be discussed in Chapter 16), which is located in the mitochondrial matrix. Following a protocol for the purification, you proceed through the steps below. As you work, a more experienced student questions you about the rationale for each procedure. Supply the answers. (Hint: see Chapter 2 for information about osmolarity; see p. 7 for information on separation of organelles from cells.)

(a) You pick up 20 kg of beef hearts from a nearby slaughterhouse (muscle cells are rich in mitochondria, which supply energy for muscle contraction). You transport the hearts on ice, and perform each step of the purification on ice or in a walk-in cold room. You homogenize the beef heart tissue in a high-speed blender in a medium containing 0.2 M sucrose, buffered to a pH of 7.2. *Why do you use beef heart tissue, and in such large quantity? What is the purpose of keeping the tissue cold and suspending it in 0.2 M sucrose, at pH 7.2? What happens to the tissue when it is homogenized?*

(b) You subject the resulting heart homogenate, which is dense and opaque, to a series of differential centrifugation steps. *What does this accomplish?*

(c) You proceed with the purification using the supernatant fraction that contains mostly intact mitochondria. Next, you osmotically lyse the mitochondria. The lysate, which is less dense than the homogenate, but still opaque, consists primarily of mitochondrial membranes and internal mitochondrial contents. To this lysate you add ammonium sulfate, a highly soluble salt, to a specific concentration. You centrifuge the solution, decant the supernatant, and discard the pellet. To the supernatant, which is clearer than the lysate, you add *more* ammonium sulfate. Once again, you centrifuge the sample, but this time you save the pellet because it contains the citrate synthase. *What is the rationale for the two-step addition of the salt?*

(d) You solubilize the ammonium sulfate pellet containing the mitochondrial proteins and dialyze it overnight against large volumes of buffered (pH 7.2) solution. *Why isn't ammonium sulfate included in the dialysis buffer? Why do you use the buffer solution instead of water?*

(e) You run the dialyzed solution over a size-exclusion chromatographic column. Following the protocol, you collect the *first* protein fraction that exits the column and discard the fractions that elute from the column later. You detect the protein by measuring UV absorbance (at 280 nm) in the fractions. *What does the instruction to collect the first fraction tell you about the protein? Why is UV absorbance at 280 nm a good way to monitor for the presence of protein in the eluted fractions?*

(f) You place the fraction collected in (e) on a cation-exchange chromatographic column. After discarding the initial solution that exits the column (the flowthrough), you add a washing solution of higher pH to the column and collect the protein fraction that immediately elutes. *Explain what you are doing*.

(g) You run a small sample of your fraction, now very reduced in volume and quite clear (though tinged pink), on an isoelectric focusing gel. When stained, the gel shows three sharp bands. According to the protocol, the citrate synthase is the protein with a pI of 5.6, but you decide to do one more assay of the protein's purity. You cut out the pI 5.6 band and subject it to SDS polyacrylamide gel electrophoresis. The protein resolves as a single band. *Why were you unconvinced of the purity of the "single" protein band on your isoelectric focusing gel? What did the results of the SDS gel tell you? Why is it important to do the SDS gel electrophoresis* after *the isoelectric focusing?*

Answer

(a) *Why do you use beef heart tissue, and why do you need so much of it?* The protein you are to isolate and purify (citrate synthase [CS]) is found in mitochondria, which are abundant in cells with high metabolic activity such as heart muscle cells. Beef hearts are relatively cheap and easy to get at the local slaughterhouse. You begin with a large amount of tissue because cells contain thousands of different proteins, and no single protein is present in high concentration—that is, the specific activity of CS is low. To purify a significant quantity, you must start with a large excess of tissue.

Why do you need to keep the tissues cold? The cold temperatures inhibit the action of lysosomal enzymes that would destroy the sample.

Why is the tissue suspended in 0.2 M sucrose, at pH 7.2? Sucrose is used in the homogenization buffer to create a medium that is isotonic with the organelles. This prevents diffusion of water into the organelles, causing them to swell, burst, and spill their contents. A pH of 7.2 helps to decrease the activity of lysosomal enzymes and maintain the native structure of proteins in the sample.

What happens to the tissue when it is homogenized? Homogenization breaks open the heart muscle cells, releasing the organelles and cytosol.

(b) *What does differential centrifugation accomplish?* Organelles differ in size and therefore sediment at different rates during centrifugation. Larger organelles and pieces of cell debris sediment first, and progressively smaller cellular components can be isolated in a series of centrifugation steps at increasing speed. The contents of each fraction can be determined microscopically or by enzyme assay.

(c) *What is the rationale for the two-step addition of ammonium sulfate?* Proteins have characteristic solubilities at different salt concentrations, depending on the functional groups in the protein. In a concentration of ammonium sulfate just below the precipitation point of CS, some unwanted proteins can be precipitated (salted out). The ammonium sulfate concentration is then increased so that CS is salted out. It can then be recovered by centrifugation.

(d) *Why is a buffer solution without ammonium sulfate used for the dialysis step?* Osmolarity (as well as pH and temperature) affects the conformation and stability of proteins. To solubilize and renature the protein, the ammonium sulfate must be removed. In dialysis against a buffered solution containing no ammonium sulfate, the ammonium sulfate in the sample moves into the buffer until its concentration is equal in both solutions. By dialyzing against large volumes of buffer that are changed frequently, the concentration of ammonium sulfate in the sample can be reduced to almost zero. This procedure usually takes a long time (typically overnight). The dialysate must be buffered to keep the pH (and ionic strength) of the sample in a range that promotes the native conformation of the protein.

(e) *What does the instruction to collect the first fraction tell you about the protein?* The CS molecule is larger than the pore size of the chromatographic gel. Size-exclusion columns retard the flow of smaller molecules, which enter the pores of the column matrix material. Larger molecules flow around the matrix, taking a direct route through the column.

Why is UV absorbance at 280 nm a good way to monitor for the presence of protein in the eluted fractions? The aromatic side chains of Tyr and Trp residues strongly absorb at 280 nm.

(f) *Explain the procedure on the cation-exchange chromatography column.* CS has a positive charge (at the pH of the separation) and binds to the negatively charged beads of the cation-exchange column, while negatively charged and neutral proteins pass through. CS is displaced from the column by raising the pH of the mobile phase and thus altering the charge on the CS molecules.

(g) *Why were you unconvinced of the purity of the "single" protein band on your isoelectric focusing gel?* Several different proteins, all with the same pI, could be focused in the "single" band. SDS polyacrylamide gel electrophoresis separates on the basis of mass and therefore would separate any polypeptides in the pI 5.6 band.

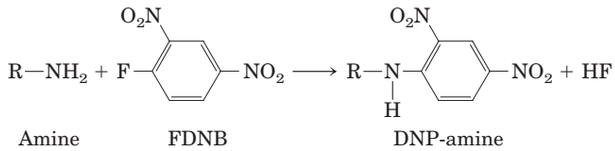
Why is it important to do the SDS gel electrophoresis after the isoelectric focusing? SDS is a highly negatively charged detergent that binds tightly and uniformly along the length of a polypeptide. Removing SDS from a protein is difficult, and a protein with only traces of SDS no longer has its native acid-base properties, including its native pI.

Data Analysis Problem

23. Determining the Amino Acid Sequence of Insulin Figure 3–24 shows the amino acid sequence of the hormone insulin. This structure was determined by Frederick Sanger and his coworkers. Most of this work is described in a series of articles published in the *Biochemical Journal* from 1945 to 1955.

When Sanger and colleagues began their work in 1945, it was known that insulin was a small protein consisting of two or four polypeptide chains linked by disulfide bonds. Sanger and his coworkers had developed a few simple methods for studying protein sequences.

Treatment with FDNB. FDNB (1-fluoro-2,4-dinitrobenzene) reacted with free amino (but not amido or guanidino) groups in proteins to produce dinitrophenyl (DNP) derivatives of amino acids:



Acid Hydrolysis. Boiling a protein with 10% HCl for several hours hydrolyzed all of its peptide and amide bonds. Short treatments produced short polypeptides; the longer the treatment, the more complete the breakdown of the protein into its amino acids.

Oxidation of Cysteines. Treatment of a protein with performic acid cleaved all the disulfide bonds and converted all Cys residues to cysteic acid residues (Fig. 3–26).

Paper Chromatography. This more primitive version of thin-layer chromatography (see Fig. 10–24) separated compounds based on their chemical properties, allowing identification of single amino acids and, in some cases, dipeptides. Thin-layer chromatography also separates larger peptides.

As reported in his first paper (1945), Sanger reacted insulin with FDNB and hydrolyzed the resulting protein. He found many free amino acids, but only three DNP–amino acids: α-DNP-glycine (DNP group attached to the α-amino group); α-DNP-phenylalanine; and ε-DNP-lysine (DNP attached to the ε-amino group). Sanger interpreted these results as showing that insulin had two protein chains: one with Gly at its amino terminus and one with Phe at its amino terminus. One of the two chains also contained a Lys residue, not at the amino terminus. He named the chain beginning with a Gly residue "A" and the chain beginning with Phe "B."

(a) Explain how Sanger's results support his conclusions.

(b) Are the results consistent with the known structure of insulin (Fig. 3–24)?

In a later paper (1949), Sanger described how he used these techniques to determine the first few amino acids (amino-terminal end) of each insulin chain. To analyze the B chain, for example, he carried out the following steps:

1. Oxidized insulin to separate the A and B chains.
2. Prepared a sample of pure B chain with paper chromatography.
3. Reacted the B chain with FDNB.
4. Gently acid-hydrolyzed the protein so that some small peptides would be produced.
5. Separated the DNP-peptides from the peptides that did not contain DNP groups.
6. Isolated four of the DNP-peptides, which were named B1 through B4.
7. Strongly hydrolyzed each DNP-peptide to give free amino acids.
8. Identified the amino acids in each peptide with paper chromatography.

The results were as follows:

B1: α-DNP-phenylalanine only

B2: α-DNP-phenylalanine; valine

B3: aspartic acid; α-DNP-phenylalanine; valine

B4: aspartic acid; glutamic acid; α-DNP-phenylalanine; valine

(c) Based on these data, what are the first four (amino-terminal) amino acids of the B chain? Explain your reasoning.

(d) Does this result match the known sequence of insulin (Fig. 3–24)? Explain any discrepancies.

Sanger and colleagues used these and related methods to determine the entire sequence of the A and B chains. Their sequence for the A chain was as follows (amino terminus on left):

1 5 10 15 20

Gly–Ile–Val–Glx–Glx–Cys–Cys–Ala–Ser–Val– Cys–Ser–Leu–Tyr–Glx–Leu–Glx–Asx–Tyr–Cys–Asx

Because acid hydrolysis had converted all Asn to Asp and all Gln to Glu, these residues had to be designated Asx and Glx, respectively (exact identity in the peptide unknown). Sanger solved this problem by using protease enzymes that cleave peptide bonds, but not the amide bonds in Asn and Gln residues, to prepare short peptides. He then determined the number of amide groups present in each peptide by measuring the NH_4^+ released when the peptide was acid-hydrolyzed. Some of the results for the A chain are shown below. The peptides may not have been completely pure, so the numbers were approximate—but good enough for Sanger's purposes.

Peptide name	Peptide sequence	Number of amide groups in peptide
Ac1	Cys–Asx	0.7
Ap15	Tyr–Glx–Leu	0.98
Ap14	Tyr–Glx–Leu–Glx	1.06
Ap3	Asx–Tyr–Cys–Asx	2.10
Ap1	Glx–Asx–Tyr–Cys–Asx	1.94
Ap5pa1	Gly–Ile–Val–Glx	0.15
Ap5	Gly–Ile–Val–Glx–Glx–Cys–Cys–Ala–Ser–Val–Cys–Ser–Leu	1.16

(e) Based on these data, determine the amino acid sequence of the A chain. Explain how you reached your answer. Compare it with Figure 3–24.

Answer

(a) Any linear polypeptide chain has only two kinds of free amino groups: a single α-amino group at the amino terminus, and an ε-amino group on each Lys residue present. These amino groups react with FDNB to form a DNP–amino acid derivative. Insulin gave two different α-amino-DNP derivatives, suggesting that it has two amino termini and thus two polypeptide chains—one with an amino-terminal Gly and the other with an amino-terminal Phe. Because the DNP-lysine product is ε-DNP-lysine, the Lys is not at an amino terminus.

(b) Yes. The A chain has amino-terminal Gly; the B chain has amino-terminal Phe; and (nonterminal) residue 29 in the B chain is Lys.

(c) Phe–Val–Asp–Glu–. Peptide B1 shows that the amino-terminal residue is Phe. Peptide B2 also includes Val, but since no DNP-Val is formed, Val is not at the amino terminus; it must be on the carboxyl side of Phe. Thus the sequence of B2 is DNP-Phe–Val. Similarly, the sequence of B3 must be DNP-Phe–Val–Asp, and the sequence of the A chain must begin Phe–Val–Asp–Glu–.

(d) No. The known amino-terminal sequence of the A chain is Phe–Val–Asn–Gln–. The Asn and Gln appear in Sanger's analysis as Asp and Glu because the vigorous hydrolysis in step ⑦ hydrolyzed the amide bonds in Asn and Gln (as well as the peptide bonds), forming Asp and Glu. Sanger et al. could not distinguish Asp from Asn or Glu from Gln at this stage in their analysis.

(e) The sequence exactly matches that in Figure 3–24. Each peptide in the table gives specific information about which Asx residues are Asn or Asp and which Glx residues are Glu or Gln.

Ac1: residues 20–21. This is the only Cys–Asx sequence in the A chain; there is ~1 amido group in this peptide, so it must be Cys–Asn:

N–Gly–Ile–Val–Glx–Glx–Cys–Cys–Ala–Ser–Val–Cys–Ser–Leu–Tyr–Glx–Leu–Glx–Asx–Tyr–Cys–**Asn**–*C*
1 5 10 15 20

Ap15: residues 14–15–16. This is the only Tyr–Glx–Leu in the A chain; there is ~1 amido group, so the peptide must be Tyr–Gln–Leu:

N–Gly–Ile–Val–Glx–Glx–Cys–Cys–Ala–Ser–Val–Cys–Ser–Leu–Tyr–**Gln**–Leu–Glx–Asx–Tyr–Cys–Asn–*C*
1 5 10 15 20

Ap14: residues 14–15–16–17. It has ~1 amido group, and we already know that residue 15 is Gln, so residue 17 must be Glu:

N–Gly–Ile–Val–Glx–Glx–Cys–Cys–Ala–Ser–Val–Cys–Ser–Leu–Tyr–Gln–Leu–**Glu**–Asx–Tyr–Cys–Asn–*C*
1 5 10 15 20

Ap3: residues 18–19–20–21. It has ~2 amido groups, and we know that residue 21 is Asn, so residue 18 must be Asn:

N–Gly–Ile–Val–Glx–Glx–Cys–Cys–Ala–Ser–Val–Cys–Ser–Leu–Tyr–Gln–Leu–Glu–**Asn**–Tyr–Cys–Asn–*C*
1 5 10 15 20

Ap1: residues 17–18–19–20–21, which is consistent with residues 18 and 21 being Asn.
Ap5pa1: residues 1–2–3–4. It has ~0 amido group, so residue 4 must be Glu:

N–Gly–Ile–Val–**Glu**–Glx–Cys–Cys–Ala–Ser–Val–Cys–Ser–Leu–Tyr–Gln–Leu–Glu–Asn–Tyr–Cys–Asn–*C*
1 5 10 15 20

Ap5: residues 1 through 13. It has ~1 amido group, and we know that residue 14 is Glu, so residue 5 must be Gln:

N–Gly–Ile–Val–Glu–**Gln**–Cys–Cys–Ala–Ser–Val–Cys–Ser–Leu–Tyr–Gln–Leu–Glu–Asn–Tyr–Cys–Asn–*C*
1 5 10 15 20

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Sanger, F. (1949) The terminal peptides of insulin. *Biochem. J.* **45,** 563–574.

chapter

4 The Three-Dimensional Structure of Proteins

1. **Properties of the Peptide Bond** In x-ray studies of crystalline peptides, Linus Pauling and Robert Corey found that the C—N bond in the peptide link is intermediate in length (1.32 Å) between a typical C—N single bond (1.49 Å) and a C═N double bond (1.27 Å). They also found that the peptide bond is planar (all four atoms attached to the C—N group are located in the same plane) and that the two α-carbon atoms attached to the C—N are always trans to each other (on opposite sides of the peptide bond).

   (a) What does the length of the C—N bond in the peptide linkage indicate about its strength and its bond order (i.e., whether it is single, double, or triple)?

   (b) What do the observations of Pauling and Corey tell us about the ease of rotation about the C—N peptide bond?

   Answer

   (a) The higher the bond order (double or triple vs. single), the shorter and stronger are the bonds. Thus, bond length is an indication of bond order. For example, the C═N bond is shorter (1.27 Å) and has a higher order (n = 2.0) than a typical C—N bond (length = 1.49 Å, n = 1.0). The length of the C—N bond of the peptide link (1.32 Å) indicates that it is intermediate in strength and bond order between a single and double bond.

   (b) Rotation about a double bond is generally impossible at physiological temperatures, and the steric relationship of the groups attached to the two atoms involved in the double bond is spatially "fixed." Since the peptide bond has considerable double-bond character, there is essentially no rotation, and the —C═O and —N—H groups are fixed in the trans configuration.

2. **Structural and Functional Relationships in Fibrous Proteins** William Astbury discovered that the x-ray diffraction pattern of wool shows a repeating structural unit spaced about 5.2 Å along the length of the wool fiber. When he steamed and stretched the wool, the x-ray pattern showed a new repeating structural unit at a spacing of 7.0 Å. Steaming and stretching the wool and then letting it shrink gave an x-ray pattern consistent with the original spacing of about 5.2 Å. Although these observations provided important clues to the molecular structure of wool, Astbury was unable to interpret them at the time.

   (a) Given our current understanding of the structure of wool, interpret Astbury's observations.

   (b) When wool sweaters or socks are washed in hot water or heated in a dryer, they shrink. Silk, on the other hand, does not shrink under the same conditions. Explain.

Answer

(a) The principal structural units in the wool fiber polypeptide, α-keratin, are successive turns of the α helix, which are spaced at 5.4 Å intervals; two α-keratin strands twisted into a coiled coil produce the 5.2 Å spacing. The intrinsic stability of the helix (and thus the fiber) results from *intra* chain hydrogen bonds (see Fig. 4–4a). Steaming and stretching the fiber yields an extended polypeptide chain with the β conformation, in which the distance between adjacent R groups is about 7.0 Å. Upon resteaming, the polypeptide chains again assume the less-extended α-helix conformation.

(b) Freshly sheared wool is primarily in its α-keratin (α-helical coiled coil) form (see Fig. 4–10). Because raw wool is crimped or curly, it is combed and stretched to straighten it before being spun into fibers for clothing. This processing converts the wool from its native α-helical conformation to a more extended β form. Moist heat triggers a conformational change back to the native α-helical structure, which shrinks both the fiber and the clothing. Under conditions of mechanical tension and moist heat, wool can be stretched back to a fully extended form. In silk, by contrast, the polypeptide chains have a very stable β-pleated sheet structure, fully extended along the axis of the fiber (see Fig. 4–6), and have small, closely packed amino acid side chains (see Fig. 4–13). These characteristics make silk resistant to stretching and shrinking.

3. Rate of Synthesis of Hair α-Keratin Hair grows at a rate of 15 to 20 cm/yr. All this growth is concentrated at the base of the hair fiber, where α-keratin filaments are synthesized inside living epidermal cells and assembled into ropelike structures (see Fig. 4–10). The fundamental structural element of α-keratin is the α helix, which has 3.6 amino acid residues per turn and a rise of 5.4 Å per turn (see Fig. 4–4a). Assuming that the biosynthesis of α-helical keratin chains is the rate-limiting factor in the growth of hair, calculate the rate at which peptide bonds of α-keratin chains must be synthesized (peptide bonds per second) to account for the observed yearly growth of hair.

Answer Because there are 3.6 amino acids (AAs) per turn and the rise is 5.4 Å/turn, the length per AA of the α helix is

$$\frac{5.4\ \text{Å/turn}}{3.6\ \text{AA/turn}} = 1.5\ \text{Å/AA} = 1.5 \times 10^{-10}\ \text{m/AA}$$

A growth rate of 20 cm/yr is equivalent to

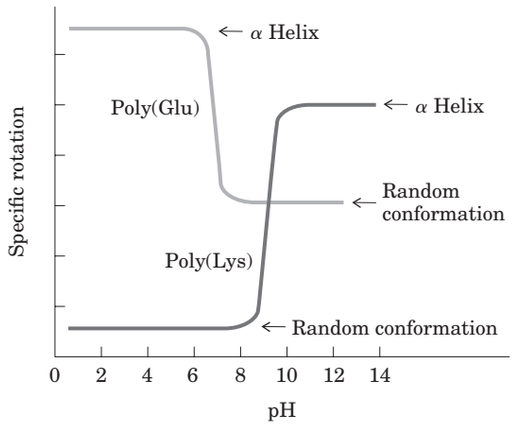
$$\frac{20\ \text{cm/year}}{(365\ \text{days/yr})(24\ \text{h/day})(60\ \text{min/h})(60\ \text{s/min})} = 6.3 \times 10^{-7}\ \text{cm/s} = 6.3 \times 10^{-9}\ \text{m/s}$$

Thus, the rate at which amino acids are added is

$$\frac{6.3 \times 10^{-9}\ \text{m/s}}{1.5 \times 10^{-10}\ \text{m/AA}} = 42\ \text{AA/s} = 42\ \text{peptide bonds per second}$$

4. Effect of pH on the Conformation of α-Helical Secondary Structures The unfolding of the α helix of a polypeptide to a randomly coiled conformation is accompanied by a large decrease in a property called specific rotation, a measure of a solution's capacity to rotate plane-polarized light. Polyglutamate, a polypeptide made up of only L-Glu residues, has the α-helical conformation at pH 3.

When the pH is raised to 7, there is a large decrease in the specific rotation of the solution. Similarly, polylysine (L-Lys residues) is an α helix at pH 10, but when the pH is lowered to 7 the specific rotation also decreases, as shown by the following graph.



What is the explanation for the effect of the pH changes on the conformations of poly(Glu) and poly(Lys)? Why does the transition occur over such a narrow range of pH?

Answer At pH values above 6, deprotonation of the carboxylate side chains of poly(Glu) leads to repulsion between adjacent negatively charged groups, which destabilizes the α helix and results in unfolding. Similarly, at pH 7 protonation of the amino-group side chains of poly(Lys) causes repulsion between positively charged groups, which leads to unfolding.

5. Disulfide Bonds Determine the Properties of Many Proteins Some natural proteins are rich in disulfide bonds, and their mechanical properties (tensile strength, viscosity, hardness, etc.) are correlated with the degree of disulfide bonding.

(a) Glutenin, a wheat protein rich in disulfide bonds, is responsible for the cohesive and elastic character of dough made from wheat flour. Similarly, the hard, tough nature of tortoise shell is due to the extensive disulfide bonding in its α-keratin. What is the molecular basis for the correlation between disulfide-bond content and mechanical properties of the protein?

(b) Most globular proteins are denatured and lose their activity when briefly heated to 65 °C. However, globular proteins that contain multiple disulfide bonds often must be heated longer at higher temperatures to denature them. One such protein is bovine pancreatic trypsin inhibitor (BPTI), which has 58 amino acid residues in a single chain and contains three disulfide bonds. On cooling a solution of denatured BPTI, the activity of the protein is restored. What is the molecular basis for this property?

Answer

(a) Disulfide bonds are covalent bonds, which are much stronger than the noncovalent interactions (hydrogen bonds, hydrophobic interactions, van der Waals interactions) that stabilize the three-dimensional structure of most proteins. Disulfide bonds serve to cross-link protein chains, increasing stiffness, hardness, and mechanical strength.

(b) As the temperature is raised, the increased thermal motion of the polypeptide chains and the vibrational motions of hydrogen bonds ultimately lead to thermal denaturation (unfolding) of a protein. Cystine residues (disulfide bonds) can, depending on their location in the protein structure, prevent or restrict the movement of folded protein domains, block access of solvent water to the interior of the protein, and prevent the complete unfolding of the protein. Refolding to the native structure from a random conformation is seldom spontaneous, owing to the very large number of conformations

possible. Disulfide bonds limit the number of conformations by allowing only a few minimally unfolded structures, and hence the protein returns to its native conformation more easily upon cooling.

6. Amino Acid Sequence and Protein Structure Our growing understanding of how proteins fold allows researchers to make predictions about protein structure based on primary amino acid sequence data. Consider the following amino acid sequence.

Ile	Ala	His	Thr	Tyr	Gly	Pro	Phe	Glu	Ala	Ala	Met	Cys	Lys	Trp	Glu	Ala	Gln	Pro	Asp	Gly	Met	Glu	Cys	Ala	Phe	His	Arg
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28

(a) Where might bends or β turns occur?

(b) Where might intrachain disulfide cross-linkages be formed?

(c) Assuming that this sequence is part of a larger globular protein, indicate the probable location (the external surface or interior of the protein) of the following amino acid residues: Asp, Ile, Thr, Ala, Gln, Lys. Explain your reasoning. (Hint: See the hydropathy index in Table 3–1.)

Answer

(a) Bends or turns are most likely to occur at residues 7 and 19 because Pro residues are often (but not always) found at bends in globular folded proteins. A bend may also occur at the Thr residue (residue 4) and, assuming that this is a portion of a larger polypeptide, at the Ile residue (residue 1).

(b) Intrachain disulfide cross-linkages can form only between residues 13 and 24 (Cys residues).

(c) Amino acids with ionic (charged) or strongly polar neutral groups (e.g., Asp, Gln, and Lys in this protein) are located on the external surface, where they interact optimally with solvent water. Residues with nonpolar side chains (such as Ala and Ile) are situated in the interior, where they escape the polar environment. Thr is of intermediate polarity and could be found either in the interior or on the exterior surface (see Table 3–1).

7. Bacteriorhodopsin in Purple Membrane Proteins Under the proper environmental conditions, the salt-loving bacterium *Halobacterium halobium* synthesizes a membrane protein (M_r 26,000) known as bacteriorhodopsin, which is purple because it contains retinal (see Fig. 10–21). Molecules of this protein aggregate into "purple patches" in the cell membrane. Bacteriorhodopsin acts as a light-activated proton pump that provides energy for cell functions. X-ray analysis of this protein reveals that it consists of seven parallel α-helical segments, each of which traverses the bacterial cell membrane (thickness 45 Å). Calculate the minimum number of amino acids necessary for one segment of α helix to traverse the membrane completely. Estimate the fraction of the bacteriorhodopsin protein that is involved in membrane-spanning helices. (Use an average amino acid residue weight of 110.)

Answer Using the parameters from Problem 3 (3.6 AA/turn, 5.4 Å/turn), we can calculate that there are 0.67 AA/Å along the axis of a helix. Thus, a helix of length 45 Å (sufficient to span the membrane) requires a minimum of (45 Å)(0.67 AA/Å) = 30 amino acid residues.

The membrane protein has an M_r of 26,000 and average AA M_r of 110. Thus the protein contains 26,000/110 = 240 AA. Of these, (30 AA/helix)(7 helices) = 210 AA are involved in membrane-spanning helices, which is 210/240 = 0.87, or 87%, of the protein.

8. Protein Structure Terminology Is myoglobin a motif, a domain, or a complete three-dimensional structure?

Answer Myoglobin is all three. The folded structure, the "globin fold," is a motif found in all globins. The polypeptide folds into a single domain, which for this protein represents the entire three-dimensional structure.

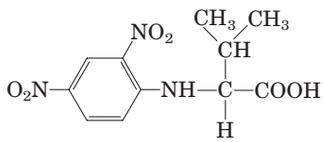
9. Pathogenic Action of Bacteria That Cause Gas Gangrene The highly pathogenic anaerobic bacterium *Clostridium perfringens* is responsible for gas gangrene, a condition in which animal tissue structure is destroyed. This bacterium secretes an enzyme that efficiently catalyzes the hydrolysis of the peptide bond indicated by an asterisk:

$$\text{—X—}\overset{*}{\text{C}}\text{O—NH—Gly—Pro—Y—} \xrightarrow{H_2O} \text{—X—COO}^- + \overset{+}{\text{H}}_3\text{N—Gly—Pro—Y—}$$

where X and Y are any of the 20 common amino acids. How does the secretion of this enzyme contribute to the invasiveness of this bacterium in human tissues? Why does this enzyme not affect the bacterium itself?

Answer Collagen is distinctive in its amino acid composition, having a very high proportion of Gly (35%) and Pro residues. The enzyme secreted by the bacterium is a collagenase, which breaks down collagen at the X–Gly bonds and damages the connective-tissue barrier (skin, hide, etc.) of the host; this allows the bacterium to invade the host tissues. Bacteria do not contain collagen and thus are unaffected by collagenase.

10. Number of Polypeptide Chains in a Multisubunit Protein A sample (660 mg) of an oligomeric protein of M_r 132,000 was treated with an excess of 1-fluoro-2,4-dinitrobenzene (Sanger's reagent) under slightly alkaline conditions until the chemical reaction was complete. The peptide bonds of the protein were then completely hydrolyzed by heating it with concentrated HCl. The hydrolysate was found to contain 5.5 mg of the following compound:



2,4-Dinitrophenyl derivatives of the α-amino groups of other amino acids could not be found.

(a) Explain how this information can be used to determine the number of polypeptide chains in an oligomeric protein.

(b) Calculate the number of polypeptide chains in this protein.

(c) What other protein analysis technique could you employ to determine whether the polypeptide chains in this protein are similar or different?

Answer

(a) Because only a single 2,4-dinitrophenyl (DNP) amino acid derivative is found, there is only one kind of amino acid at the amino terminus (i.e., all the polypeptide chains have the same amino-terminal residue). Comparing the number of moles of this derivative to the number of moles of protein gives the number of polypeptide chains.

(b) The amount of protein = (0.66 g)/(132,000 g/mol) = 5×10^{-6} mol.
Because M_r for DNP-Val ($C_{11}H_{13}O_6N_3$) = 283, the amount of DNP-Val = (0.0055 g)/(283 g/mol) = 1.9×10^{-5} mol.

The ratio of moles of DNP-Val to moles of protein gives the number of amino-terminal residues and thus the number of chains per oligomer:

$$\frac{1.9 \times 10^{-5} \text{ mol DNP-Val}}{5 \times 10^{-6} \text{ mol protein}} = 4 \text{ polypeptide chains}$$

An alternative approach to the problem is through the proportionality (n = number of polypeptide chains):

$$\frac{n(283 \text{ g/mol})}{132{,}000 \text{ g/mol}} = \frac{5.5 \text{ mg}}{660 \text{ mg}}$$

$$n = \frac{(5.5 \text{ mg})(132{,}000 \text{ g/mol})}{(660 \text{ mg})(283 \text{ g/mol})} = 3.9 \approx 4$$

(c) Polyacrylamide gel electrophoresis in the presence of a detergent (such as sodium dodecylsulfate [SDS]) and an agent that prevents the formation of disulfide bonds (such as β-mercaptoethanol) would provide information on subunit structure of a protein. In the example here, an oligomeric protein of M_r 132,000 that had four *identical* subunits would produce a single band on the electrophoretic gel, with apparent M_r ~33,000 (132,000/33,000 = 4). If the protein were made up of different polypeptide subunits, they would likely appear as multiple discrete bands on the gel.

11. Predicting Secondary Structure Which of the following peptides is more likely to take up an α-helical structure, and why?

(a) LKAENDEAARAMSEA

(b) CRAGGFPWDQPGTSN

Answer By cursory inspection, peptide **(a)** has five Ala residues (most likely to take up an α-helical conformation), and peptide **(b)** has five Pro and Gly residues (least often found in an α helix). This suggests that **(a)** is more likely than **(b)** to form an α helix. Referring to Table 4–1, **(a)** has 15 residues with a total $\Delta\Delta G^\circ$ of 13 kJ/mol, and **(b)** has 15 residues with a total $\Delta\Delta G^\circ$ of 41 kJ/mol. Given that a lower $\Delta\Delta G^\circ$ indicates a greater tendency to take up an α-helical structure, this confirms that peptide **(a)** is much more likely to form an α helix.

12. Amyloid Fibers in Disease Several small aromatic molecules, such as phenol red (used as a non-toxic drug model), have been shown to inhibit the formation of amyloid in laboratory model systems. A goal of the research on these small aromatic compounds is to find a drug that would efficiently inhibit the formation of amyloid in the brain in people with incipient Alzheimer's disease.

(a) Suggest why molecules with aromatic substituents would disrupt the formation of amyloid.

(b) Some researchers have suggested that a drug used to treat Alzheimer's disease may also be effective in treating type 2 (adult onset) diabetes mellitus. Why might a single drug be effective in treating these two different conditions?

Answer

(a) Aromatic residues seem to play an important role in stabilizing amyloid fibrils. Thus, molecules with aromatic substituents may inhibit amyloid formation by interfering with the stacking or association of the aromatic side chains.

(b) Amyloid is formed in the pancreas in association with type 2 diabetes, as it is in the brain in Alzheimer's disease. Although the amyloid fibrils in the two diseases involve different proteins, the fundamental structure of the amyloid is similar and similarly stabilized in both, and thus they are potential targets for similar drugs designed to disrupt this structure.

Biochemistry on the Internet

13. Protein Modeling on the Internet A group of patients with Crohn's disease (an inflammatory bowel disease) underwent biopsies of their intestinal mucosa in an attempt to identify the causative agent. Researchers identified a protein that was present at higher levels in patients with Crohn's disease than in patients with an unrelated inflammatory bowel disease or in unaffected controls. The protein was isolated, and the following *partial* amino acid sequence was obtained (reads left to right):

EAELCPDRCI HSFQNLGIQC VKKRDLEQAI SQRIQTNNNP FQVPIEEQRG
DYDLNAVRLC FQVTVRDPSG RPLRLPPVLP HPIFDNRAPN TAELKICRVN
RNSGSCLGGD EIFLLCDKVQ KEDIEVYFTG PGWEARGSFS QADVHRQVAI
VFRTPPYADP SLQAPVRVSM QLRRPSDREL SEPMEFQYLP DTDDRHRIEE
KRKRTYETFK SIMKKSPFSG PTDPRPPPRR IAVPSRSSAS VPKPAPQPYP

(a) You can identify this protein using a protein database on the Internet. Some good places to start include Protein Information Resource (PIR; http://pir.georgetown.edu), Structural Classification of Proteins (SCOP; http://scop.mrc-lmb.cam.ac.uk/scop), and Prosite (http://expasy.org/prosite).

At your selected database site, follow links to the sequence comparison engine. Enter about 30 residues from the protein sequence in the appropriate search field and submit it for analysis. What does this analysis tell you about the identity of the protein?

(b) Try using different portions of the amino acid sequence. Do you always get the same result?

(c) A variety of websites provide information about the three-dimensional structure of proteins. Find information about the protein's secondary, tertiary, and quaternary structures using database sites, such as the Protein Data Bank (PDB; www.rcsb.org) or SCOP.

(d) In the course of your Web searches, what did you learn about the cellular function of the protein?

Answer

(a) At the PIR—International Protein Sequence Database (http://pir.georgetown.edu), click on "Search/Analysis" and choose "BLAST search." Paste the first 30 amino acid residues of the sequence into the search box and submit the sequence for comparison. The table that returns includes many proteins that have 100% sequence identity with these 30 residues. Among the human proteins are RelA and the transcription factor NFκB. Proteins from other species match as well. Click on the "Help" button for explanations of the various options and table items.

(b) As more proteins are sequenced, the number of hits returned from a 30-residue sequence increases. Sequence matching based on the first 30 residues brings up several proteins that contain this sequence (identity = 100%). Using sequence segments from different parts of the protein will return some different results, but the proteins with high sequence identities will likely be similar. Even when the *entire* sequence is entered in the search field, similar proteins from cattle, mouse, and rat match with very high scores, and several hundred hits are returned. When the entire sequence is used, the human protein with the best match is the p65 subunit of nuclear transcription factor kappa B (NFκB). A synonym for this protein is RelA transforming protein.

(c) At the PDB (rcsb.org) search on "NF-kappa-B p65." You will get more than a dozen hits. Adding "human" to the search limits the results further. Go back to the more general search on "NF-kappa-B p65" and scan through the returned items. NFκB has two subunits. There are multiple variants of the subunits, with the best-characterized being 50, 52, and 65 kDa (p50, p52, and p65, respectively). These pair with each other to form a variety of homodimers and heterodimers.

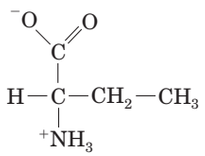
(d) The various proteins that predominate in this search are eukaryotic transcription factors, which stimulate transcription of genes involved in development and some immune responses. The proteins have two distinct domains, including an amino-terminal Rel homology domain 300 amino acid residues long and a carboxyl-terminal domain involved in gene activation. A search of the various links in the databases will reveal much additional information about the proteins' structure and function.

Data Analysis Problem

14. Mirror-Image Proteins As noted in Chapter 3, "The amino acid residues in protein molecules are exclusively L stereoisomers." It is not clear whether this selectivity is necessary for proper protein function or is an accident of evolution. To explore this question, Milton and colleagues (1992) published a study of an enzyme made entirely of D stereoisomers. The enzyme they chose was HIV protease, a proteolytic enzyme made by HIV that converts inactive viral pre-proteins to their active forms.

Previously, Wlodawer and coworkers (1989) had reported the complete chemical synthesis of HIV protease from L-amino acids (the L-enzyme), using the process shown in Figure 3–29. Normal HIV protease contains two Cys residues at positions 67 and 95. Because chemical synthesis of proteins containing Cys is technically difficult, Wlodawer and colleagues substituted the synthetic amino acid L-α-amino-n-butyric acid (Aba) for the two Cys residues in the protein. In the authors' words, this was done so as to "reduce synthetic difficulties associated with Cys deprotection and ease product handling."

(a) The structure of Aba is shown below. Why was this a suitable substitution for a Cys residue? Under what circumstances would it not be suitable?



L-α-Amino-n-butyric acid

Wlodawer and coworkers denatured the newly synthesized protein by dissolving it in 6 M guanidine HCl, and then allowed it to fold slowly by dialyzing away the guanidine against a neutral buffer (10% glycerol, 25 mM $NaPO_4$, pH 7).

(b) There are many reasons to predict that a protein synthesized, denatured, and folded in this manner would not be active. Give three such reasons.

(c) Interestingly, the resulting L-protease was active. What does this finding tell you about the role of disulfide bonds in the native HIV protease molecule?

In their new study, Milton and coworkers synthesized HIV protease from D-amino acids, using the same protocol as the earlier study (Wlodawer et al.). Formally, there are three possibilities for the folding of the D-protease: it would give (1) the same shape as the L-protease, (2) the mirror image of the L-protease, or (3) something else, possibly inactive.

(d) For each possibility, decide whether or not it is a likely outcome and defend your position.

In fact, the D-protease was active: it cleaved a particular synthetic substrate and was inhibited by specific inhibitors. To examine the structure of the D- and L-enzymes, Milton and coworkers tested both forms for activity with D and L forms of a chiral peptide substrate and for inhibition by D and L forms of a chiral peptide-analog inhibitor. Both forms were also tested for inhibition by the achiral inhibitor Evans blue. The findings are given in the table.

HIV protease	Substrate hydrolysis		Inhibition		
			Peptide inhibitor		Evans blue (achiral)
	D-substrate	L-protease	D-inhibitor	L-inhibitor	
L-protease	−	+	−	+	+
D-protease	+	−	+	−	+

(e) Which of the three models proposed above is supported by these data? Explain your reasoning.

(f) Why does Evans blue inhibit both forms of the protease?

(g) Would you expect chymotrypsin to digest the D-protease? Explain your reasoning.

(h) Would you expect total synthesis from D-amino acids followed by renaturation to yield active enzyme for any enzyme? Explain your reasoning.

Answer

(a) Aba is a suitable replacement because Aba and Cys have approximately the same sized side chain and are similarly hydrophobic. However, Aba cannot form disulfide bonds so it will not be a suitable replacement if these are required.

(b) There are many important differences between the synthesized protein and HIV protease produced by a human cell, any of which could result in an inactive synthetic enzyme: (1) Although Aba and Cys have similar size and hydrophobicity, Aba may not be similar enough for the protein to fold properly. (2) HIV protease may require disulfide bonds for proper functioning. (3) Many proteins synthesized by ribosomes fold as they are produced; the protein in this study folded only after the chain was complete. (4) Proteins synthesized by ribosomes may interact with the ribosomes as they fold; this is not possible for the protein in the study. (5) Cytosol is a more complex solution than the buffer used in the study; some proteins may require specific, unknown proteins for proper folding. (6) Proteins synthesized in cells often require chaperones for proper folding; these are not present in the study buffer. (7) In cells, HIV protease is synthesized as part of a larger chain that is then proteolytically processed; the protein in the study was synthesized as a single molecule.

(c) Because the enzyme *is* functional with Aba substituted for Cys, disulfide bonds do not play an important role in the structure of HIV protease.

(d) *Model 1*: it would fold like the L-protease. *Argument for*: the covalent structure is the same (except for chirality), so it should fold like the L-protease. *Argument against*: chirality is not a trivial detail; three-dimensional shape is a key feature of biological molecules. The synthetic enzyme will not fold like the L-protease. *Model 2*: it would fold to the mirror image of the L-protease. *For*: because the individual components are mirror images of those in the biological protein, it will fold in the mirror-image shape. *Against*: the interactions involved in protein folding are very complex, so the synthetic protein will most likely fold in another form. *Model* 3: it would fold to something else. *For*: the interactions involved in protein folding are very complex, so the synthetic protein will most likely fold in another form. *Against*: because the individual components are mirror images of those in the biological protein, it will fold in the mirror-image shape.

(e) Model 1. The enzyme is active, but with the enantiomeric form of the biological substrate, and it is inhibited by the enantiomeric form of the biological inhibitor. This is consistent with the D-protease being the mirror image of the L-protease.

(f) Evans blue is achiral; it binds to both forms of the enzyme.

(g) No. Because proteases contain only L-amino acids and recognize only L-peptides, chymotrypsin would not digest the D-protease.

(h) Not necessarily. Depending on the individual enzyme, any of the problems listed in **(b)** could result in an inactive enzyme.

References

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chapter

5 Protein Function

1. **Relationship between Affinity and Dissociation Constant** Protein A has a binding site for ligand X with a K_d of 10^{-6} M. Protein B has a binding site for ligand X with a K_d of 10^{-9} M. Which protein has a higher affinity for ligand X? Explain your reasoning. Convert the K_d to K_a for both proteins.

   Answer Protein B has a higher affinity for ligand X. The lower K_d indicates that protein B will be half-saturated with bound ligand X at a much lower concentration of X than will protein A. Because $K_a = 1/K_d$, protein A has $K_a = 10^6\ M^{-1}$; protein B has $K_a = 10^9\ M^{-1}$.

2. **Negative Cooperativity** Which of the following situations would produce a Hill plot with $n_H < 1.0$? Explain your reasoning in each case.
   (a) The protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site decreases the binding affinity of other sites for the ligand.
   (b) The protein is a single polypeptide with two ligand-binding sites, each having a different affinity for the ligand.
   (c) The protein is a single polypeptide with a single ligand-binding site. As purified, the protein preparation is heterogeneous, containing some protein molecules that are partially denatured and thus have a lower binding affinity for the ligand.

   Answer All three situations would produce $n_H < 1.0$. An n_H (Hill coefficient) of <1.0 generally suggests situation **(a)**—the classic case of negative cooperativity. However, closer examination of the properties of a protein exhibiting apparent negative cooperativity in ligand binding often reveals situation **(b)** or **(c).** When two or more types of ligand-binding sites with different affinities for the ligand are present on the same or different proteins in the same solution, apparent negative cooperativity is observed. In **(b),** the higher-affinity ligand-binding sites bind the ligand first. As the ligand concentration is increased, binding to the lower-affinity sites produces an $n_H < 1.0$, even though binding to the two ligand-binding sites is completely independent. Even more common is situation **(c),** in which the protein preparation is heterogeneous. Unsuspected proteolytic digestion by contaminating proteases and partial denaturation of the protein under certain solvent conditions are common artifacts of protein purification. There are few well-documented cases of *true* negative cooperativity.

3. **Affinity for Oxygen of Hemoglobin** What is the effect of the following changes on the O_2 affinity of hemoglobin? **(a)** A drop in the pH of blood plasma from 7.4 to 7.2. **(b)** A decrease in the partial pressure of CO_2 in the lungs from 6 kPa (holding one's breath) to 2 kPa (normal). **(c)** An increase in the BPG level from 5 mM (normal altitudes) to 8 mM (high altitudes). **(d)** An increase in CO from 1.0 parts per million (ppm) in a normal indoor atmosphere to 30 ppm in a home that has a malfunctioning or leaking furnace.

Answer The affinity of hemoglobin for O_2 is regulated by the binding of the ligands H^+, CO_2, and BPG. The binding of each ligand shifts the O_2-saturation curve to the right—that is, the O_2 affinity of hemoglobin is reduced in the presence of ligand. **(a)** decreases the affinity; **(b)** increases the affinity; **(c)** decreases the affinity; **(d)** decreases the affinity.

4. Reversible Ligand Binding The protein calcineurin binds to the protein calmodulin with an association rate of $8.9 \times 10^3\ \text{M}^{-1}\text{s}^{-1}$ and an overall dissociation constant, K_d, of 10 nM. Calculate the dissociation rate, k_d, including appropriate units.

Answer K_d, the dissociation constant, is the ratio of k_d, the rate constant for the dissociation reaction, to k_a, the rate constant for the association reaction.

$$K_d = k_d/k_a$$

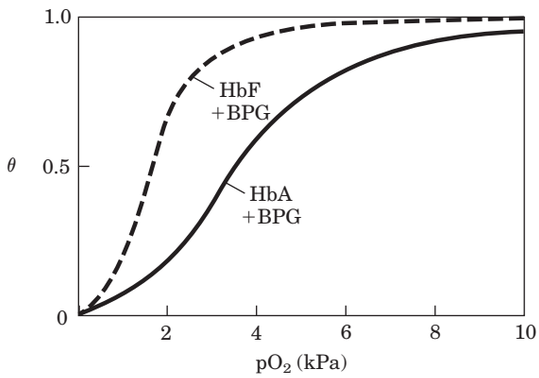
Rearrange to solve for k_d and substitute the known values.

$$k_d = K_d \times k_a = (10 \times 10^{-9}\ \text{M})(8.9 \times 10^3\ \text{M}^{-1}\text{s}^{-1}) = 8.9 \times 10^{-5}\ \text{s}^{-1}$$

5. Cooperativity in Hemoglobin Under appropriate conditions, hemoglobin dissociates into its four subunits. The isolated α subunit binds oxygen, but the O_2-saturation curve is hyperbolic rather than sigmoid. In addition, the binding of oxygen to the isolated α subunit is not affected by the presence of H^+, CO_2, or BPG. What do these observations indicate about the source of the cooperativity in hemoglobin?

Answer These observations indicate that the cooperative behavior—the sigmoid O_2-binding curve and the positive cooperativity in ligand binding—of hemoglobin arises from interaction between subunits.

6. Comparison of Fetal and Maternal Hemoglobins Studies of oxygen transport in pregnant mammals show that the O_2-saturation curves of fetal and maternal blood are markedly different when measured under the same conditions. Fetal erythrocytes contain a structural variant of hemoglobin, HbF, consisting of two α and two γ subunits ($\alpha_2\gamma_2$), whereas maternal erythrocytes contain HbA ($\alpha_2\beta_2$).



(a) Which hemoglobin has a higher affinity for oxygen under physiological conditions, HbA or HbF? Explain.

(b) What is the physiological significance of the different O_2 affinities?

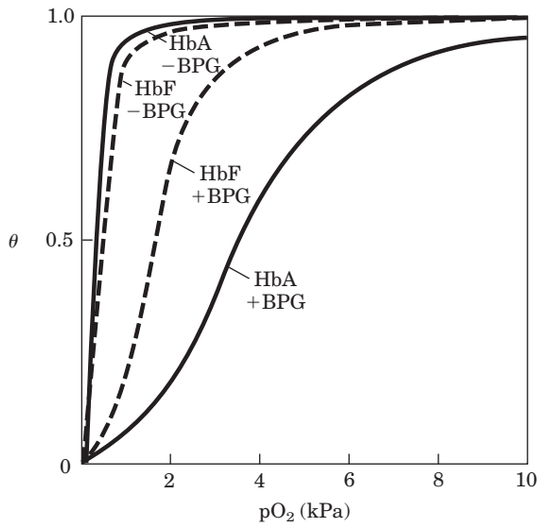
(c) When all the BPG is carefully removed from samples of HbA and HbF, the measured O_2-saturation curves (and consequently the O_2 affinities) are displaced to the left. However, HbA now has a greater affinity for oxygen than does HbF. When BPG is reintroduced, the O_2-saturation curves return to normal, as shown in the graph. What is the effect of BPG on the O_2 affinity of hemoglobin? How can the above information be used to explain the different O_2 affinities of fetal and maternal hemoglobin?

Answer

(a) The observation that hemoglobin A (HbA; maternal) is about 60% saturated at $pO_2 = 4$ kPa (the pO_2 in tissues), whereas hemoglobin F (HbF; fetal) is more than 90% saturated under the same physiological conditions, indicates that HbF has a higher O_2 affinity than HbA. In other words, at identical O_2 concentrations, HbF binds more oxygen than does HbA. Thus, HbF must bind oxygen more tightly (with higher affinity) than HbA under physiological conditions.

(b) The higher O_2 affinity of HbF ensures that oxygen will flow from maternal blood to fetal blood in the placenta. For maximal O_2 transport, the oxygen pressure at which fetal blood approaches full saturation must be in the region where the O_2 affinity of HbA is low. This is indeed the case.

(c)



Binding of BPG to hemoglobin reduces the affinity of hemoglobin for O_2, as shown in the graph. The O_2-saturation curve for HbA shifts far to the right when BPG binds (solid curves)—that is, the O_2 affinity is dramatically lowered. The O_2-saturation curve for HbF also shifts to the right when BPG binds (dashed curves), but not as far. Because the O_2-saturation curve of HbA undergoes a larger shift on BPG binding than does that of HbF, we can conclude that HbA binds BPG more tightly than does HbF. Differential binding of BPG to the two hemoglobins may determine the difference in their O_2 affinities.

7. Hemoglobin Variants There are almost 500 naturally occurring variants of hemoglobin. Most are the result of a single amino acid substitution in a globin polypeptide chain. Some variants produce clinical illness, though not all variants have deleterious effects. A brief sample follows:

HbS (sickle-cell Hb): substitutes a Val for a Glu on the surface

Hb Cowtown: eliminates an ion pair involved in T-state stabilization

Hb Memphis: substitutes one uncharged polar residue for another of similar size on the surface

Hb Bibba: substitutes a Pro for a Leu involved in an α helix

Hb Milwaukee: substitutes a Glu for a Val

Hb Providence: substitutes an Asn for a Lys that normally projects into the central cavity of the tetramer

Hb Philly: substitutes a Phe for a Tyr, disrupting hydrogen bonding at the $\alpha_1\beta_1$ interface

Explain your choices for each of the following:

(a) The Hb variant *least* likely to cause pathological symptoms.

(b) The variant(s) most likely to show pI values different from that of HbA on an isoelectric focusing gel.

(c) The variant(s) most likely to show a decrease in BPG binding and an increase in the overall affinity of the hemoglobin for oxygen.

Answer

(a) Hb Memphis; it has a conservative substitution that is unlikely to have a significant effect on function.

(b) HbS, Hb Milwaukee, and Hb Providence; all have substitutions that alter the net charge on the protein, which will change the pI. The loss of an ion pair in Hb Cowtown may indicate loss of a charged residue, which would also change the pI, but there is not enough information to be sure.

(c) Hb Providence; it has an Asn residue in place of a Lys that normally projects into the central cavity of hemoglobin. Loss of the positively charged Lys that normally interacts with the negative charges on BPG results in Hb Providence having lower affinity for BPG and thus higher affinity for O_2.

8. Oxygen Binding and Hemoglobin Structure A team of biochemists uses genetic engineering to modify the interface region between hemoglobin subunits. The resulting hemoglobin variants exist in solution primarily as $\alpha\beta$ dimers (few, if any, $\alpha_2\beta_2$ tetramers form). Are these variants likely to bind oxygen more weakly or more tightly? Explain your answer.

Answer More tightly. An inability to form tetramers would limit the cooperativity of these variants, and the binding curve would become more hyperbolic. Also, the BPG-binding site would be disrupted. Oxygen binding would probably be tighter, because the default state in the absence of bound BPG is the tight-binding R state.

9. Reversible (but Tight) Binding to an Antibody An antibody binds to an antigen with a K_d of 5×10^{-8} M. At what concentration of antigen will θ be **(a)** 0.2, **(b)** 0.5, **(c)** 0.6, **(d)** 0.8?

Answer (a) 1×10^{-8} M, **(b)** 5×10^{-8} M, **(c)** 8×10^{-8} M, **(d)** 2×10^{-7} M. These are calculated from a rearrangement of Equation 5–8 to give $[L] = \theta K_d/(1 - \theta)$, and for this antigen-antibody binding, $[L] = \theta(5 \times 10^{-8}\ \text{M})/(1 - \theta)$. For example, for **(a)** $[L] = 0.2(5 \times 10^{-8}\ \text{M})/(0.8) = 1 \times 10^{-8}$ M.

10. Using Antibodies to Probe Structure-Function Relationships in Proteins A monoclonal antibody binds to G-actin but not to F-actin. What does this tell you about the epitope recognized by the antibody?

Answer The epitope is likely to be a structure that is buried when G-actin polymerizes to form F-actin.

11. The Immune System and Vaccines A host organism needs time, often days, to mount an immune response against a new antigen, but memory cells permit a rapid response to pathogens previously encountered. A vaccine to protect against a particular viral infection often consists of weakened or killed virus or isolated proteins from a viral protein coat. When injected into a human patient, the vaccine generally does not cause an infection and illness, but it effectively "teaches" the immune system what the viral particles look like, stimulating the production of memory cells. On subsequent infection, these cells can bind to the virus and trigger a rapid immune response. Some pathogens, including HIV, have developed mechanisms to evade the immune system, making it difficult or impossible to develop effective vaccines against them. What strategy could a pathogen use to evade the immune system? Assume that a host's antibodies and/or T-cell receptors are available to bind to any structure that might appear on the surface of a pathogen and that, once bound, the pathogen is destroyed.

Answer Many pathogens, including HIV, have evolved mechanisms by which they can repeatedly alter the surface proteins to which immune system components initially bind. Thus the host organism regularly faces new antigens and requires time to mount an immune response to each one. As the immune system responds to one variant, new variants are created. Some

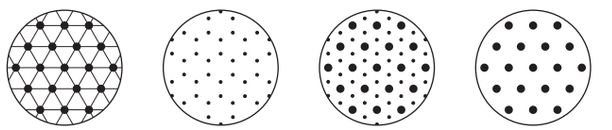
molecular mechanisms that are used to vary viral surface proteins are described in Part III of the text. HIV uses an additional strategy to evade the immune system: it actively infects and destroys immune system cells.

12. How We Become a "Stiff" When a vertebrate dies, its muscles stiffen as they are deprived of ATP, a state called rigor mortis. Explain the molecular basis of the rigor state.

Answer Binding of ATP to myosin triggers dissociation of myosin from the actin thin filament. In the absence of ATP, actin and myosin bind tightly to each other.

13. Sarcomeres from Another Point of View The symmetry of thick and thin filaments in a sarcomere is such that six thin filaments ordinarily surround each thick filament in a hexagonal array. Draw a cross section (transverse cut) of a myofibril at the following points: **(a)** at the M line; **(b)** through the I band; **(c)** through the dense region of the A band; **(d)** through the less dense region of the A band, adjacent to the M line (see Fig. 5–29b, c).

Answer



(a) At M line **(b)** At I band **(c)** At dense region of A band **(d)** At less dense region of A band

The less dense region of the A band, also known as the H zone (not shown in Fig. 5–29b), is the region in which the myosin thick filaments do not overlap the actin thin filaments. When the sarcomere contracts (see Fig. 5–29c), the H zone and the I band decrease in width.

Biochemistry on the Internet

14. Lysozyme and Antibodies To fully appreciate how proteins function in a cell, it is helpful to have a three-dimensional view of how proteins interact with other cellular components. Fortunately, this is possible using Web-based protein databases and three-dimensional molecular viewing utilities. Some molecular viewers require that you download a program or plug-in; some can be problematic when used with certain operating systems or browsers; some require the use of command-line code; some have a more user-friendly interface. We suggest you go to www.umass.edu/microbio/rasmol and look at the information about RasMol, Protein Explorer, and Jmol FirstGlance. Choose the viewer most compatible with your operating system, browser, and level of expertise. Then download and install any software or plug-ins you may need.

In this exercise you will examine the interactions between the enzyme lysozyme (Chapter 4) and the Fab portion of the anti-lysozyme antibody. Use the PDB identifier 1FDL to explore the structure of the IgG1 Fab fragment–lysozyme complex (antibody-antigen complex). To answer the following questions, use the information on the Structure Summary page at the Protein Data Bank (www.rcsb.org), and view the structure using RasMol, Protein Explorer, or FirstGlance in Jmol.

(a) Which chains in the three-dimensional model correspond to the antibody fragment and which correspond to the antigen, lysozyme?

(b) What type of secondary structure predominates in this Fab fragment?

(c) How many amino acid residues are in the heavy and light chains of the Fab fragment? In lysozyme? Estimate the percentage of the lysozyme that interacts with the antigen-binding site of the antibody fragment.

(d) Identify the specific amino acid residues in lysozyme and in the variable regions of the Fab heavy and light chains that are situated at the antigen-antibody interface. Are the residues contiguous in the primary sequence of the polypeptide chains?

Answer

(a) Chain L is the light chain and chain H is the heavy chain of the Fab fragment of this antibody molecule. Chain Y is lysozyme.

(b) At the PDB, the SCOP and CATH data show that the proteins have predominantly β secondary structure forming immunoglobulin-like β-sandwich folds. Use the Jmol viewing utility at the PDB to view the complex. You should be able to identify the β structures in the variable and constant regions of both the light and heavy chains.

(c) The heavy chain of the Fab fragment has 218 amino acid residues, the light chain fragment has 214, and lysozyme has 129. Viewing the structure in the spacefill mode shows that less than 15% of the total lysozyme molecule is in contact with the combined V_L and V_H domains of the antibody fragment.

(d) To answer this question you may wish to use FirstGlance in Jmol (http://firstglance.jmol.org). Enter the PDB ID 1FDL. When the molecule appears, check the "Spin" box to stop the molecule from spinning. Next, click "Contacts." With "Chains" selected as the target, click on the lysozyme portion of the complex (Chain Y). The atoms will have asterisks when they are selected. Click "Show Atoms Contacting Target." Only the atoms (in the immunoglobulin chains) that are in contact with lysozyme will remain in space-filling mode. A quick click on each atom will bring up identifying information. Repeat the process with each of the immunoglobulin chains selected to find the lysozyme residues at the interface. In the H chain these residues include Gly^{31}, Tyr^{32}, Asp^{100}, and Tyr^{101}; in the L chain, Tyr^{32}, Tyr^{49}, Tyr^{50}, and Trp^{92}. In lysozyme, residues Asn^{19}, Gly^{22}, Tyr^{23}, Ser^{24}, Lys^{116}, Gly^{117}, Thr^{118}, Asp^{119}, Gln^{121}, and Arg^{125} appear to be situated at the antigen-antibody interface. Not all these residues are adjacent in the primary structure. In any antibody, the residues in the V_L and V_H domains that come into contact with the antigen are located primarily in the loops connecting the β strands of the β-sandwich supersecondary structure. Folding of the polypeptide chain into higher levels of structure brings the nonconsecutive residues together to form the antigen-binding site.

15. Exploring Reversible Interactions of Proteins and Ligands with Living Graphs Use the living graphs for Equations 5–8, 5–11, 5–14, and 5–16 to work through the following exercises.

(a) Reversible binding of a ligand to a simple protein, without cooperativity. For Equation 5–8, set up a plot of θ versus [L] (vertical and horizontal axes, respectively). Examine the plots generated when K_d is set at 5, 10, 20, and 100 μM. Higher affinity of the protein for the ligand means more binding at lower ligand concentrations. Suppose that four different proteins exhibit these four different K_d values for ligand L. Which protein would have the highest affinity for L?

Examine the plot generated when $K_d = 10$ μM. How much does θ increase when [L] increases from 0.2 to 0.4 μM? How much does θ increase when [L] increases from 40 to 80 μM?

You can do the same exercise for Equation 5–11. Convert [L] to pO_2 and K_d to P_{50}. Examine the curves generated when P_{50} is set at 0.5, 1, 2, and 10 kPa. For the curve generated when $P_{50} = 1$ kPa, how much does θ change when the pO_2 increases from 0.02 to 0.04 kPa? From 4 to 8 kPa?

(b) Cooperative binding of a ligand to a multisubunit protein. Using Equation 5–14, generate a binding curve for a protein and ligand with $K_d = 10$ μM and $n = 3$. Note the altered definition of K_d in Equation 5–16. On the same plot, add a curve for a protein with $K_d = 20$ μM and $n = 3$. Now see how both curves change when you change to $n = 4$. Generate Hill plots (Eqn 5–16) for each of these cases. For $K_d = 10$ μM and $n = 3$, what is θ when [L] = 20 μM?

(c) Explore these equations further by varying all the parameters used above.

Answer

(a) The plots should be a series of hyperbolic curves, with $\theta = 1.0$ as the limit. Each curve passes through $\theta = 0.5$ at the point on the x axis where [L] = K_d. The protein with $K_d = 5$ μM has the highest affinity for ligand L. When $K_d = 10$ μM, doubling [L] from 0.2 to 0.4 μM (values well below K_d) nearly doubles θ (the actual increase factor is 1.96).

This is a property of the hyperbolic curve; at low ligand concentrations, θ is an almost linear function of [L]. By contrast, doubling [L] from 40 to 80 μM (well above K_d, where the binding curve is approaching its asymptotic limit) increases θ by a factor of only 1.1. The increase factors are identical for the curves generated from Equation 5–11.

(b) The curves generated from Equation 5–14 should be sigmoidal. Increasing the Hill coefficient (n) increases the slope of the curves at the inflection point. Using Equation 5–14, with [L] = 20 μM, K_d = 10 μM, and $n = 3$, you will find that $\theta = 0.998$.

(c) A variety of answers will be obtained depending on the values entered for the different parameters.

Data Analysis Problem

16. Protein Function During the 1980s, the structures of actin and myosin were known only at the resolution shown in Figure 5–28a, b. Although researchers knew that the S1 portion of myosin binds to actin and hydrolyzes ATP, there was a substantial debate about where in the myosin molecule the contractile force was generated. At the time, two competing models were proposed for the mechanism of force generation in myosin.

In the "hinge" model, S1 bound to actin, but the pulling force was generated by contraction of the "hinge region" in the myosin tail. The hinge region is in the heavy meromyosin portion of the myosin molecule, near where trypsin cleaves off light meromyosin (see Fig. 5–27b). This is roughly the point labeled "Two supercoiled α helices" in Figure 5–27a. In the "S1" model, the pulling force was generated in the S1 "head" itself and the tail was just for structural support.

Many experiments had been performed but provided no conclusive evidence. In 1987, James Spudich and his colleagues at Stanford University published a study that, although not conclusive, went a long way toward resolving this controversy.

Recombinant DNA techniques were not sufficiently developed to address this issue in vivo, so Spudich and colleagues used an interesting in vitro motility assay. The alga *Nitella* has extremely long cells, often several centimeters in length and about 1 mm in diameter. These cells have actin fibers that run along their long axes, and the cells can be cut open along their length to expose the actin fibers. Spudich and his group had observed that plastic beads coated with myosin would "walk" along these fibers in the presence of ATP, just as myosin would do in contracting muscle.

For these experiments, they used a more well-defined method for attaching the myosin to the beads. The "beads" were clumps of killed bacterial (*Staphylococcus aureus*) cells. These cells have a protein on their surface that binds to the Fc region of antibody molecules (Fig. 5–21a). The antibodies, in turn, bind to several (unknown) places along the tail of the myosin molecule. When bead-antibody-myosin complexes were prepared with intact myosin molecules, they would move along *Nitella* actin fibers in the presence of ATP.

(a) Sketch a diagram showing what a bead-antibody-myosin complex might look like at the molecular level.

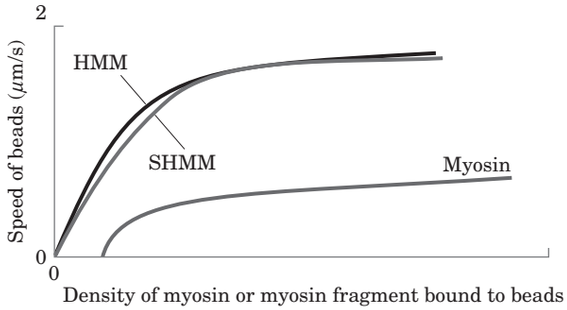
(b) Why was ATP required for the beads to move along the actin fibers?

(c) Spudich and coworkers used antibodies that bound to the myosin tail. Why would this experiment have failed if they had used an antibody that bound to the part of S1 that normally binds to actin? Why would this experiment have failed if they had used an antibody that bound to actin?

To help focus in on the part of myosin responsible for force production, Spudich and his colleagues used trypsin to produce two partial myosin molecules (see Fig. 5–27): (1) heavy meromyosin (HMM), made by briefly digesting myosin with trypsin; HMM consists of S1 and the part of the tail that includes the hinge; and (2) short heavy meromyosin (SHMM), made from a more extensive digestion of HMM with trypsin; SHMM consists of S1 and a shorter part of the tail that does not include the hinge. Brief digestion of myosin with trypsin produces HMM and light meromyosin (Fig. 5–27), by cleavage of a single specific peptide bond in the myosin molecule.

(d) Why might trypsin attack this peptide bond first rather than other peptide bonds in myosin?

Spudich and colleagues prepared bead-antibody-myosin complexes with varying amounts of myosin, HMM, and SHMM, and measured their speeds along *Nitella* actin fibers in the presence of ATP. The graph below sketches their results.



(e) Which model ("S1" or "hinge") is consistent with these results? Explain your reasoning.

(f) Provide a plausible explanation for why the speed of the beads increased with increasing myosin density.

(g) Provide a plausible explanation for why the speed of the beads reached a plateau at high myosin density.

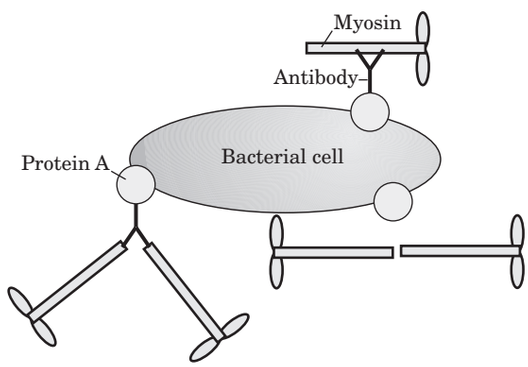
The more extensive trypsin digestion required to produce SHMM had a side effect: another specific cleavage of the myosin polypeptide backbone in addition to the cleavage in the tail. This second cleavage was in the S1 head.

(h) Based on this information, why is it surprising that SHMM was still capable of moving beads along actin fibers?

(i) As it turns out, the tertiary structure of the S1 head remains intact in SHMM. Provide a plausible explanation of how the protein remains intact and functional even though the polypeptide backbone has been cleaved and is no longer continuous.

Answer

(a)



The drawing is not to scale; any given cell would have many more myosin molecules on its surface.

(b) ATP is needed to provide the chemical energy to drive the motion (see Chapter 13).

(c) An antibody that bound to the myosin tail, the actin-binding site, would block actin binding and prevent movement. An antibody that bound to actin would also prevent actin-myosin interaction and thus movement.

(d) There are two possible explanations: (1) Trypsin cleaves only at Lys and Arg residues (see Table 3–7) so would not cleave at many sites in the protein. (2) Not all Arg or Lys residues are equally accessible to trypsin; the most-exposed sites would be cleaved first.

(e) The S1 model. The hinge model predicts that bead-antibody-HMM complexes (with the hinge) would move, but bead-antibody-SHMM complexes (no hinge) would not. The S1 model predicts that because both complexes include S1, both would move. The finding that the beads move with SHMM (no hinge) is consistent only with the S1 model.

(f) With fewer myosin molecules bound, the beads could temporarily fall off the actin as a myosin let go of it. The beads would then move more slowly, as time is required for a second myosin to bind. At higher myosin density, as one myosin lets go another quickly binds, leading to faster motion.

(g) Above a certain density, what limits the rate of movement is the intrinsic speed with which myosin molecules move the beads. The myosin molecules are moving at a maximum rate and adding more will not increase speed.

(h) Because the force is produced in the S1 head, damaging the S1 head would probably inactivate the resulting molecule, and SHMM would be incapable of producing movement.

(i) The S1 head must be held together by noncovalent interactions that are strong enough to retain the active shape of the molecule.

Reference

Hynes, T.R., Block, S.M., White, B.T., & Spudich, J.A. (1987) Movement of myosin fragments in vitro: domains involved in force production. *Cell* **48,** 953–963.

chapter

6

Enzymes

1. Keeping the Sweet Taste of Corn The sweet taste of freshly picked corn (maize) is due to the high level of sugar in the kernels. Store-bought corn (several days after picking) is not as sweet, because about 50% of the free sugar is converted to starch within one day of picking. To preserve the sweetness of fresh corn, the husked ears can be immersed in boiling water for a few minutes ("blanched") then cooled in cold water. Corn processed in this way and stored in a freezer maintains its sweetness. What is the biochemical basis for this procedure?

Answer After an ear of corn has been removed from the plant, the enzyme-catalyzed conversion of sugar to starch continues. Inactivation of these enzymes slows down the conversion to an imperceptible rate. One of the simplest techniques for inactivating enzymes is heat denaturation. Freezing the corn lowers any remaining enzyme activity to an insignificant level.

2. Intracellular Concentration of Enzymes To approximate the actual concentration of enzymes in a bacterial cell, assume that the cell contains equal concentrations of 1,000 different enzymes in solution in the cytosol and that each protein has a molecular weight of 100,000. Assume also that the bacterial cell is a cylinder (diameter 1.0 μm, height 2.0 μm), that the cytosol (specific gravity 1.20) is 20% soluble protein by weight, and that the soluble protein consists entirely of enzymes. Calculate the *average* molar concentration of each enzyme in this hypothetical cell.

Answer There are three different ways to approach this problem.

(i) The concentration of total protein in the cytosol is

$$\frac{(1.2\text{ g/mL})(0.20)}{100{,}000\text{ g/mol}} = 0.24 \times 10^{-5}\text{ mol/mL} = 2.4 \times 10^{-3}\text{ M}$$

Thus, for 1 enzyme in 1,000, the enzyme concentration is

$$\frac{2.4 \times 10^{-3}\text{ M}}{1000} = 2.4 \times 10^{-6}\text{ M}$$

(ii) The average molar concentration $= \dfrac{\text{moles of each enzyme in cell}}{\text{volume of cell in liters}}$

Volume of bacterial cytosol $= \pi r^2 h = (3.14)(0.50\ \mu\text{m})^2(2.0\ \mu\text{m}) = 1.6\ \mu\text{m}^3$
$= 1.6 \times 10^{-12}\text{ cm}^3 = 1.6 \times 10^{-12}\text{ mL}$
$= 1.6 \times 10^{-15}\text{ L}$

Amount (in moles) of each enzyme in cell is

$$\frac{(0.20)(1.2\text{ g/cm}^3)(1.6\ \mu\text{m}^3)(10^{-12}\text{ cm}^3/\mu\text{m}^3)}{(100{,}000\text{ g/mol})(1000)} = 3.8 \times 10^{-21}\text{ mol}$$

$$\text{Average molar concentration} = \frac{3.8 \times 10^{-21}\text{ mol}}{1.6 \times 10^{-15}\text{ L}}$$

$$= 2.4 \times 10^{-6}\text{ mol/L} = 2.4 \times 10^{-6}\text{ M}$$

(iii) Volume of bacterial cytosol = $\pi r^2 h$

$= (3.14)(0.50\ \mu\text{m})^2(2.0\ \mu\text{m}) = 1.6\ \mu\text{m}^3 = 1.6 \times 10^{-12}$ mL

Weight of cytosol = (specific gravity)(volume)

$= (1.2\ \text{g/mL})(1.6 \times 10^{-12}\ \text{mL}) = 1.9 \times 10^{-12}$ g

Average weight of each protein (1 in 1,000, 20% wt/wt protein)

$= (1.9 \times 10^{-12}\ \text{g})(0.20)/(1{,}000) = 3.8 \times 10^{-16}$ g

Average molar concentration of each protein

= (average weight)/(M_r)(volume)

$= (3.8 \times 10^{-16}\ \text{g})/(10^5\ \text{g/mol})(1.6 \times 10^{-12}\ \text{mL})(1\ \text{L}/1000\ \text{mL})$

$= 2.4 \times 10^{-6}\ \text{mol/L} = 2.4 \times 10^{-6}\ \text{M}$

3. Rate Enhancement by Urease The enzyme urease enhances the rate of urea hydrolysis at pH 8.0 and 20 °C by a factor of 10^{14}. If a given quantity of urease can completely hydrolyze a given quantity of urea in 5.0 min at 20 °C and pH 8.0, how long would it take for this amount of urea to be hydrolyzed under the same conditions in the absence of urease? Assume that both reactions take place in sterile systems so that bacteria cannot attack the urea.

Answer

Time to hydrolyze urea

$$= \frac{(5.0\ \text{min})(10^{14})}{(60\ \text{min/hr})(24\ \text{hr/day})(365\ \text{days/yr})}$$

$= 9.5 \times 10^8$ yr

= 950 million years!

4. Protection of an Enzyme against Denaturation by Heat When enzyme solutions are heated, there is a progressive loss of catalytic activity over time due to denaturation of the enzyme. A solution of the enzyme hexokinase incubated at 45 °C lost 50% of its activity in 12 min, but when incubated at 45 °C in the presence of a very large concentration of one of its substrates, it lost only 3% of its activity in 12 min. Suggest why thermal denaturation of hexokinase was retarded in the presence of one of its substrates.

Answer One possibility is that the ES complex is more stable than the free enzyme. This implies that the ground state for the ES complex is at a lower energy level than that for the free enzyme, thus *increasing the height of the energy barrier* to be crossed in passing from the native to the denatured or unfolded state.

An alternative view is that an enzyme denatures in two stages: reversible conversion of active native enzyme (N) to an inactive unfolded state (U), followed by irreversible conversion to inactivated enzyme (I):

$$\text{N} \rightleftharpoons \text{U} \longrightarrow \text{I}$$

If substrate, S, binds only to N, saturation with S to form NS would leave less free N available for conversion to U or I, as the N $\rightleftharpoons$ U equilibrium is perturbed toward N. If N but not NS is converted to U or I, then substrate binding will cause stabilization.

5. Requirements of Active Sites in Enzymes Carboxypeptidase, which sequentially removes carboxyl-terminal amino acid residues from its peptide substrates, is a single polypeptide of 307 amino acids. The two essential catalytic groups in the active site are furnished by Arg^{145} and Glu^{270}.

(a) If the carboxypeptidase chain were a perfect α helix, how far apart (in Å) would Arg^{145} and Glu^{270} be? (Hint: see Fig. 4–4a.)

(b) Explain how the two amino acid residues can catalyze a reaction occurring in the space of a few angstroms.

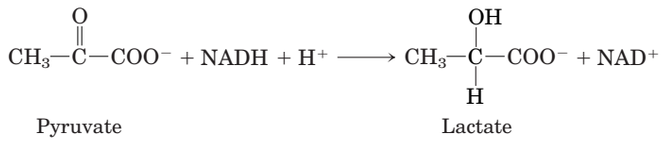
Answer

(a) Arg^{145} is separated from Glu^{270} by $270 - 145 = 125$ amino acid (AA) residues. From Figure 4–4a we see that the α helix has 3.6 AA/turn and increases in length along the major axis by 5.4 Å/turn. Thus, the distance between the two residues is

$$\frac{(125 \text{ AA})(5.4 \text{ Å/turn})}{3.6 \text{ AA/turn}} = 190 \text{ Å}$$

(b) Three-dimensional folding of the enzyme brings the two amino acid residues into close proximity.

6. Quantitative Assay for Lactate Dehydrogenase The muscle enzyme lactate dehydrogenase catalyzes the reaction



NADH and NAD^+ are the reduced and oxidized forms, respectively, of the coenzyme NAD. Solutions of NADH, but *not* NAD^+, absorb light at 340 nm. This property is used to determine the concentration of NADH in solution by measuring spectrophotometrically the amount of light absorbed at 340 nm by the solution. Explain how these properties of NADH can be used to design a quantitative assay for lactate dehydrogenase.

Answer The reaction rate can be measured by following the decrease in absorption at 340 nm (as NADH is converted to NAD^+) as the reaction proceeds. The researcher needs to obtain three pieces of information to develop a good quantitative assay for lactate dehydrogenase:

(i) Determine K_m values (see Box 6–1).

(ii) Measure the initial rate at several known concentrations of enzyme with saturating concentrations of NADH and pyruvate.

(iii) Plot the initial rates as a function of [E]; the plot should be linear, with a slope that provides a measure of lactate dehydrogenase concentration.

7. Effect of Enzymes on Reactions Which of the following effects would be brought about by any enzyme catalyzing the simple reaction

$$S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P \quad \text{where} \quad K'_{eq} = \frac{[P]}{[S]}?$$

(a) Decreased K'_{eq}; (b) Increased k_1; (c) Increased K'_{eq}; (d) Increased $\Delta G^{\ddagger}$; (e) Decreased $\Delta G^{\ddagger}$; (f) More negative $\Delta G'^{\circ}$; (g) Increased k_2.

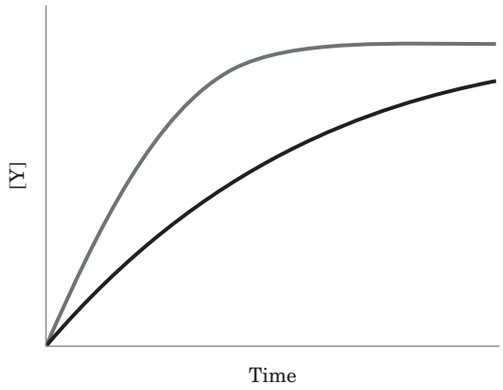
Answer (b), (e), (g). Enzymes do not change a reaction's equilibrium constant and thus catalyze the reaction in both directions, making (b) and (g) correct. Enzymes increase the rate of a reaction by lowering the activation energy, hence (e) is correct.

8. Relation between Reaction Velocity and Substrate Concentration: Michaelis-Menten Equation

(a) At what substrate concentration would an enzyme with a k_{cat} of 30.0 s^{-1} and a K_m of 0.0050 M operate at one-quarter of its maximum rate?

(b) Determine the fraction of V_{max} that would be obtained at the following substrate concentrations [S]: $\frac{1}{2}K_m$, $2K_m$, and $10K_m$.

(c) An enzyme that catalyzes the reaction X ⇌ Y is isolated from two bacterial species. The enzymes have the same V_{max}, but different K_m values for the substrate X. Enzyme A has a K_m of 2.0 μM, while enzyme B has a K_m of 0.5 μM. The plot below shows the kinetics of reactions carried out with the same concentration of each enzyme and with [X] = 1 μM. Which curve corresponds to which enzyme?



Answer

(a) Here we want to find the value of [S] when $V_0 = 0.25\ V_{max}$. The Michaelis-Menten equation is

$$V_0 = V_{max}[S]/(K_m + [S])$$

so $V_0 = V_{max}$ when $[S]/(K_m + [S]) = 0.25$; or

$$[S] = 0.33K_m = 0.33(0.0050\ \text{M}) = 1.7 \times 10^{-3}\ \text{M}$$

(b) The Michaelis-Menten equation can be rearranged to

$$V_0/V_{max} = [S]/(K_m + [S])$$

Substituting $[S] = \frac{1}{2} K_m$ into the equation gives

$$V_0/V_{max} = 0.5\ K_m/1.5K_m = 0.33$$

Similarly, substituting $[S] = 2K_m$ gives

$$V_0/V_{max} = 0.67$$

And substituting $[S] = 10K_m$ gives

$$V_0/V_{max} = 0.91$$

(c) The upper curve corresponds to enzyme B ([X] is greater than the K_m for this enzyme), and the lower curve corresponds to enzyme A. When the initial concentration of substrate is greater than K_m, the rate of the reaction is less sensitive to the depletion of substrate at early stages of the reaction and the rate remains approximately linear for a longer time.

9. Applying the Michaelis-Menten Equation I A research group discovers a new version of happyase, which they call happyase*, that catalyzes the chemical reaction

$$\text{HAPPY} \rightleftharpoons \text{SAD}$$

The researchers begin to characterize the enzyme.

(a) In the first experiment, with $[E_t]$ at 4 nM, they find that the V_{max} is 1.6 μM s^{-1}. Based on this experiment, what is the k_{cat} for happyase*? (Include appropriate units.)

(b) In another experiment, with $[E_t]$ at 1 nM and [HAPPY] at 30 μM, the researchers find that V_0 = 300 nM s^{-1}. What is the measured K_m of happyase* for its substrate HAPPY? (Include appropriate units.)

(c) Further research shows that the purified happyase* used in the first two experiments was actually contaminated with a reversible inhibitor called ANGER. When ANGER is carefully removed from the happyase* preparation, and the two experiments repeated, the measured V_{max} in **(a)** is increased to 4.8 μM s^{-1}, and the measured K_m in **(b)** is now 15 μM. For the inhibitor ANGER, calculate the values of α and α'.

(d) Based on the information given above, what type of inhibitor is ANGER?

Answer

(a) Use the equation $k_{cat} = V_{max}/[E_t]$. k_{cat} = 1600 nM s^{-1}/4 nM = 400 s^{-1}.

(b) Use the equation $V_{max} = k_{cat}[E_t]$. When $[E_t]$ = 1 nM, V_{max} = 400 nM s^{-1}.

$$V_0/V_{max} = 300 \text{ nM s}^{-1}/400 \text{ nM s}^{-1} = \frac{3}{4}$$

Rearrange the Michaelis-Menten equation, substitute for V_0/V_{max}, and solve for K_m.

$$V_0/V_{max} = [S]/(K_m + [S])$$
$$\frac{3}{4} = [S]/(K_m + [S])$$
$$K_m = [S]/3$$

In this experiment, the concentration of the substrate, HAPPY, was 30 μM, so K_m = 10 μM.

(c) As shown in Table 6–9, V_{max} varies as a function of V_{max}/α'. Because V_{max} increased by a factor of 3, α' = 3. Similarly, K_m varies as a function of $\alpha K_m/\alpha'$. Given that K_m increased by a factor of 1.5 when ANGER was removed (that is, the inhibitor decreased the observed K_m by $\frac{2}{3}$) and α' = 3, then α = 2.

(d) Because both α and α' are affected, ANGER is a mixed inhibitor.

10. Applying the Michaelis-Menten Equation II Another enzyme is found that catalyzes the reaction

$$A \rightleftharpoons B$$

Researchers find that the K_m for the substrate A is 4 μM, and the k_{cat} is 20 min^{-1}.

(a) In an experiment, [A] = 6 mM, and the initial velocity, V_0 was 480 nM min^{-1}. What was the $[E_t]$ used in the experiment?

(b) In another experiment, $[E_t]$ = 0.5 μM, and the measured V_0 = 5 μM min^{-1}. What was the [A] used in the experiment?

(c) The compound Z is found to be a very strong competitive inhibitor of the enzyme, with an α of 10. In an experiment with the same $[E_t]$ as in part **(a),** but a different [A], an amount of Z is added that reduces the rate V_0 to 240 nM min^{-1}. What is the [A] in this experiment?

(d) Based on the kinetic parameters given above, has this enzyme evolved to achieve catalytic perfection? Explain your answer briefly, using the kinetic parameter(s) that define catalytic perfection.

Answer

(a) Because [S] is much greater than (more than 1000-fold) K_m, assume that the measured rate of the reaction reflects V_{max}. Use the equation $V_{max} = k_{cat}[E_t]$, and solve for $[E_t]$. $[E_t] = V_{max}/k_{cat}$ = 480 nM min^{-1}/20 min^{-1} = 24 nM.

(b) At this $[E_t]$, the calculated $V_{max} = k_{cat}[E_t]$ = 20 min^{-1} × 0.5 μM = 10 μM min^{-1}. Recall that K_m equals the substrate concentration at which $V_0 = \frac{1}{2}V_{max}$. The measured V_0 is exactly half V_{max}, so [A] = K_m = 4 μM.

(c) Given the same $[E_t]$ as in **(a),** $V_{max} = 480$ nM min^{-1}. The V_0 is again exactly half V_{max} ($V_0 = 240$ nM min^{-1}), so [A] = the apparent or measured K_m. In the presence of an inhibitor with $\alpha = 10$, the measured $K_m = 40$ μM = [S].

(d) No. $k_{cat}/K_m = 0.33/(4 \times 10^{-6}\ M^{-1}\ s^{-1}) = 8.25 \times 10^4\ M^{-1}\ s^{-1}$, well below the diffusion-controlled limit.

11. Estimation of V_{max} and K_m by Inspection Although graphical methods are available for accurate determination of the V_{max} and K_m of an enzyme-catalyzed reaction (see Box 6–1), sometimes these quantities can be quickly estimated by inspecting values of V_0 at increasing [S]. Estimate the V_{max} and K_m of the enzyme-catalyzed reaction for which the following data were obtained.

[S] (M)	V_0 (μM/min)	[S] (M)	V_0 (μM/min)
2.5×10^{-6}	28	4×10^{-5}	112
4.0×10^{-6}	40	1×10^{-4}	128
1×10^{-5}	70	2×10^{-3}	139
2×10^{-5}	95	1×10^{-2}	140

Answer Notice how little the velocity changes as the substrate concentration increases by five-fold from 2 to 10 mM. Thus, we can estimate a V_{max} of 140 μM/min. K_m is defined as the substrate concentration that produces a velocity of $\frac{1}{2}V_{max}$, or 70 μM/min. Inspection of the table indicates that this V_0 occurs at $[S] = 1 \times 10^{-5}$ M, thus $K_m \approx 1 \times 10^{-5}$ M.

12. Properties of an Enzyme of Prostaglandin Synthesis Prostaglandins are a class of eicosanoids, fatty acid derivatives with a variety of extremely potent actions on vertebrate tissues. They are responsible for producing fever and inflammation and its associated pain. Prostaglandins are derived from the 20-carbon fatty acid arachidonic acid in a reaction catalyzed by the enzyme prostaglandin endoperoxide synthase. This enzyme, a cyclooxygenase, uses oxygen to convert arachidonic acid to PGG_2, the immediate precursor of many different prostaglandins (prostaglandin synthesis is described in Chapter 21).

(a) The kinetic data given below are for the reaction catalyzed by prostaglandin endoperoxide synthase. Focusing here on the first two columns, determine the V_{max} and K_m of the enzyme.

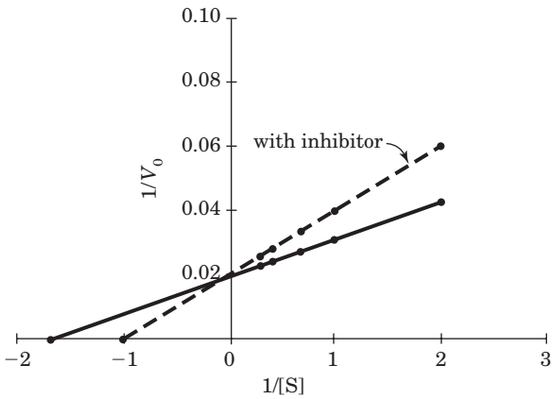
[Arachidonic acid] (mM)	Rate of formation of PGG_2 (mM/min)	Rate of formation of PGG_2 with 10 mg/mL ibuprofen (mM/min)
0.5	23.5	16.67
1.0	32.2	25.25
1.5	36.9	30.49
2.5	41.8	37.04
3.5	44.0	38.91

(b) Ibuprofen is an inhibitor of prostaglandin endoperoxide synthase. By inhibiting the synthesis of prostaglandins, ibuprofen reduces inflammation and pain. Using the data in the first and third columns of the table, determine the type of inhibition that ibuprofen exerts on prostaglandin endoperoxide synthase.

Answer

(a) Calculate the reciprocal values for the data, as in parentheses below, and prepare a double-reciprocal plot to determine the kinetic parameters.

[S] (mM) (1/[S] (mM^{-1}))	V_0 (mM/min) ($1/V_0$ (min/mM))	V_0 with 10 mg/mL ibuprofen (mM/min) ($1/V_0$ (min/mM))
0.5 (2.0)	23.5 (0.043)	16.67 (0.0600)
1.0 (1.0)	32.2 (0.031)	25.25 (0.0396)
1.5 (0.67)	36.9 (0.027)	30.49 (0.0328)
2.5 (0.40)	41.8 (0.024)	37.04 (0.0270)
3.5 (0.28)	44.0 (0.023)	38.91 (0.0257)



The intercept on the vertical axis = $-1/V_{max}$ and the intercept on the horizontal axis = $-1/K_m$. From these values, we can calculate V_{max} and K_m.

$-1/V_{max} = -0.0194$, and $V_{max} = 51.5$ mM/min

$-1/K_m = -1.7$, and $K_m = 0.59$ mM

(b) Ibuprofen acts as a competitive inhibitor. The double-reciprocal plot (with inhibitor) shows that, in the presence of ibuprofen, the V_{max} of the reaction is unchanged (the intercept on the $1/V_0$ axis is the same) and K_m is increased ($-1/K_m$ is closer to the origin).

13. Graphical Analysis of V_{max} and K_m The following experimental data were collected during a study of the catalytic activity of an intestinal peptidase with the substrate glycylglycine:

$$\text{Glycylglycine} + H_2O \longrightarrow 2 \text{ glycine}$$

[S] (mM)	Product formed (μmol/min)	[S] (mM)	Product formed (μmol/min)
1.5	0.21	4.0	0.33
2.0	0.24	8.0	0.40
3.0	0.28	16.0	0.45

Use graphical analysis (see Box 6–1) to determine the K_m and V_{max} for this enzyme preparation and substrate.

Answer As described in Box 6–1, the standard method is to use V_0 versus [S] data to calculate $1/V_0$ and 1/[S].

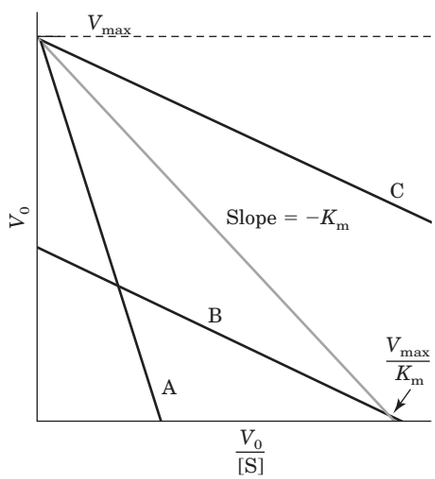
V_0 (μmol/min)	$1/V_0$ (min/μmol)	[S] (mM)	1/[S] (mM^{-1})
0.21	4.8	1.5	0.67
0.24	4.2	2.0	0.50
0.28	3.6	3.0	0.33
0.33	3.0	4.0	0.25
0.40	2.5	8.0	0.13
0.45	2.2	16.0	0.06

Graphing these values gives a Lineweaver-Burk plot. From the best straight line through the data, the intercept on the horizontal axis $= -1/K_m$ and the intercept on the vertical axis $= 1/V_{max}$. From these values, we can calculate K_m and V_{max}:
$-1/K_m = -0.45$, and $K_m = 2.2$ mM
$-1/V_{max} = -2.0$, and $V_{max} = 0.50$ μmol/min

14. The Eadie-Hofstee Equation One transformation of the Michaelis-Menten equation is the Lineweaver-Burk, or double-reciprocal, equation. Multiplying both sides of the Lineweaver-Burk equation by V_{max} and rearranging gives the Eadie-Hofstee equation:

$$V_0 = (-K_m)\frac{V_0}{[S]} + V_{max}$$

A plot of V_0 vs. $V_0/[S]$ for an enzyme-catalyzed reaction is shown below. The curve labeled "Slope = $-K_m$" was obtained in the absence of inhibitor. Which of the other curves (A, B, or C) shows the enzyme activity when a competitive inhibitor is added to the reaction mixture? Hint: See Equation 6–30.



Answer Curve A shows competitive inhibition. V_{max} for A is the same as for the normal curve, as seen by the identical intercepts on the V_0 axis. And, for every value of [S] (until maximal velocity is reached at saturating substrate levels), V_0 is lower for curve A than for the normal curve, indicating competitive inhibition. Note that as [S] increases, V_0/[S] decreases, so that V_{max}—that is, the V_0 at the highest (saturating) [S]—is found at the intersection of the curve at the y axis. Curve C, while also having an identical V_{max}, shows higher V_0 values for every [S] (and for every V_0/[S]) than the normal curve, which is not indicative of inhibition. The lower V_{max} for curve B rules out competitive inhibition.

15. The Turnover Number of Carbonic Anhydrase Carbonic anhydrase of erythrocytes (M_r 30,000) has one of the highest turnover numbers we know of. It catalyzes the reversible hydration of CO_2:

$$H_2O + CO_2 \rightleftharpoons H_2CO_3$$

This is an important process in the transport of CO_2 from the tissues to the lungs. If 10.0 μg of pure carbonic anhydrase catalyzes the hydration of 0.30 g of CO_2 in 1 min at 37 °C at V_{max}, what is the turnover number (k_{cat}) of carbonic anhydrase (in units of min^{-1})?

Answer The turnover number of an enzyme is the number of substrate molecules transformed per unit time by a single enzyme molecule (or a single catalytic site) when the enzyme is saturated with substrate:

$$k_{cat} = V_{max}/E_t$$

where E_t = total moles of active sites.

We can convert the values given in the problem into a turnover number (min^{-1}) by converting the weights of enzyme and substrate to molar amounts:

$$V_{max} \text{ (moles of } CO_2\text{/min)} = \frac{0.30 \text{ g/min}}{44 \text{ g/mol}} = 6.8 \times 10^{-3} \text{ mol/min}$$

$$\text{Amount of enzyme (moles)} = \frac{(10.0\ \mu\text{g})(1\ \text{g}/10^6\ \mu\text{g})}{30{,}000 \text{ g/mol}} = 3.3 \times 10^{-10} \text{ mol}$$

The turnover number is obtained by dividing moles of CO_2/min by moles of enzyme:

$$k_{cat} = \frac{6.8 \times 10^{-3} \text{ mol/min}}{3.3 \times 10^{-10} \text{ mol}} = 2.0 \times 10^7 \text{ min}^{-1}$$

16. Deriving a Rate Equation for Competitive Inhibition The rate equation for an enzyme subject to competitive inhibition is

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]}$$

Beginning with a new definition of total enzyme as

$$[E_t] = [E] + [ES] + [EI]$$

and the definitions of α and K_I provided in the text, derive the rate equation above. Use the derivation of the Michaelis-Menten equation as a guide.

Answer The basic assumptions used to derive the Michaelis-Menten equation still hold. The reaction is at steady state, and the overall rate is determined by

$$V_0 = k_2[ES] \qquad \text{(a)}$$

With the competitive inhibitor, I, now to be added, the goal again is to describe V_0 in terms of the measurable quantities [E_t], [S], and [I]. In the presence of inhibitor,

$$[E_t] = [ES] + [E] + [EI] \qquad (b)$$

We first solve for [EI]. As we have seen,

$$K_I = \frac{[E][I]}{[EI]}; \text{ so } [EI] = \frac{[E][I]}{K_I}$$

Substituting for [EI] in (b) gives

$$[E_t] = [ES] + [E] + \frac{[E][I]}{K_I} \qquad (c)$$

and simplifying gives

$$[E_t] = [ES] + [E]\left(1 + \frac{[I]}{K_I}\right) = [ES] + [E]\alpha \qquad (d)$$

where α describes the effect of the competitive inhibitor. [E] in the absence of inhibitor can be obtained from a rearrangement of Equation 6–19 (remembering that $[E_t] = [ES] + [E]$), to give

$$[E] = \frac{[ES]K_m}{[S]} \qquad (e)$$

Substituting (e) into (d) gives

$$[E_t] = [ES] + \left(\frac{[ES]K_m}{[S]}\right)\alpha \qquad (f)$$

and rearranging and solving for [ES] gives

$$[ES] = \frac{[E_t][S]}{\alpha K_m + [S]} \qquad (g)$$

Next, substituting (g) into (a), and defining $k_2[E_t] = V_{max}$, we get the final equation for reaction velocity in the presence of a competitive inhibitor:

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]}$$

17. Irreversible Inhibition of an Enzyme Many enzymes are inhibited irreversibly by heavy metal ions such as Hg^{2+}, Cu^{2+}, or Ag^+, which can react with essential sulfhydryl groups to form mercaptides:

$$\text{Enz—SH} + Ag^+ \longrightarrow \text{Enz—S—Ag} + H^+$$

The affinity of Ag^+ for sulfhydryl groups is so great that Ag^+ can be used to titrate —SH groups quantitatively. To 10.0 mL of a solution containing 1.0 mg/mL of a pure enzyme, an investigator added just enough $AgNO_3$ to completely inactivate the enzyme. A total of 0.342 μmol of $AgNO_3$ was required. Calculate the minimum molecular weight of the enzyme. Why does the value obtained in this way give only the *minimum* molecular weight?

Answer An equivalency exists between millimoles of $AgNO_3$ required for inactivation and millimoles of —SH group and thus, assuming one —SH group per enzyme molecule, millimoles of enzyme:

$$0.342 \times 10^{-3} \text{ mmol} = \frac{(1.0 \text{ mg/mL})(10.0 \text{ mL})}{(\text{minimum } M_r)(\text{mg/mmol})}$$

$$\text{Thus, the minimum } M_r = \frac{(1.0 \text{ mg/mL})(10.0 \text{ mL})}{0.342 \times 10^{-3} \text{ mmol}} = 2.9 \times 10^4 = 29{,}000$$

This is the *minimum* molecular weight because it assumes only one titratable —SH group per enzyme molecule.

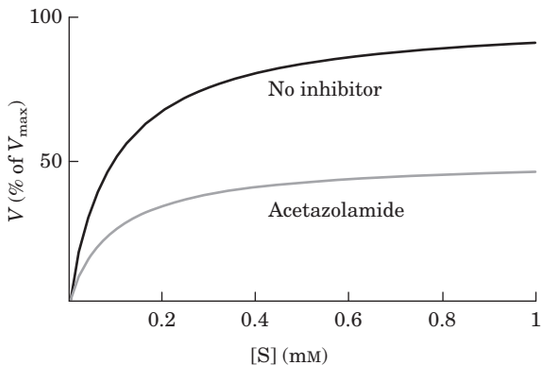
18. Clinical Application of Differential Enzyme Inhibition Human blood serum contains a class of enzymes known as acid phosphatases, which hydrolyze biological phosphate esters under slightly acidic conditions (pH 5.0):

$$R{-}O{-}PO_3^{2-} + H_2O \longrightarrow R{-}OH + HO{-}PO_3^{2-}$$

Acid phosphatases are produced by erythrocytes, the liver, kidney, spleen, and prostate gland. The enzyme of the prostate gland is clinically important because its increased activity in the blood can be an indication of prostate cancer. The phosphatase from the prostate gland is strongly inhibited by tartrate ion, but acid phosphatases from other tissues are not. How can this information be used to develop a specific procedure for measuring the activity of the acid phosphatase of the prostate gland in human blood serum?

Answer First, measure the *total* acid phosphatase activity in a blood sample in units of μmol of phosphate ester hydrolyzed per mL of serum. Next, remeasure this activity in the presence of tartrate ion at a concentration sufficient to completely inhibit the enzyme from the prostate gland. The difference between the two activities represents the activity of acid phosphatase from the prostate gland.

19. Inhibition of Carbonic Anhydrase by Acetazolamide Carbonic anhydrase is strongly inhibited by the drug acetazolamide, which is used as a diuretic (i.e., to increase the production of urine) and to lower excessively high pressure in the eye (due to accumulation of intraocular fluid) in glaucoma. Carbonic anhydrase plays an important role in these and other secretory processes because it participates in regulating the pH and bicarbonate content of several body fluids. The experimental curve of initial reaction velocity (as percentage of V_{max}) versus [S] for the carbonic anhydrase reaction is illustrated below (upper curve). When the experiment is repeated in the presence of acetazolamide, the lower curve is obtained. From an inspection of the curves and your knowledge of the kinetic properties of competitive and mixed enzyme inhibitors, determine the nature of the inhibition by acetazolamide. Explain your reasoning.



Answer The graph gives us several pieces of information. First, the inhibitor prevents the enzyme from achieving the same V_{max} as in the absence of inhibitor. Second, the overall shape of the two curves is very similar: at any [S] the ratio of the two velocities (±inhibitor) is the same. Third, the velocity does not change very much above [S] = 1 mM, so at much higher [S] the observed velocity is essentially V_{max} for each curve. Fourth, if we estimate the [S] at which $\frac{1}{2}V_{max}$ is achieved, this value is nearly identical for both curves. Noncompetitive inhibition, a special form of mixed inhibition that is rarely observed, alters the V_{max} of enzymes but leaves K_m unchanged. Thus, acetazolamide acts as a noncompetitive (mixed) inhibitor of carbonic anhydrase.

20. The Effects of Reversible Inhibitors Derive the expression for the effect of a reversible inhibitor on observed K_m (apparent $K_m = \alpha K_m/\alpha'$). Start with Equation 6–30 and the statement that apparent K_m is equivalent to the [S] at which $V_0 = V_{max}/2\alpha'$.

Answer Equation 6–30 is

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}$$

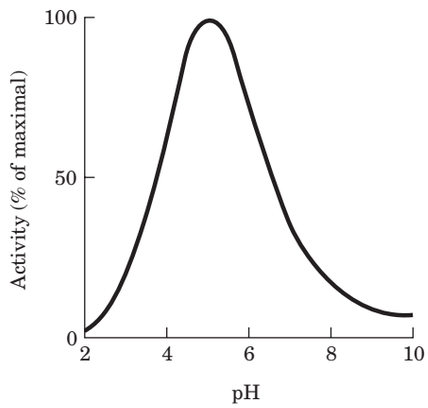
Or $V_0 = V_{max} \times [S]/(\alpha K_m + \alpha'[S])$. Thus, the [S] at which $V_0 = V_{max}/2\alpha'$ is obtained when all the terms on the right side of the equation except V_{max} equal $\frac{1}{2}\alpha'$:

$$[S]/(\alpha K_m + \alpha'[S]) = \tfrac{1}{2}\alpha'$$

We can now solve this equation for [S]:

$2\alpha'[S] = \alpha K_m + \alpha'[S]$
$2\alpha'[S] - \alpha'[S] = \alpha K_m$
$\alpha'[S] = \alpha K_m$
$[S] = \alpha K_m/\alpha'$
Thus, observed $K_m = \alpha K_m/\alpha'$.

21. pH Optimum of Lysozyme The active site of lysozyme contains two amino acid residues essential for catalysis: Glu^{35} and Asp^{52}. The pK_a values of the carboxyl side chains of these residues are 5.9 and 4.5, respectively. What is the ionization state (protonated or deprotonated) of each residue at pH 5.2, the pH optimum of lysozyme? How can the ionization states of these residues explain the pH-activity profile of lysozyme shown below?



Answer At a pH midway between the two pK_a values (pH 5.2), the side-chain carboxyl group of Asp^{52}, with the lower pK_a (4.5), is mainly deprotonated (—COO^-), whereas Glu^{35}, with the higher pK_a (5.9; the stronger base), is protonated (—COOH). At pH values below 5.2, Asp^{52} becomes protonated and the activity decreases. Similarly, at pH values above 5.2, Glu^{35} becomes deprotonated and the activity also decreases. The pH-activity profile suggests that maximum catalytic activity occurs at a pH midway between the pK_a values of the two acidic groups, when Glu^{35} is protonated and Asp^{52} is deprotonated.

22. Working with Kinetics Go to the Living Graphs for Chapter 6.

(a) Using the Living Graph for Equation 6–9, create a V versus [S] plot. Use $V_{max} = 100\ \mu M\ s^{-1}$, and $K_m = 10\ \mu M$. How much does V_0 increase when [S] is doubled, from 0.2 to 0.4 μM? What is V_0 when [S] = 10 μM? How much does the V_0 increase when [S] increases from 100 to 200 μM? Observe how the graph changes when the values for V_{max} or K_m are halved or doubled.

(b) Using the Living Graph for Equation 6–30 and the kinetic parameters in **(a),** create a plot in which both α and α' are 1.0. Now observe how the plot changes when $\alpha = 2.0$; when $\alpha' = 3.0$; and when $\alpha = 2.0$ and $\alpha' = 3.0$.

(c) Using the Living Graphs for Equation 6–30 and the Lineweaver-Burk equation in Box 6–1, create Lineweaver-Burk (double-reciprocal) plots for all the cases in **(a)** and **(b).** When $\alpha = 2.0$, does the x intercept move to the right or to the left? If $\alpha = 2.0$ and $\alpha' = 3.0$, does the x intercept move to the right or to the left?

Answer

(a) When [S] increases from 0.2 to 0.4 μM, V_0 increases by a factor of 1.96. When [S] = 10 μM, $V_0 = 50$ μM s^{-1}. When [S] increases from 100 to 200 μM, V_0 increases by a factor of 1.048.

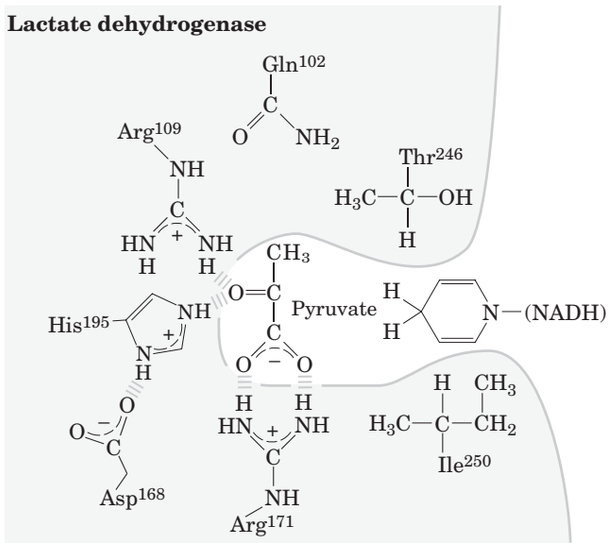
(b) When $\alpha = 2.0$, the curve is shifted to the right as the K_m is increased by a factor of 2. When $\alpha' = 3.0$, the asymptote of the curve (the V_{max}) declines by a factor of 3. When $\alpha = 2.0$ and $\alpha' = 3.0$, the curve briefly rises above the curve where both α and $\alpha' = 1.0$, due to a decline in K_m. However, the asymptote is lower because V_{max} declines by a factor of 3.

(c) When $\alpha = 2.0$, the x intercept moves to the right. When $\alpha = 2.0$ and $\alpha' = 3.0$, the x intercept moves to the left.

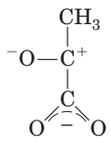
Data Analysis Problem

23. Exploring and Engineering Lactate Dehydrogenase Examining the structure of an enzyme results in hypotheses about the relationship between different amino acids in the protein's structure and the protein's function. One way to test these hypotheses is to use recombinant DNA technology to generate mutant versions of the enzyme and then examine the structure and function of these altered forms. The technology used to do this is described in Chapter 9.

One example of this kind of analysis is the work of Clarke and colleagues on the enzyme lactate dehydrogenase, published in 1989. Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate with NADH to form lactate (see Section 14.3). A schematic of the enzyme's active site is shown below; the pyruvate is in the center:



The reaction mechanism is similar to many NADH reductions (Fig. 13–24); it is approximately the reverse of steps 2 and 3 of Figure 14–7. The transition state involves a strongly polarized carbonyl group of the pyruvate molecule as shown below:



(a) A mutant form of LDH in which Arg^{109} is replaced with Gln shows only 5% of the pyruvate binding and 0.07% of the activity of wild-type enzyme. Provide a plausible explanation for the effects of this mutation.

(b) A mutant form of LDH in which Arg^{171} is replaced with Lys shows only 0.05% of the wild-type level of substrate binding. Why is this dramatic effect surprising?

(c) In the crystal structure of LDH, the guanidinium group of Arg^{171} and the carboxyl group of pyruvate are aligned as shown in a co-planar "forked" configuration. Based on this, provide a plausible explanation for the dramatic effect of substituting Arg^{171} with Lys.

(d) A mutant form of LDH in which Ile^{250} is replaced with Gln shows reduced binding of NADH. Provide a plausible explanation for this result.

Clarke and colleagues also set out to engineer a mutant version of LDH that would bind and reduce oxaloacetate rather than pyruvate. They made a single substitution, replacing Gln^{102} with Arg; the resulting enzyme would reduce oxaloacetate to malate and would no longer reduce pyruvate to lactate. They had therefore converted LDH to malate dehydrogenase.

(e) Sketch the active site of this mutant LDH with oxaloacetate bound.

(f) Provide a plausible explanation for why this mutant enzyme now "prefers" oxaloacetate instead of pyruvate.

(g) The authors were surprised that substituting a larger amino acid in the active site allowed a larger substrate to bind. Provide a plausible explanation for this result.

Answer

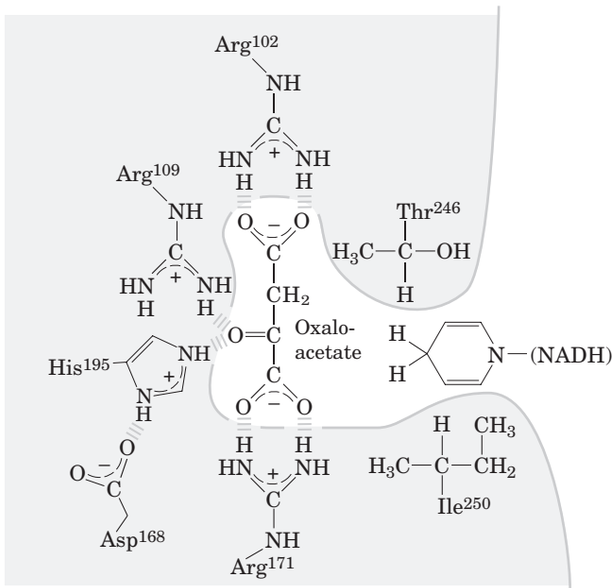
(a) In the wild-type enzyme, the substrate is held in place by a hydrogen bond and an ion-dipole interaction between the charged side chain of Arg^{109} and the polar carbonyl of pyruvate. During catalysis, the charged Arg^{109} side chain also stabilizes the polarized carbonyl transition state. In the mutant, the binding is reduced to just a hydrogen bond, substrate binding is weaker, and ionic stabilization of the transition state is lost, reducing catalytic activity.

(b) Because Lys and Arg are roughly the same size and have a similar positive charge, they probably have very similar properties. Furthermore, because pyruvate binds to Arg^{171} by (presumably) an ionic interaction, an Arg to Lys mutation would probably have little effect on substrate binding.

(c) The "forked" arrangement aligns two positively charged groups of Arg residues with the negatively charged oxygens of pyruvate and facilitates two combined hydrogen-bond and ion-dipole interactions. When Lys is present, only one such combined hydrogen-bond and ion-dipole interaction is possible, thus reducing the strength of the interaction. The positioning of the substrate is less precise.

(d) Ile^{250} interacts hydrophobically with the ring of NADH. This type of interaction is not possible with the hydrophilic side chain of Gln.

(e) The structure is shown below.



(f) The mutant enzyme rejects pyruvate because pyruvate's hydrophobic methyl group will not interact with the highly hydrophilic guanidinium group of Arg^{102}. The mutant binds oxaloacetate because of the strong ionic interaction between the Arg^{102} side chain and the carboxyl of oxaloacetate.

(g) The protein must be flexible enough to accommodate the added bulk of the side chain and the larger substrate.

References

Clarke, A.R., Atkinson, T., & Holbrook, J.J. (1989) From analysis to synthesis: new ligand binding sites on the lactate dehydrogenase framework, Part I. *Trends Biochem. Sci*. **14,** 101–105.

Clarke, A.R., Atkinson, T., & Holbrook, J.J. (1989) From analysis to synthesis: new ligand binding sites on the lactate dehydrogenase framework, Part II. *Trends Biochem. Sci*. **14,** 145–148.

chapter

7 Carbohydrates and Glycobiology

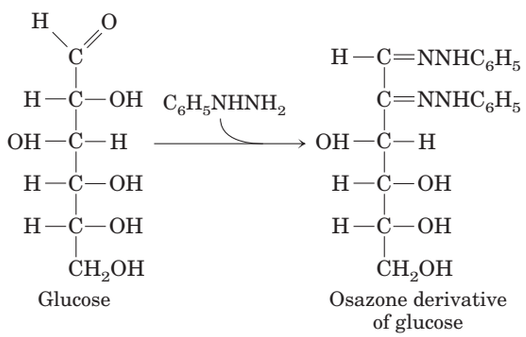
1. **Sugar Alcohols** In the monosaccharide derivatives known as sugar alcohols, the carbonyl oxygen is reduced to a hydroxyl group. For example, D-glyceraldehyde can be reduced to glycerol. However, this sugar alcohol is no longer designated D or L. Why?

   Answer With reduction of the carbonyl oxygen to a hydroxyl group, the stereochemistry at C-1 and C-3 is the same; the glycerol molecule is not chiral.

2. **Recognizing Epimers** Using Figure 7–3, identify the epimers of **(a)** D-allose, **(b)** D-gulose, and **(c)** D-ribose at C-2, C-3, and C-4.

   Answer Epimers differ by the configuration about only *one* carbon.
   (a) D-altrose (C-2), D-glucose (C-3), D-gulose (C-4)
   (b) D-idose (C-2), D-galactose (C-3), D-allose (C-4)
   (c) D-arabinose (C-2), D-xylose (C-3)

3. **Melting Points of Monosaccharide Osazone Derivatives** Many carbohydrates react with phenylhydrazine ($C_6H_5NHNH_2$) to form bright yellow crystalline derivatives known as osazones:



Glucose — Osazone derivative of glucose

The melting temperatures of these derivatives are easily determined and are characteristic for each osazone. This information was used to help identify monosaccharides before the development of HPLC or gas-liquid chromatography. Listed below are the melting points (MPs) of some aldose-osazone derivatives:

Monosaccharide	MP of anhydrous monosaccharide (°C)	MP of osazone derivative (°C)
Glucose	146	205
Mannose	132	205
Galactose	165–168	201
Talose	128–130	201

As the table shows, certain pairs of derivatives have the same melting points, although the underivatized monosaccharides do not. Why do glucose and mannose, and similarly galactose and talose, form osazone derivatives with the same melting points?

Answer The configuration at C-2 of an aldose is lost in its osazone derivative, so aldoses differing only at the C-2 configuration (C-2 epimers) give the same derivative, with the same melting point. Glucose and mannose are C-2 epimers and thus form the same osazone; the same is true for galactose and talose (see Fig. 7–3).

4. Interconversion of D-Glucose Forms A solution of one enantiomer of a given monosaccharide rotates plane-polarized light to the left (counterclockwise) and is called the levorotatory isomer, designated (−); the other enantiomer rotates plane-polarized light to the same extent but to the right (clockwise) and is called the dextrorotatory isomer, designated (+). An equimolar mixture of the (+) and (−) forms does not rotate plane-polarized light.

The optical activity of a stereoisomer is expressed quantitatively by its *optical rotation*, the number of degrees by which plane-polarized light is rotated on passage through a given path length of a solution of the compound at a given concentration. The *specific rotation* $[\alpha]_\lambda^t$ of an optically active compound is defined thus:

$$[\alpha]_\lambda^t = \frac{\text{observed optical rotation (°)}}{\text{optical path length (dm)} \times \text{concentration (g/mL)}}$$

The temperature (t) and the wavelength of the light (λ) employed (usually, as here, the D line of sodium, 589 nm) must be specified.

A freshly prepared solution of α-D-glucose shows a specific rotation of +112°. Over time, the rotation of the solution gradually decreases and reaches an equilibrium value corresponding to $[\alpha]_D^{25°C} = +52.5°$. In contrast, a freshly prepared solution of β-D-glucose has a specific rotation of +19°. The rotation of this solution increases over time to the same equilibrium value as that shown by the α anomer.

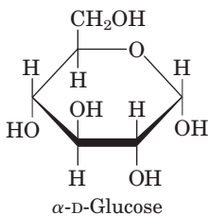
(a) Draw the Haworth perspective formulas of the α and β forms of D-glucose. What feature distinguishes the two forms?

(b) Why does the specific rotation of a freshly prepared solution of the α form gradually decrease with time? Why do solutions of the α and β forms reach the same specific rotation at equilibrium?

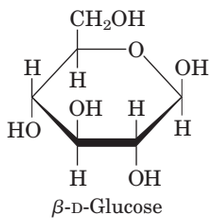
(c) Calculate the percentage of each of the two forms of D-glucose present at equilibrium.

Answer

(a)



α-D-Glucose



β-D-Glucose

The α and β forms of D-glucose differ only at the hemiacetal carbon (C-1; the anomeric carbon).

(b) A fresh solution of the α form of glucose undergoes mutarotation to an equilibrium mixture containing both the α and β forms. The same applies to a fresh solution of the β form.

(c) The change in specific rotation of a solution in changing from 100% α form ($[\alpha]_D^{25°C}$ 112°) to 100% β form ($[\alpha]_D^{25°C}$ 19°) is 93°. For an equilibrium mixture having $[\alpha]_D^{25°C}$ 52.5°, the fraction of D-glucose in the α form is

$$\frac{52.5° - 19°}{112° - 19°} = \frac{33.5°}{93°} = 0.36 = 36\%$$

Thus, ignoring the small portions of furanose forms (~0.5% each), the mixture contains about 36% α-D-glucose and 64% β-D-glucose.

5. **Configuration and Conformation** Which bond(s) in α-D-glucose must be broken to change its configuration to β-D-glucose? Which bond(s) to convert D-glucose to D-mannose? Which bond(s) to convert one "chair" form of D-glucose to the other?

   Answer To convert α-D-glucose to β-D-glucose, the bond between C-1 and the hydroxyl on C-5 must be broken and reformed in the opposite configuration (as in Fig. 7–6). To convert D-glucose to D-mannose, either the —H or the —OH on C-2 must be broken and reformed in the opposite configuration. Conversion between chair conformations does not require bond breakage; this is the critical distinction between configuration and conformation.

6. **Deoxysugars** Is D-2-deoxygalactose the same chemical as D-2-deoxyglucose? Explain.

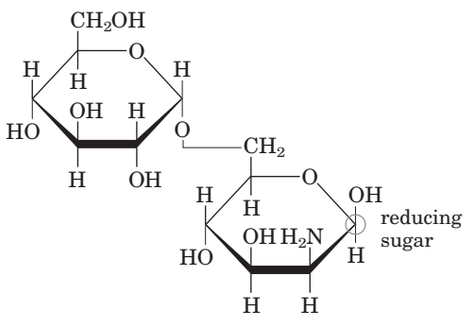
   Answer No; glucose and galactose differ in their configuration at C-4.

7. **Sugar Structures** Describe the common structural features and the differences for each pair: **(a)** cellulose and glycogen; **(b)** D-glucose and D-fructose; **(c)** maltose and sucrose.

   Answer (a) Both are polymers of D-glucose, but they differ in the glycosidic linkage: (β1→4) for cellulose, (α1→4) for glycogen. **(b)** Both are hexoses, but glucose is an aldohexose, fructose a ketohexose. **(c)** Both are disaccharides, but maltose has two (α1→4)-linked D-glucose units; sucrose has (α1↔2β)-linked D-glucose and D-fructose.

8. **Reducing Sugars** Draw the structural formula for α-D-glucosyl-(1→6)-D-mannosamine and circle the part of this structure that makes the compound a reducing sugar.

   Answer



9. **Hemiacetal and Glycosidic Linkages** Explain the difference between a hemiacetal and a glycoside.

   Answer A hemiacetal is formed when an aldose or ketose condenses with an alcohol; a glycoside is formed when a hemiacetal condenses with an alcohol (see Fig. 7–5, p. 238).

10. **A Taste of Honey** The fructose in honey is mainly in the β-D-pyranose form. This is one of the sweetest carbohydrates known, about twice as sweet as glucose; the β-D-furanose form of fructose is much less sweet. The sweetness of honey gradually decreases at a high temperature. Also, high-fructose corn syrup (a commercial product in which much of the glucose in corn syrup is converted to fructose) is used for sweetening *cold* but not *hot* drinks. What chemical property of fructose could account for both these observations?

    Answer Straight-chain fructose can cyclize to yield either the pyranose or the furanose structure. Increasing the temperature shifts the equilibrium in the direction of the furanose form, reducing the sweetness of the solution. The higher the temperature, the less sweet is the fructose solution.

11. Reducing Disaccharide A disaccharide, which you know to be either maltose or sucrose, is treated with Fehling's solution, and a red color is formed. Which sugar is it, and how do you know?

Answer Maltose; sucrose has no reducing (oxidizable) group, as the anomeric carbons of both monosaccharides are involved in the glycosidic bond.

12. Glucose Oxidase in Determination of Blood Glucose The enzyme glucose oxidase isolated from the mold *Penicillium notatum* catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone. This enzyme is highly specific for the β anomer of glucose and does not affect the α anomer. In spite of this specificity, the reaction catalyzed by glucose oxidase is commonly used in a clinical assay for total blood glucose—that is, for solutions consisting of a mixture of β- and α-D-glucose. What are the circumstances required to make this possible? Aside from allowing the detection of smaller quantities of glucose, what advantage does glucose oxidase offer over Fehling's reagent for the determination of blood glucose?

Answer The rate of mutarotation (interconversion of the α and β anomers) is sufficiently high that, as the enzyme consumes β-D-glucose, more α-D-glucose is converted to the β form, and, eventually, all the glucose is oxidized. Glucose oxidase is specific for glucose and does not detect other reducing sugars (such as galactose). Fehling's reagent reacts with any reducing sugar.

13. Invertase "Inverts" Sucrose The hydrolysis of sucrose (specific rotation +66.5°) yields an equimolar mixture of D-glucose (specific rotation +52.5°) and D-fructose (specific rotation −92°). (See Problem 4 for details of specific rotation.)

(a) Suggest a convenient way to determine the rate of hydrolysis of sucrose by an enzyme preparation extracted from the lining of the small intestine.

(b) Explain why, in the food industry, an equimolar mixture of D-glucose and D-fructose formed by hydrolysis of sucrose is called invert sugar.

(c) The enzyme invertase (now commonly called sucrase) is allowed to act on a 10% (0.1 g/mL) solution of sucrose until hydrolysis is complete. What will be the observed optical rotation of the solution in a 10 cm cell? (Ignore a possible small contribution from the enzyme.)

Answer

(a) An equimolar mixture of D-glucose and D-fructose, such as that formed from sucrose hydrolysis, has optical rotation = 52.5° + (−92.0°) = −39.5°. Enzyme (sucrase) activity can be assayed by observing the change in optical rotation of a solution of 100% sucrose (specific rotation = +66.5°) as it is converted to a 1:1 mixture of D-glucose and D-fructose.

(b) The optical rotation of the hydrolysis mixture is negative (inverted) relative to that of the unhydrolyzed sucrose solution.

(c) The addition of 1 mol of water (M_r 18) in the hydrolysis of 1 mol of sucrose (M_r 342) gives the products an increase in weight of (18/342)(100%) = 5.26% with respect to the starting sugar. Accordingly, a 10% sucrose solution yields a [10 + (0.053 × 10)]% = 10.5% solution of invert sugar. Of this 10.5%, 5.25% (0.0525 g/mL) is D-glucose and 5.25% is D-fructose. By rearranging the equation in Problem 4,

$$[\alpha]_D^{25°C} = \frac{\text{observed optical rotation (°)}}{\text{optical path length (dm)} \times \text{concentration (g/mL)}}$$

we can determine the optical rotation of each sugar in the mixture in a 10 cm cell:

Optical rotation of glucose = (52.5°)(1 dm)(0.0525 g/mL) = 2.76°

Optical rotation of fructose = (92°)(1 dm)(0.0525 g/mL) = −4.8°

The observed optical rotation of the solution is 2.76° + (−4.8°) = −2.0°

14. Manufacture of Liquid-Filled Chocolates The manufacture of chocolates containing a liquid center is an interesting application of enzyme engineering. The flavored liquid center consists largely of an aqueous solution of sugars rich in fructose to provide sweetness. The technical dilemma is the following: the chocolate coating must be prepared by pouring hot melted chocolate over a solid (or almost solid) core, yet the final product must have a liquid, fructose-rich center. Suggest a way to solve this problem. (Hint: Sucrose is much less soluble than a mixture of glucose and fructose.)

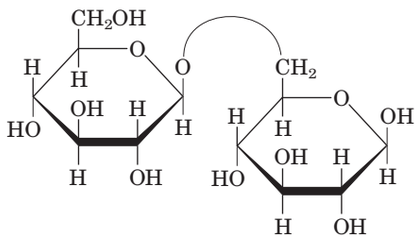
Answer Prepare the core as a semisolid slurry of sucrose and water. Add a small amount of sucrase (invertase), and quickly coat the semisolid mixture with chocolate. After the chocolate coat has cooled and hardened, the sucrase hydrolyzes enough of the sucrose to form a more liquid center: a mixture of fructose, glucose, and sucrose.

15. Anomers of Sucrose? Lactose exists in two anomeric forms, but no anomeric forms of sucrose have been reported. Why?

Answer Lactose (Gal(β1→4)Glc) has a free anomeric carbon (on the glucose residue). In sucrose (Glc(α1↔2β)Fru), the anomeric carbons of both monosaccharide units are involved in the glycosidic bond, and the disaccharide has no free anomeric carbon to undergo mutarotation.

16. Gentiobiose Gentiobiose (D-Glc(β1→6)D-Glc) is a disaccharide found in some plant glycosides. Draw the structure of gentobiose based on its abbreviated name. Is it a reducing sugar? Does it undergo mutarotation?

Answer



It is a reducing sugar; it undergoes mutarotation.

17. Identifying Reducing Sugars Is *N*-acetyl-β-D-glucosamine (Fig. 7–9) a reducing sugar? What about D-gluconate? Is the disaccharide GlcN(α1↔1α)Glc a reducing sugar?

Answer *N*-Acetyl-β-D-glucosamine is a reducing sugar; its C-1 can be oxidized (see Fig. 7–10, p. 241). D-Gluconate is not a reducing sugar; its C-1 is already at the oxidation state of a carboxylic acid. GlcN(α1↔1α)Glc is not a reducing sugar; the anomeric carbons of both monosaccharides are involved in the glycosidic bond.

18. Cellulose Digestion Cellulose could provide a widely available and cheap form of glucose, but humans cannot digest it. Why not? If you were offered a procedure that allowed you to acquire this ability, would you accept? Why or why not?

Answer Humans cannot break down cellulose to its monosaccharides because they lack cellulases, a family of enzymes, produced chiefly by fungi, bacteria, and protozoans, that catalyze the hydrolysis of cellulose to glucose. In ruminant animals (such as cows and sheep), the rumen (one of four stomach compartments) acts as an anaerobic fermenter in which bacteria and protozoa degrade cellulose, making its glucose available as a nutrient to the animal. If cellulase were present in the human digestive tract, we could use foods rich in cellulose as nutrients. This would greatly increase the forms of biomass that could be used for human nutrition. This change might require some changes in the teeth that would allow cellulosic materials to be ground into small pieces to serve as cellulase substrates.

19. Physical Properties of Cellulose and Glycogen The almost pure cellulose obtained from the seed threads of *Gossypium* (cotton) is tough, fibrous, and completely insoluble in water. In contrast, glycogen obtained from muscle or liver disperses readily in hot water to make a turbid solution. Despite their markedly different physical properties, both substances are (1→4)-linked D-glucose polymers of comparable molecular weight. What structural features of these two polysaccharides underlie their different physical properties? Explain the biological advantages of their respective properties.

Answer Native cellulose consists of glucose units linked by (β1→4) glycosidic bonds. The β linkages force the polymer chain into an extended conformation. Parallel series of these extended chains can form *intermolecular* hydrogen bonds, thus aggregating into long, tough, insoluble fibers. Glycogen consists of glucose units linked by (α1→4) glycosidic bonds. The α linkages cause bends in the chain, and glycogen forms helical structures with *intramolecular* hydrogen bonding; it cannot form long fibers. In addition, glycogen is highly branched and, because many of its hydroxyl groups are exposed to water, is highly hydrated and therefore very water-soluble. It can be extracted as a dispersion in hot water.

The physical properties of the two polymers are well suited to their biological roles. Cellulose serves as a structural material in plants, consistent with the side-by-side aggregation of long molecules into tough, insoluble fibers. Glycogen is a storage fuel in animals. The highly hydrated glycogen granules, with their abundance of free, nonreducing ends, can be rapidly hydrolyzed by glycogen phosphorylase to release glucose 1-phosphate, available for oxidation and energy production.

20. Dimensions of a Polysaccharide Compare the dimensions of a molecule of cellulose and a molecule of amylose, each of M_r 200,000.

Answer Cellulose is several times longer; it assumes an extended conformation, whereas amylose has a helical structure.

21. Growth Rate of Bamboo The stems of bamboo, a tropical grass, can grow at the phenomenal rate of 0.3 m/day under optimal conditions. Given that the stems are composed almost entirely of cellulose fibers oriented in the direction of growth, calculate the number of sugar residues per second that must be added enzymatically to growing cellulose chains to account for the growth rate. Each D-glucose unit contributes ~0.5 nm to the length of a cellulose molecule.

Answer First, calculate the growth per second:

$$\frac{0.3 \text{ m/day}}{(24 \text{ h/day})(60 \text{ min/h})(60 \text{ s/min})} = 3 \times 10^{-6} \text{ m/s}$$

Given that each glucose residue increases the length of the cellulose chain by 0.5 nm (5×10^{-10} m), the number of residues added per second is

$$\frac{3 \times 10^{-6} \text{ m/s}}{5 \times 10^{-10} \text{ m/residue}} = 6{,}000 \text{ residues/s}$$

22. Glycogen as Energy Storage: How Long Can a Game Bird Fly? Since ancient times it has been observed that certain game birds, such as grouse, quail, and pheasants, are easily fatigued. The Greek historian Xenophon wrote, "The bustards . . . can be caught if one is quick in starting them up, for they will fly only a short distance, like partridges, and soon tire; and their flesh is delicious." The flight muscles of game birds rely almost entirely on the use of glucose 1-phosphate for energy, in the form of ATP (Chapter 14). The glucose 1-phosphate is formed by the breakdown of stored muscle glycogen, catalyzed by the enzyme glycogen phosphorylase. The rate of ATP production is limited by the rate at which glycogen can be broken down. During a "panic flight," the game bird's rate of glycogen breakdown is quite high, approximately 120 μmol/min of glucose 1-phosphate produced per gram of fresh tissue. Given that the flight muscles usually contain about 0.35% glycogen by weight, calculate how long a game bird can fly. (Assume the average molecular weight of a glucose residue in glycogen is 162 g/mol.)

Answer Given the average molecular weight of a glucose residue = 162, the amount of usable glucose (as glycogen) in 1 g of tissue is

$$\frac{3.5 \times 10^{-3}\ \text{g}}{162\ \text{g/mol}} = 2.2 \times 10^{-5}\ \text{mol}$$

In 1 min, 120 μmol of glucose 1-phosphate is produced, so 120 μmol of glucose is hydrolyzed. Thus, depletion of the glycogen would occur in

$$\frac{(2.2 \times 10^{-5}\ \text{mol})(60\ \text{s/min})}{120 \times 10^{-6}\ \text{mol/min}} = 11\ \text{s}$$

23. Relative Stability of Two Conformers Explain why the two structures shown in Figure 7–19 are so different in energy (stability). Hint: See Figure 1–21.

Answer The ball-and-stick model of the disaccharide in Figure 7–19 shows no steric interactions, but a space-filling model, showing atoms with their real relative sizes, would show several strong steric hindrances in the −170°,−170° conformer that are not present in the 30°,−40° conformer.

24. Volume of Chondroitin Sulfate in Solution One critical function of chondroitin sulfate is to act as a lubricant in skeletal joints by creating a gel-like medium that is resilient to friction and shock. This function seems to be related to a distinctive property of chondroitin sulfate: the volume occupied by the molecule is much greater in solution than in the dehydrated solid. Why is the volume so much larger in solution?

Answer In solution, the negative charges on chondroitin sulfate repel each other and force the molecule into an extended conformation. The polar molecule also attracts many water molecules (water of hydration), further increasing the molecular volume. In the dehydrated solid, each negative charge is counterbalanced by a counterion, such as Na^+, and the molecule collapses into its condensed form.

25. Heparin Interactions Heparin, a highly negatively charged glycosaminoglycan, is used clinically as an anticoagulant. It acts by binding several plasma proteins, including antithrombin III, an inhibitor of blood clotting. The 1:1 binding of heparin to antithrombin III seems to cause a conformational change in the protein that greatly increases its ability to inhibit clotting. What amino acid residues of antithrombin III are likely to interact with heparin?

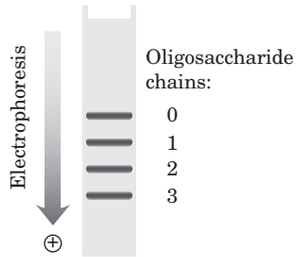
Answer Positively charged amino acid residues would be the best candidates to bind to the highly negatively charged groups on heparin. In fact, Lys residues of antithrombin III interact with heparin.

26. Permutations of a Trisaccharide Think about how one might estimate the number of possible trisaccharides composed of *N*-acetylglucosamine 4-sulfate (GlcNAc4S) and glucuronic acid (GlcA), and draw 10 of them.

Answer If GlcNAc4S is represented as A, and GlcA as B, the trimer could have any of these sequences: AAA, AAB, ABB, ABA, BBB, BBA, BAA, or BAB (8 possible sequences). The connections between each pair of monosaccharides could be 1→6, 1→4, 1→3, or 1→1 (4 possibilities for each of two bonds, or $4 \times 4 = 16$ possible linkages in all), and each linkage could involve either the α or the β anomer of each sugar (2 possibilities for each of two bonds, so $2 \times 2 = 4$ stereochemical possibilities). Therefore there are $8 \times 16 \times 4 = 512$ possible permutations!

27. Effect of Sialic Acid on SDS Polyacrylamide Gel Electrophoresis Suppose you have four forms of a protein, all with identical amino acid sequence but containing zero, one, two, or three oligosaccharide chains, each ending in a single sialic acid residue. Draw the gel pattern you would expect when a mixture of these four glycoproteins is subjected to SDS polyacrylamide gel electrophoresis (see Fig. 3–18) and stained for protein. Identify any bands in your drawing.

Answer

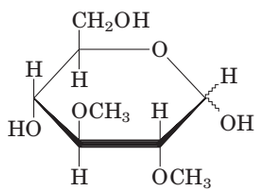


The significant feature of sialic acids is the negative charge of their carboxyl group. The four glycoproteins would have the same charge except for the additional 1, 2, or 3 negative charges of the sialic acid residues. In SDS gel electrophoresis, the proteins are coated uniformly with a layer of sodium dodecyl sulfate (which is negatively charged, p. 89) and thus move toward the positive electrode. The glycoproteins with 1, 2, or 3 extra negative charges will move progressively faster than the form without sialic acid.

28. Information Content of Oligosaccharides The carbohydrate portion of some glycoproteins may serve as a cellular recognition site. In order to perform this function, the oligosaccharide moiety of glycoproteins must have the potential to exist in a large variety of forms. Which can produce a greater variety of structures: oligopeptides composed of five different amino acid residues or oligosaccharides composed of five different monosaccharide residues? Explain.

Answer Oligosaccharides; their monosaccharide residues can be combined in more ways than the amino acid residues of oligopeptides. Each of the several hydroxyl groups of each monosaccharide can participate in glycosidic bonds, and the configuration of each glycosidic bond can be either α or β. Furthermore, the polymer can be linear or branched. Oligopeptides are unbranched polymers, with all amino acid residues linked through identical peptide bonds.

29. Determination of the Extent of Branching in Amylopectin The amount of branching (number of (α1→6) glycosidic bonds) in amylopectin can be determined by the following procedure. A sample of amylopectin is exhaustively methylated—treated with a methylating agent (methyl iodide) that replaces the hydrogen of every sugar hydroxyl with a methyl group, converting —OH to —OCH_3. All the glycosidic bonds in the treated sample are then hydrolyzed in aqueous acid, and the amount of 2,3-di-*O*-methylglucose so formed is determined.



2,3-Di-*O*-methylglucose

(a) Explain the basis of this procedure for determining the number of (α1→6) branch points in amylopectin. What happens to the unbranched glucose residues in amylopectin during the methylation and hydrolysis procedure?

(b) A 258 mg sample of amylopectin treated as described above yielded 12.4 mg of 2,3-di-*O*-methylglucose. Determine what percentage of the glucose residues in amylopectin contain an (α1→6) branch. (Assume the average molecular weight of a glucose residue in amylopectin is 162 g/mol.)

Answer

(a) In glucose residues at branch points, the hydroxyl of C-6 is protected from methylation because it is involved in a glycosidic linkage. During complete methylation and subsequent hydrolysis, the branch-point residues yield 2,3-di-*O*-methylglucose and the unbranched residues yield 2,3,6-tri-*O*-methylglucose.

(b) Given the average molecular weight of a glucose residue = 162, then 258 mg of amylopectin contains

$$\frac{258 \times 10^{-3}\ \text{g}}{162\ \text{g/mol}} = 1.59 \times 10^{-3}\ \text{mol of glucose}$$

The 12.4 mg yield of 2,3-di-*O*-methylglucose (M_r 208) is equivalent to

$$\frac{12.4 \times 10^{-3}\ \text{g}}{208\ \text{g/mol}} = 5.96 \times 10^{-5}\ \text{mol of glucose}$$

Thus, the percentage of glucose residues in amylopectin that yield 2,3-di-*O*-methylglucose is

$$\frac{(5.96 \times 10^{-5}\ \text{mol})(100\%)}{1.59 \times 10^{-3}\ \text{mol}} = 3.75\%$$

30. Structural Analysis of a Polysaccharide A polysaccharide of unknown structure was isolated, subjected to exhaustive methylation, and hydrolyzed. Analysis of the products revealed three methylated sugars: 2,3,4-tri-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose, in the ratio 20:1:1. What is the structure of the polysaccharide?

Answer The polysaccharide is a branched glucose polymer. Because the predominant product is 2,3,4-tri-*O*-methyl-D-glucose, the predominant glycosidic linkage must be (1→6). The formation of 2,4-di-*O*-methyl-D-glucose indicates that branch points occur through C-3. The ratio of these two methylated sugars indicates that a branch occurs at an average frequency of once every 20 residues. The 2,3,4,6-tetra-*O*-methyl-D-glucose is derived from nonreducing chain ends, which compose about $\frac{1}{20}$, or 5%, of the residues, consistent with a high degree of branching. Thus, the polysaccharide has chains of (1→6)-linked D-glucose residues with (1→3)-linked branches, about one branch every 20 residues.

Data Analysis Problem

31. Determining the Structure of ABO Blood Group Antigens The human ABO blood group system was first discovered in 1901, and in 1924 this trait was shown to be inherited at a single gene locus with three alleles. In 1960, W. T. J. Morgan published a paper summarizing what was known at that time about the structure of the ABO antigen molecules. When the paper was published, the complete structures of the A, B, and O antigens were not yet known; this paper is an example of what scientific knowledge looks like "in the making."

In any attempt to determine the structure of an unknown biological compound, researchers must deal with two fundamental problems: (1) If you don't know what *it* is, how do you know if *it* is pure? (2) If you don't know what *it* is, how do you know that your extraction and purification conditions have not changed *its* structure? Morgan addressed problem 1 through several methods. One method is described in his paper as observing "constant analytical values after fractional solubility tests" (p. 312). In this case, "analytical values" are measurements of chemical composition, melting point, and so forth.

(a) Based on your understanding of chemical techniques, what could Morgan mean by "fractional solubility tests"?

(b) Why would the analytical values obtained from fractional solubility tests of a *pure* substance be constant, and those of an *impure* substance not be constant?

Morgan addressed problem 2 by using an assay to measure the immunological activity of the substance present in different samples.

(c) Why was it important for Morgan's studies, and especially for addressing problem 2, that this activity assay be quantitative (measuring a level of activity) rather than simply qualitative (measuring only the presence or absence of a substance)?

The structure of the blood group antigens is shown in Figure 10–15. In his paper (p. 314), Morgan listed several properties of the three antigens, A, B, and O, that were known at that time:

1. Type B antigen has a higher content of galactose than A or O.
2. Type A antigen contains more total amino sugars than B or O.
3. The glucosamine/galactosamine ratio for the A antigen is roughly 1.2; for B, it is roughly 2.5.

(d) Which of these findings is (are) consistent with the known structures of the blood group antigens?

(e) How do you explain the discrepancies between Morgan's data and the known structures?

In later work, Morgan and his colleagues used a clever technique to obtain structural information about the blood group antigens. Enzymes had been found that would specifically degrade the antigens. However, these were available only as crude enzyme preparations, perhaps containing more than one enzyme of unknown specificity. Degradation of the blood type antigens by these crude enzymes could be inhibited by the addition of particular sugar molecules to the reaction. Only sugars found in the blood type antigens would cause this inhibition. One enzyme preparation, isolated from the protozoan *Trichomonas foetus*, would degrade all three antigens and was inhibited by the addition of particular sugars. The results of these studies are summarized in the table below, showing the percentage of substrate remaining unchanged when the *T. foetus* enzyme acted on the blood group antigens in the presence of sugars.

	Unchanged substrate (%)		
Sugar added	**A antigen**	**B antigen**	**O antigen**
Control—no sugar	3	1	1
L-Fucose	3	1	100
D-Fucose	3	1	1
L-Galactose	3	1	3
D-Galactose	6	100	1
N-Acetylglucosamine	3	1	1
N-Acetylgalactosamine	100	6	1

For the O antigen, a comparison of the control and L-fucose results shows that L-fucose inhibits the degradation of the antigen. This is an example of product inhibition, in which an excess of reaction product shifts the equilibrium of the reaction, preventing further breakdown of substrate.

(f) Although the O antigen contains galactose, *N*-acetylglucosamine, and *N*-acetylgalactosamine, none of these sugars inhibited the degradation of this antigen. Based on these data, is the enzyme preparation from *T. foetus* an endo- or exoglycosidase? (Endoglycosidases cut bonds between interior residues; exoglycosidases remove one residue at a time from the end of a polymer.) Explain your reasoning.

(g) Fucose is also present in the A and B antigens. Based on the structure of these antigens, why does fucose fail to prevent their degradation by the *T. foetus* enzyme? What structure would be produced?

(h) Which of the results in **(f)** and **(g)** are consistent with the structures shown in Figure 10–15? Explain your reasoning.

Answer

(a) The tests involve trying to dissolve only part of the sample in a variety of solvents, then analyzing both dissolved and undissolved materials to see whether their compositions differ.

(b) For a pure substance, all molecules are the same and any dissolved fraction will have the same composition as any undissolved fraction. An impure substance is a mixture of more than one compound. When treated with a particular solvent, more of one component may dissolve, leaving more of the other component(s) behind. As a result, the dissolved and undissolved fractions have different compositions.

(c) A quantitative assay allows researchers to be sure that none of the activity has been lost through degradation. When determining the structure of a molecule, it is important that the sample under analysis consist only of intact (undegraded) molecules. If the sample is contaminated with degraded material, this will give confusing and perhaps uninterpretable structural results. A qualitative assay would detect the presence of activity, even if it had become significantly degraded.

(d) Results 1 and 2. Result 1 is consistent with the known structure, because type B antigen has three molecules of galactose; types A and O each have only two. Result 2 is also consistent, because type A has two amino sugars (*N*-acetylgalactosamine and *N*-acetylglucosamine); types B and O have only one (*N*-acetylglucosamine). Result 3 is *not* consistent with the known structure: for type A, the glucosamine:galactosamine ratio is 1:1; for type B, it is 1:0.

(e) The samples were probably impure and/or partly degraded. The first two results were correct possibly because the method was only roughly quantitative and thus not as sensitive to inaccuracies in measurement. The third result is more quantitative and thus more likely to differ from predicted values, because of impure or degraded samples.

(f) An exoglycosidase. If it were an endoglycosidase, one of the products of its action on O antigen would include galactose, *N*-acetylglucosamine, or *N*-acetylgalactosamine, and at least one of those sugars would be able to inhibit the degradation. Given that the enzyme is not inhibited by any of these sugars, it must be an exoglycosidase, removing only the terminal sugar from the chain. The terminal sugar of O antigen is fucose, so fucose is the only sugar that could inhibit the degradation of O antigen.

(g) The exoglycosidase removes *N*-acetylgalactosamine from A antigen and galactose from B antigen. Because fucose is not a product of either reaction, it will not prevent removal of these sugars, and the resulting substances will no longer be active as A or B antigen. However, the products should be active as O antigen, because degradation stops at fucose.

(h) All the results are consistent with Figure 10–15. (1) D-Fucose and L-galactose, which would protect against degradation, are not present in any of the antigens. (2) The terminal sugar of A antigen is *N*-acetylgalactosamine, and this sugar alone protects this antigen from degradation. (3) The terminal sugar of B antigen is galactose, which is the only sugar capable of protecting this antigen.

Reference

Morgan, W.T. (1960) The Croonian Lecture: a contribution to human biochemical genetics; the chemical basis of blood-group specificity. *Proc. R. Soc. Lond. B Biol. Sci.* **151,** 308–347.

chapter

8

Nucleotides and Nucleic Acids

1. Nucleotide Structure Which positions in the purine ring of a purine nucleotide in DNA have the potential to form hydrogen bonds but are not involved in Watson-Crick base pairing?

Answer All purine ring nitrogens (N-1, N-3, N-7, and N-9) have the potential to form hydrogen bonds (see Figs. 8–1, 8–11, and 2–3). However, N-1 is involved in Watson-Crick hydrogen bonding with a pyrimidine, and N-9 is involved in the *N*-glycosyl linkage with deoxyribose and has very limited hydrogen-bonding capacity. Thus, N-3 and N-7 are available to form further hydrogen bonds.

2. Base Sequence of Complementary DNA Strands One strand of a double-helical DNA has the sequence (5′)GCGCAATATTTCTCAAAATATTGCGC(3′). Write the base sequence of the complementary strand. What special type of sequence is contained in this DNA segment? Does the double-stranded DNA have the potential to form any alternative structures?

Answer The complementary strand is

(5′)GCGCAATATTTTGAGAAATATTGCGC(3′)

(Note that the sequence of a single strand is always written in the 5′→3′ direction.) This sequence has a palindrome, an inverted repeat with twofold symmetry:

(5′)GCGCAATATTTCTCAAAATATTGCGC(3′)
(3′)CGCGTTATAAAGAGTTTTATAACGCG(5′)

Because this sequence is self-complementary, the individual strands have the potential to form hairpin structures. The two strands together may also form a cruciform.

3. DNA of the Human Body Calculate the weight in grams of a double-helical DNA molecule stretching from the Earth to the moon (~320,000 km). The DNA double helix weighs about 1×10^{-18} g per 1,000 nucleotide pairs; each base pair extends 3.4 Å. For an interesting comparison, your body contains about 0.5 g of DNA!

Answer

The length of the DNA is

$$(3.2 \times 10^5 \text{ km})(10^{12} \text{ nm/km})(10 \text{ Å/nm}) = 3.2 \times 10^{18} \text{ Å}$$

The number of base pairs (bp) is

$$\frac{3.2 \times 10^{18} \text{Å}}{3.4 \text{ Å/bp}} = 9.4 \times 10^{17} \text{ bp}$$

Thus, the weight of the DNA molecule is

$$(9.4 \times 10^{17} \text{ bp})(1 \times 10^{-18} \text{ g}/10^3 \text{ bp}) = 9.4 \times 10^{-4} \text{ g} = 0.00094 \text{ g}$$

4. **DNA Bending** Assume that a poly(A) tract five base pairs long produces a 20° bend in a DNA strand. Calculate the total (net) bend produced in a DNA if the center base pairs (the third of five) of two successive $(dA)_5$ tracts are located **(a)** 10 base pairs apart; **(b)** 15 base pairs apart. Assume 10 base pairs per turn in the DNA double helix.

   Answer When bending elements are repeated in phase with the helix turn (i.e., every 10 base pairs) as in **(a),** the total bend is additive; when bending elements are repeated out of phase by one half-turn as in **(b),** they cancel each other out. Thus, the net bend is **(a)** 40°; **(b)** 0°.

5. **Distinction between DNA Structure and RNA Structure** Hairpins may form at palindromic sequences in single strands of either RNA or DNA. How is the helical structure of a long and fully base-paired (except at the end) hairpin in RNA different from that of a similar hairpin in DNA?

   Answer The RNA helix assumes the A conformation; the DNA helix generally assumes the B conformation. (The presence of the 2′-OH group on ribose makes it sterically impossible for double-helical RNA to assume the B-form helix.)

6. **Nucleotide Chemistry** The cells of many eukaryotic organisms have highly specialized systems that specifically repair G–T mismatches in DNA. The mismatch is repaired to form a G≡C (not A═T) base pair. This G–T mismatch repair mechanism occurs in addition to a more general system that repairs virtually all mismatches. Can you suggest why cells might require a specialized system to repair G–T mismatches?

   Answer Many C residues of CpG sequences in eukaryotic DNA are methylated at the 5′ position to 5-methylcytosine. (About 5% of all C residues are methylated.) Spontaneous deamination of 5-methylcytosine yields thymine, T, and a G–T mismatch resulting from spontaneous deamination of 5-methylcytosine in a G≡C base pair is one of the most common mismatches in eukaryotic cells. The specialized repair mechanism to convert G–T back to G≡C is directed at this common class of mismatch.

7. **Spontaneous DNA Damage** Hydrolysis of the *N*-glycosyl bond between deoxyribose and a purine in DNA creates an AP site. An AP site generates a thermodynamic destabilization greater than that created by any DNA mismatched base pair. This effect is not completely understood. Examine the structure of an AP site (see Fig. 8–33b) and describe some chemical consequences of base loss.

   Answer Without the base, the ribose ring can be opened to generate the noncyclic aldehyde form. This, and the loss of base-stacking interactions, could contribute significant flexibility to the DNA backbone.

8. **Nucleic Acid Structure** Explain why the absorption of UV light by double-stranded DNA increases (the hyperchromic effect) when the DNA is denatured.

   Answer The double-helical structure is stabilized by hydrogen bonding between complementary bases on opposite strands and by base stacking between adjacent bases on the same strand. Base stacking in nucleic acids causes a decrease in the absorption of UV light (relative to the non-stacked structure). On denaturation of DNA, the base stacking is lost and UV absorption increases.

9. **Determination of Protein Concentration in a Solution Containing Proteins and Nucleic Acids** The concentration of protein or nucleic acid in a solution containing both can be estimated by using their different light absorption properties: proteins absorb most strongly at 280 nm and nucleic acids at 260 nm. Estimates of their respective concentrations in a mixture can be made by measuring the absorbance (A) of the solution at 280 nm and 260 nm and using the table that follows, which gives $R_{280/260}$, the ratio of absorbances at 280 and 260 nm; the percentage of total mass that is nucleic acid; and a factor, F, that corrects the A_{280} reading and gives a more accurate protein estimate. The protein concentration (in mg/ml) = $F \times A_{280}$ (assuming the cuvette is 1 cm wide). Calculate the protein concentration in a solution of $A_{280} = 0.69$ and $A_{260} = 0.94$.

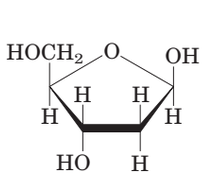
$R_{280/260}$	Proportion of nucleic acid (%)	F
1.75	0.00	1.116
1.63	0.25	1.081
1.52	0.50	1.054
1.40	0.75	1.023
1.36	1.00	0.994
1.30	1.25	0.970
1.25	1.50	0.944
1.16	2.00	0.899
1.09	2.50	0.852
1.03	3.00	0.814
0.979	3.50	0.776
0.939	4.00	0.743
0.874	5.00	0.682
0.846	5.50	0.656
0.822	6.00	0.632
0.804	6.50	0.607
0.784	7.00	0.585
0.767	7.50	0.565
0.753	8.00	0.545
0.730	9.00	0.508
0.705	10.00	0.478
0.671	12.00	0.422
0.644	14.00	0.377
0.615	17.00	0.322
0.595	20.00	0.278

Answer For this protein solution, $R_{280/260} = 0.69/0.94 = 0.73$, so (from the table) $F = 0.508$. The concentration of protein is $F \times A_{280} = (0.508 \times 0.69)$ mg/mL = 0.35 mg/mL.

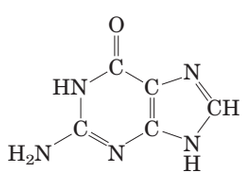
Note: the table applies to mixtures of proteins, such as might be found in a crude cellular extract, and reflects the absorption properties of average proteins. For a purified protein, the values of F would have to be altered to reflect the unique molar extinction coefficient of that protein.

10. Solubility of the Components of DNA Draw the following structures and rate their relative solubilities in water (most soluble to least soluble): deoxyribose, guanine, phosphate. How are these solubilities consistent with the three-dimensional structure of double-stranded DNA?

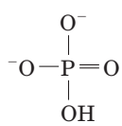
Answer



Deoxyribose



Guanine



Phosphate

Solubilities: phosphate > deoxyribose > guanine. The negatively charged phosphate is the most water-soluble; the deoxyribose, with several hydroxyl groups, is quite water-soluble; and guanine, a hydrophobic base, is relatively insoluble in water. The polar phosphate groups and sugars are on the outside of the DNA double helix, exposed to water. The hydrophobic bases are located in the interior of the double helix, away from water.

11. Sanger Sequencing Logic In the Sanger (dideoxy) method for DNA sequencing, a small amount of a dideoxynucleotide triphosphate—say, ddCTP—is added to the sequencing reaction along with a larger amount of the corresponding dCTP. What result would be observed if the dCTP were omitted?

Answer If dCTP is omitted from the reaction mixture, when the first G residue is encountered in the template, ddCTP is added and polymerization halts. Only one band will appear in the sequencing gel.

12. DNA Sequencing The following DNA fragment was sequenced by the Sanger method. The asterisk indicates a fluorescent label.

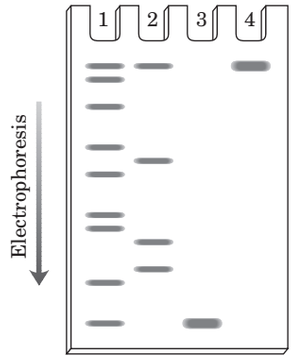
*5′ ——— 3′-OH
3′ ——— ATTACGCAAGGACATTAGAC---5′

A sample of the DNA was reacted with DNA polymerase and each of the nucleotide mixtures (in an appropriate buffer) listed below. Dideoxynucleotides (ddNTPs) were added in relatively small amounts.

1. dATP, dTTP, dCTP, dGTP, ddTTP
2. dATP, dTTP, dCTP, dGTP, ddGTP
3. dATP, dCTP, dGTP, ddTTP
4. dATP, dTTP, dCTP, dGTP

The resulting DNA was separated by electrophoresis on an agarose gel, and the fluorescent bands on the gel were located. The band pattern resulting from nucleotide mixture 1 is shown below. Assuming that all mixtures were run on the same gel, what did the remaining lanes of the gel look like?

Answer



Lane 1: The reaction mixture that generated these bands included all the deoxynucleotides, plus dideoxythymidine. The fragments are of various lengths, all terminating where a ddTTP was substituted for a dTTP. For a small portion of the strands synthesized in the experiment, ddTTP would not be inserted and the strand would thus extend to the final G. Thus, the nine products are (from top to bottom of the gel):

5′-primer-TAATGCGTTCCTGTAATCTG
5′-primer-TAATGCGTTCCTGTAATCT
5′-primer-TAATGCGTTCCTGTAAT
5′-primer-TAATGCGTTCCTGT
5′-primer-TAATGCGTTCCT
5′-primer-TAATGCGTT
5′-primer-TAATGCGT
5′-primer-TAAT
5′-primer-T

Lane 2: Similarly, this lane will have four bands (top to bottom), for the following fragments, each terminating where ddGTP was inserted in place of dGTP:

5′-primer-TAATGCGTTCCTGTAATCTG
5′-primer-TAATGCGTTCCTG
5′-primer-TAATGCG
5′-primer-TAATG

Lane 3: Because mixture 3 lacked dTTP, every fragment was terminated immediately after the primer as ddTTP was inserted, to form 5′-primer-T. The result will be a single thick band near the bottom of the gel.

Lane 4: When all the deoxynucleotides were provided, but no dideoxynucleotide, a single labeled product formed: 5′-primer-TAATGCGTTCCTGTAATCTG. This will appear as a single thick band at the top of the gel.

13. Snake Venom Phosphodiesterase An exonuclease is an enzyme that sequentially cleaves nucleotides from the end of a polynucleotide strand. Snake venom phosphodiesterase, which hydrolyzes nucleotides from the 3′ end of any oligonucleotide with a free 3′-hydroxyl group, cleaves between the 3′ hydroxyl of the ribose or deoxyribose and the phosphoryl group of the next nucleotide. It acts on single-stranded DNA or RNA and has no base specificity. This enzyme was used in sequence

determination experiments before the development of modern nucleic acid sequencing techniques. What are the products of partial digestion by snake venom phosphodiesterase of an oligonucleotide with the following sequence?

(5′)GCGCCAUUGC(3′)–OH

Answer When snake venom phosphodiesterase cleaves a nucleotide from a nucleic acid strand, it leaves the phosphoryl group attached to the 5′ position of the released nucleotide and a free 3′-OH group on the remaining strand. Partial digestion of the oligonucleotide gives a mixture of fragments of all lengths, as well as some of the original, undigested strand, so the products are (P represents the phosphate group):

(5′)P–GCGCCAUUGC(3′)–OH
(5′)P–GCGCCAUUG(3′)–OH
(5′)P–GCGCCAUU(3′)–OH
(5′)P–GCGCCAU(3′)–OH
(5′)P–GCGCCA(3′)–OH
(5′)P–GCGCC(3′)–OH
(5′)P–GCGC(3′)–OH
(5′)P–GCG(3′)–OH
(5′)P–GC(3′)–OH

and the released nucleoside 5′-phosphates, GMP, UMP, AMP, and CMP.

14. Preserving DNA in Bacterial Endospores Bacterial endospores form when the environment is no longer conducive to active cell metabolism. The soil bacterium *Bacillus subtilis*, for example, begins the process of sporulation when one or more nutrients are depleted. The end product is a small, metabolically dormant structure that can survive almost indefinitely with no detectable metabolism. Spores have mechanisms to prevent accumulation of potentially lethal mutations in their DNA over periods of dormancy that can exceed 1,000 years. *B. subtilis* spores are much more resistant than are the organism's growing cells to heat, UV radiation, and oxidizing agents, all of which promote mutations.

(a) One factor that prevents potential DNA damage in spores is their greatly decreased water content. How would this affect some types of mutations?

(b) Endospores have a category of proteins called small acid-soluble proteins (SASPs) that bind to their DNA, preventing formation of cyclobutane-type dimers. What causes cyclobutane dimers, and why do bacterial endospores need mechanisms to prevent their formation?

Answer

(a) Water is a participant in most biological reactions, including those that cause mutations. The low water content in endospores reduces the activity of mutation-causing enzymes and slows the rate of nonenzymatic depurination reactions, which are hydrolysis reactions.

(b) UV light induces the condensation of adjacent pyrimidine bases to form cyclobutane pyrimidine dimers. The spores of *B. subtilis,* a soil organism, are at constant risk of being lofted to the top of the soil or into the air, where they are subject to UV exposure, possibly for prolonged periods. Protection from UV-induced mutation is critical to spore DNA integrity.

15. Oligonucleotide Synthesis In the scheme of Figure 8–35, each new base to be added to the growing oligonucleotide is modified so that its 3′ hydroxyl is activated and the 5′ hydroxyl has a dimethoxytrityl (DMT) group attached. What is the function of the DMT group on the incoming base?

Answer DMT is a blocking group that prevents reaction of the incoming base with itself.

Biochemistry on the Internet

16. The Structure of DNA Elucidation of the three-dimensional structure of DNA helped researchers understand how this molecule conveys information that can be faithfully replicated from one generation to the next. To see the secondary structure of double-stranded DNA, go to the Protein Data Bank website (www.rcsb.org). Use the PDB identifiers listed below to retrieve the structure summaries for the two forms of DNA. Open the structures using Jmol (linked under the Display Options), and use the controls in the Jmol menu (accessed with a control-click or by clicking on the Jmol logo in the lower right corner of the image screen) to complete the following exercises. Refer to the Jmol help links as needed.

(a) Obtain the file for 141D, a highly conserved, repeated DNA sequence from the end of the HIV-1 (the virus that causes AIDS) genome. Display the molecule as a ball-and-stick structure (in the control menu, choose Select > All, then Render > Scheme > Ball and Stick). Identify the sugar–phosphate backbone for each strand of the DNA duplex. Locate and identify individual bases. Identify the 5′ end of each strand. Locate the major and minor grooves. Is this a right- or left-handed helix?

(b) Obtain the file for 145D, a DNA with the Z conformation. Display the molecule as a ball-and-stick structure. Identify the sugar–phosphate backbone for each strand of the DNA duplex. Is this a right- or left-handed helix?

(c) To fully appreciate the secondary structure of DNA, view the molecules in stereo. On the control menu, Select > All, then Render > Stereographic > Cross-eyed or Wall-eyed. You will see two images of the DNA molecule. Sit with your nose approximately 10 inches from the monitor and focus on the tip of your nose (cross-eyed) or the opposite edges of the screen (wall-eyed). In the background you should see three images of the DNA helix. Shift your focus to the middle image, which should appear three-dimensional. (Note that only one of the two authors can make this work.)

Answer

(a) The DNA fragment modeled in file 141D, from the human immunodeficiency virus, is the B form, the standard Watson-Crick structure (although this particular structure is a bent B-form DNA). This fragment has an adenine at the 5′ end and a guanine at the 3′ end; click on the bases at each end of the helix to identify which is the 5′ end. When the helix is oriented with the 5′ adenine at the upper left-hand side of the model, the *minor groove* is in the center of the model. Rotating the model so that the 5′ adenine is at the upper right-hand side positions the *major groove* in the center. The spiral of this helix runs upward in a counterclockwise direction, so this is a right-handed helix.

(b) The model of DNA in the Z conformation includes a shell of water molecules around the helix. The water molecules are visible when the complex is viewed in ball-and-stick mode. Turn off the display of the water molecules using the console controls Select > Nucleic > DNA. Then Select > Display Selected Only. The backbone of DNA in the Z conformation is very different from that in the B conformation. The helix spiral runs upward in a clockwise direction, so this is a left-handed helix.

(c) Viewing the structures in stereo takes a bit of practice, but perseverance will be rewarded! Here are some tips for successful three-dimensional viewing:

(1) Turn off or lower the room lighting.
(2) Sit directly in front of the screen.
(3) Use a ruler to make sure you are 10 to 11 inches from the screen.
(4) Position your head so that when you focus on the tip of your nose, the screen images are on either side of the tip (i.e., look down your nose at the structures).
(5) Move your head slightly closer to or farther away from the screen to bring the middle image into focus. Don't look directly at the middle image as you try to bring it into focus.

(6) If you find it uncomfortable to focus on the tip of your nose, try using the tip of a finger (positioned just beyond the tip of your nose) instead.

(7) Relax as you attempt to view the three-dimensional image.

Note that many people, including one of the text authors, have some trouble making this work!

Data Analysis Problem

17. Chargaff's Studies of DNA Structure The chapter section "DNA Is a Double Helix that Stores Genetic Information" includes a summary of the main findings of Erwin Chargaff and his coworkers, listed as four conclusions ("Chargaff's rules"; p. 278). In this problem, you will examine the data Chargaff collected in support of these conclusions.

In one paper, Chargaff (1950) described his analytical methods and some early results. Briefly, he treated DNA samples with acid to remove the bases, separated the bases by paper chromatography, and measured the amount of each base with UV spectroscopy. His results are shown in the three tables below. The *molar ratio* is the ratio of the number of moles of each base in the sample to the number of moles of phosphate in the sample—this gives the fraction of the total number of bases represented by each particular base. The *recovery* is the sum of all four bases (the sum of the molar ratios); full recovery of all bases in the DNA would give a recovery of 1.0.

	Molar ratios in ox DNA					
	Thymus			Spleen		Liver
Base	Prep. 1	Prep. 2	Prep. 3	Prep. 1	Prep. 2	Prep. 1
Adenine	0.26	0.28	0.30	0.25	0.26	0.26
Guanine	0.21	0.24	0.22	0.20	0.21	0.20
Cytosine	0.16	0.18	0.17	0.15	0.17	
Thymine	0.25	0.24	0.25	0.24	0.24	
Recovery	*0.88*	*0.94*	*0.94*	*0.84*	*0.88*	

	Molar ratios in human DNA				
	Sperm		Thymus	Liver	
Base	Prep. 1	Prep. 2	Prep. 1	Normal	Carcinoma
Adenine	0.29	0.27	0.28	0.27	0.27
Guanine	0.18	0.17	0.19	0.19	0.18
Cytosine	0.18	0.18	0.16		0.15
Thymine	0.31	0.30	0.28		0.27
Recovery	*0.96*	*0.92*	*0.91*		*0.87*

	Molar ratios in DNA of microorganisms		
	Yeast		Avian tubercle bacilli
Base	Prep. 1	Prep. 2	Prep. 1
Adenine	0.24	0.30	0.12
Guanine	0.14	0.18	0.28
Cytosine	0.13	0.15	0.26
Thymine	0.25	0.29	0.11
Recovery	*0.76*	*0.92*	*0.77*

(a) Based on these data, Chargaff concluded that "no differences in composition have so far been found in DNA from different tissues of the same species." This corresponds to conclusion 2 in this chapter. However, a skeptic looking at the data above might say, "They certainly look different to me!" If you were Chargaff, how would you use the data to convince the skeptic to change her mind?

(b) The base composition of DNA from normal and cancerous liver cells (hepatocarcinoma) was not distinguishably different. Would you expect Chargaff's technique to be capable of detecting a difference between the DNA of normal and cancerous cells? Explain your reasoning.

As you might expect, Chargaff's data were not completely convincing. He went on to improve his techniques, as described in a later paper (Chargaff, 1951), in which he reported molar ratios of bases in DNA from a variety of organisms:

Source	A:G	T:C	A:T	G:C	Purine:pyrimidine
Ox	1.29	1.43	1.04	1.00	1.1
Human	1.56	1.75	1.00	1.00	1.0
Hen	1.45	1.29	1.06	0.91	0.99
Salmon	1.43	1.43	1.02	1.02	1.02
Wheat	1.22	1.18	1.00	0.97	0.99
Yeast	1.67	1.92	1.03	1.20	1.0
Haemophilus influenzae type c	1.74	1.54	1.07	0.91	1.0
E. coli K-12	1.05	0.95	1.09	0.99	1.0
Avian tubercle bacillus	0.4	0.4	1.09	1.08	1.1
Serratia marcescens	0.7	0.7	0.95	0.86	0.9
Bacillus schatz	0.7	0.6	1.12	0.89	1.0

(c) According to Chargaff, as stated in conclusion 1 in this chapter, "The base composition of DNA generally varies from one species to another." Provide an argument, based on the data presented so far, that supports this conclusion.

(d) According to conclusion 4, "In *all* cellular DNAs, regardless of the species . . . A + G = T + C." Provide an argument, based on the data presented so far, that supports this conclusion.

Part of Chargaff's intent was to disprove the "tetranucleotide hypothesis"; this was the idea that DNA was a monotonous tetranucleotide polymer $(AGCT)_n$ and therefore not capable of containing sequence information. Although the data presented above show that DNA cannot be simply a tetranucleotide—if so, all samples would have molar ratios of 0.25 for each base—it was still possible that the DNA from different organisms was a slightly more complex, but still monotonous, repeating sequence.

To address this issue, Chargaff took DNA from wheat germ and treated it with the enzyme deoxyribonuclease for different time intervals. At each time interval, some of the DNA was converted to small fragments; the remaining, larger fragments he called the "core." In the table below, the "19% core" corresponds to the larger fragments left behind when 81% of the DNA was degraded; the "8% core" corresponds to the larger fragments left after 92% degradation.

Base	Intact DNA	19% Core	8% Core
Adenine	0.27	0.33	0.35
Guanine	0.22	0.20	0.20
Cytosine	0.22	0.16	0.14
Thymine	0.27	0.26	0.23
Recovery	*0.98*	*0.95*	*0.92*

(e) How would you use these data to argue that wheat germ DNA is not a monotonous repeating sequence?

Answer

(a) It would not be easy! The data for different samples from the same organism show significant variation, and the recovery is never 100%. The numbers for C and T show much more consistency than those for A and G, so for C and T it is much easier to make the case that samples from the same organism have the same composition. But even with the less consistent values for A and G, (1) the range of values for different tissues does overlap substantially; (2) the difference between different preparations of the same tissue is about the same as the difference between samples from different tissues; and (3) in samples for which recovery is high, the numbers are more consistent.

(b) This technique would not be sensitive enough to detect a difference between normal and cancerous cells. Cancer is caused by mutations, but these changes in DNA—a few base pairs out of several billion—would be too small to detect with these techniques.

(c) The ratios of A:G and T:C vary widely among different species. For example, in the bacterium *Serratia marcescens*, both ratios are 0.4, meaning that the DNA contains mostly G and C. In *Haemophilus influenzae*, by contrast, the ratios are 1.74 and 1.54, meaning that the DNA is mostly A and T.

(d) Conclusion 4 has three requirements:

A = T: The table shows an A:T ratio very close to 1 in all cases. Certainly, the variation in this ratio is substantially less than the variation in the A:G and T:C ratios.

G = C: Again, the G:C ratio is very close to 1, and the other ratios vary widely.

(A + G) = (T + C): This is the purine:pyrimidine ratio, which also is very close to 1.

(e) The different "core" fractions represent different regions of the wheat germ DNA. If the DNA were a monotonous repeating sequence, the base composition of all regions would be the same. Because different core regions have different sequences, the DNA sequence must be more complex.

References

Chargaff, E. (1950) Chemical specificity of nucleic acids and mechanism of their enzymic degradation. *Experientia* **6,** 201–209.

Chargaff, E. (1951) Structure and function of nucleic acids as cell constituents. *Fed. Proc.* **10,** 654–659.

chapter 10 Lipids

1. **Operational Definition of Lipids** How is the definition of "lipid" different from the types of definitions used for other biomolecules that we have considered, such as amino acids, nucleic acids, and proteins?

   Answer The term "lipid" does not specify a particular chemical structure. Whereas one can write a general formula for an amino acid, nucleic acid, or protein, lipids are much more chemically diverse. Compounds are categorized as lipids based on their greater solubility in organic solvents than in water.

2. **Melting Points of Lipids** The melting points of a series of 18-carbon fatty acids are: stearic acid, 69.6 °C; oleic acid, 13.4 °C; linoleic acid, −5 °C; and linolenic acid, −11 °C.
   (a) What structural aspect of these 18-carbon fatty acids can be correlated with the melting point?
   (b) Draw all the possible triacylglycerols that can be constructed from glycerol, palmitic acid, and oleic acid. Rank them in order of increasing melting point.
   (c) Branched-chain fatty acids are found in some bacterial membrane lipids. Would their presence increase or decrease the fluidity of the membranes (that is, give them a lower or higher melting point)? Why?

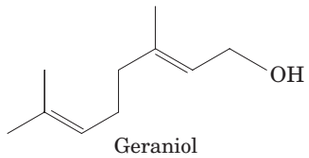
   Answer
   (a) The number of cis double bonds (stearic acid, 18:0; oleic, 18:1; linoleic, 18:2; linolenic, 18:3). Each cis double bond causes a bend in the hydrocarbon chain, and bent chains are less well packed than straight chains in a crystal lattice. The lower the extent of packing, the lower the melting temperature.
   (b) Six different triacylglycerols are possible: one with glycerol and only palmitic acid (PPP); one with glycerol and only oleic acid (OOO); and four with glycerol and a mixture of oleic and palmitic acids. Four mixed triacylglycerols are possible, because the three carbons of glycerol are not equivalent: thus OOP and OPO are positional isomers, as are POP and OPP. The greater the content of saturated fatty acid (P), the higher the melting point. Thus, the order of melting points is OOO < OOP = OPO < POP = OPP < PPP. See Table 10–1 and Figure 10–3 for information on how to draw the triacylglycerols.
   (c) Branched-chain fatty acids will increase the fluidity of membranes (i.e., lower their melting point) because they decrease the extent of packing possible within the membrane. The effect of branches is similar to that of bends caused by double bonds.

3. **Preparation of Béarnaise Sauce** During the preparation of béarnaise sauce, egg yolks are incorporated into melted butter to stabilize the sauce and avoid separation. The stabilizing agent in the egg yolks is lecithin (phosphatidylcholine). Suggest why this works.

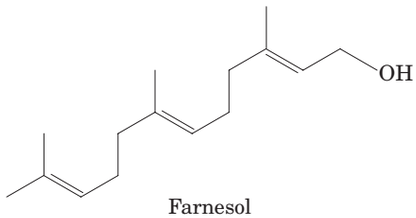
   Answer Lecithin (see Fig. 10–14 for structure), an amphipathic molecule, is an emulsifying agent, solubilizing the fat (triacylglycerols) in butter. Lecithin is such a good emulsifying agent that it

can be used to create a stable emulsion in a mixture that contains up to 75% oil. Mayonnaise, too, is an emulsion created with egg yolks, with an oil:vinegar mixture in a 3:1 ratio.

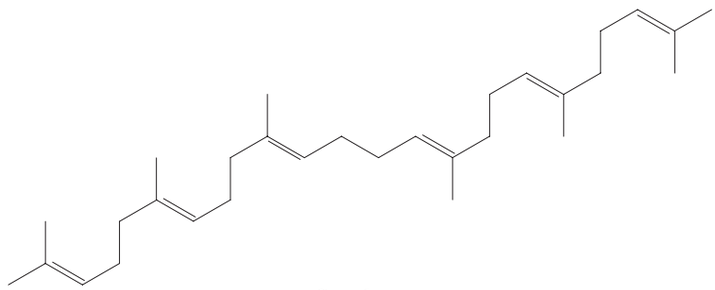
4. Isoprene Units in Isoprenoids Geraniol, farnesol, and squalene are called isoprenoids, because they are synthesized from five-carbon isoprene units. In each compound, circle the five-carbon units representing isoprene units (see Fig. 10–22).



Geraniol

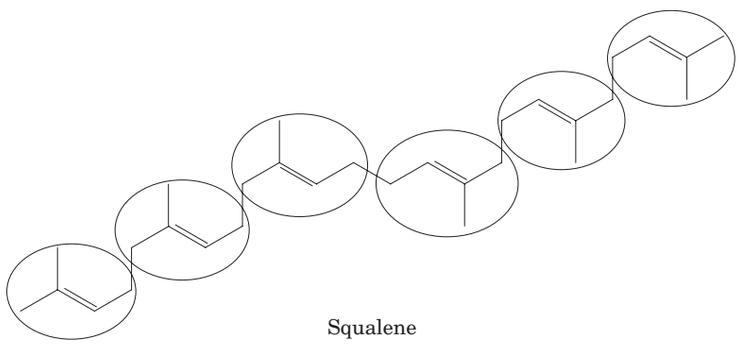


Farnesol



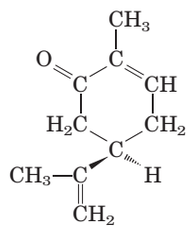
Squalene

Answer

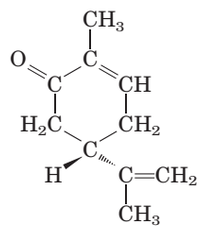


Squalene

5. Naming Lipid Stereoisomers The two compounds below are stereoisomers of carvone with quite different properties; the one on the left smells like spearmint, and that on the right, like caraway. Name the compounds using the RS system.



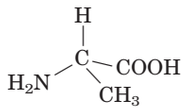
Spearmint



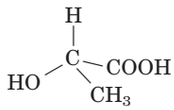
Caraway

Answer Spearmint is (*R*)-carvone; caraway is (*S*)-carvone.

6. RS Designations for Alanine and Lactate Draw (using wedge-bond notation) and label the (*R*) and (*S*) isomers of 2-aminopropanoic acid (alanine) and 2-hydroxypropanoic acid (lactic acid).

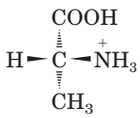


2-Aminopropanoic acid
(alanine)

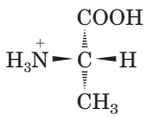


2-Hydroxypropanoic acid
(lactic acid)

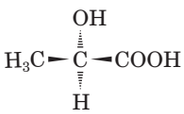
Answer



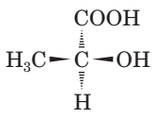
(*R*)-2-Aminopropanoic acid



(*S*)-2-Aminopropanoic acid



(*R*)-2-Hydroxypropanoic acid



(*S*)-2-Hydroxypropanoic acid

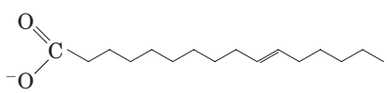
7. Hydrophobic and Hydrophilic Components of Membrane Lipids A common structural feature of membrane lipids is their amphipathic nature. For example, in phosphatidylcholine, the two fatty acid chains are hydrophobic and the phosphocholine head group is hydrophilic. For each of the following membrane lipids, name the components that serve as the hydrophobic and hydrophilic units: **(a)** phosphatidylethanolamine; **(b)** sphingomyelin; **(c)** galactosylcerebroside; **(d)** ganglioside; **(e)** cholesterol.

Answer

	Hydrophobic unit(s)	**Hydrophilic unit(s)**
(a)	2 Fatty acids	Phosphoethanolamine
(b)	1 Fatty acid and the hydrocarbon chain of sphingosine	Phosphocholine
(c)	1 Fatty acid and the hydrocarbon chain of sphingosine	D-Galactose
(d)	1 Fatty acid and the hydrocarbon chain of sphingosine	Several sugar molecules
(e)	Steroid nucleus and acyl side chain	Alcohol group

8. Structure of Omega-6 Fatty Acid Draw the structure of the omega-6 fatty acid 16:1.

Answer



9. Catalytic Hydrogenation of Vegetable Oils Catalytic hydrogenation, used in the food industry, converts double bonds in the fatty acids of the oil triacylglycerols to —CH_2—CH_2—. How does this affect the physical properties of the oils?

Answer It reduces double bonds, which increases the melting point of lipids containing the fatty acids.

10. Alkali Lability of Triacylglycerols A common procedure for cleaning the grease trap in a sink is to add a product that contains sodium hydroxide. Explain why this works.

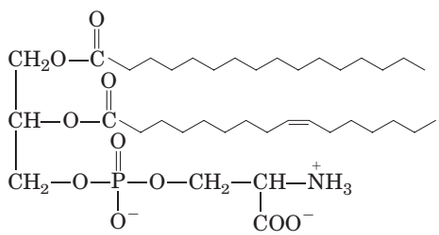
Answer Triacylglycerols, a component of grease (consisting largely of animal fats), are hydrolyzed by NaOH to form glycerol and the sodium salts of free fatty acids, a process known as saponification. The fatty acids form micelles, which are more water-soluble than triacylglycerols.

11. Deducing Lipid Structure from Composition Compositional analysis of a certain lipid shows that it has exactly one mole of fatty acid per mole of inorganic phosphate. Could this be a glycerophospholipid? A ganglioside? A sphingomyelin?

Answer It could only be a sphingolipid (sphingomyelin). Sphingomyelin has one fatty acid molecule and a phosphocholine molecule attached to the sphingosine backbone, for a ratio of fatty acid to inorganic phosphate of 1:1. Glycerophospholipids have two fatty acyl chains and a head group attached to a phosphoglycerol molecule. Unless the head group included additional phosphate groups, the ratio of fatty acid to inorganic phosphate would be 2:1. (Phosphatidylinositol 4,5-bisphosphate would have a ratio of 2:3; cardiolipin, a ratio of 4:2.) Gangliosides do not contain inorganic phosphate.

12. Deducing Lipid Structure from Molar Ratio of Components Complete hydrolysis of a glycerophospholipid yields glycerol, two fatty acids (16:1(Δ^9) and 16:0), phosphoric acid, and serine in the molar ratio 1:1:1:1:1. Name this lipid and draw its structure.

Answer



Phosphatidylserine

13. Impermeability of Waxes What property of the waxy cuticles that cover plant leaves makes the cuticles impermeable to water?

Answer Long, saturated acyl chains, nearly solid at air temperature, form a hydrophobic layer in which a polar compound such as H_2O cannot dissolve or diffuse.

14. The Action of Phospholipases The venom of the Eastern diamondback rattler and the Indian cobra contains phospholipase A_2, which catalyzes the hydrolysis of fatty acids at the C-2 position of glycerophospholipids. The phospholipid breakdown product of this reaction is lysolecithin (lecithin is phosphatidylcholine). At high concentrations, this and other lysophospholipids act as detergents, dissolving the membranes of erythrocytes and lysing the cells. Extensive hemolysis may be life-threatening.

(a) All detergents are amphipathic. What are the hydrophilic and hydrophobic portions of lysolecithin?

(b) The pain and inflammation caused by a snake bite can be treated with certain steroids. What is the basis of this treatment?

(c) Though the high levels of phospholipase A_2 can be deadly, this enzyme is necessary for a variety of normal metabolic processes. What are these processes?

Answer

(a) The free —OH group on C-2 and the phosphocholine head group on C-3 are the hydrophilic portions; the fatty acid on C-1 of the lysolecithin is the hydrophobic portion.

(b) Certain steroids such as prednisone inhibit the action of phospholipase A_2, the enzyme that releases the fatty acid arachidonate from the C-2 position of some membrane glycerophospholipids. Arachidonate is converted to a variety of eicosanoids, some of which cause inflammation and pain.

(c) Phospholipase A_2 is necessary to release arachidonate from certain membrane glycerophopholipids. Arachidonate is a precursor of other eicosanoids that have vital protective functions in the body. The enzyme is also important in digestion, breaking down dietary glycerophospholipids.

15. Lipids in Blood Group Determination We note in Figure 10–15 that the structure of glycosphingolipids determines the blood groups A, B, and O in humans. It is also true that glycoproteins determine blood groups. How can both statements be true?

Answer The part of the membrane lipid that determines blood type is the oligosaccharide in the head group of the membrane sphingolipids (see Fig. 10–15, p. 355). This same oligosaccharide is attached to certain membrane glycoproteins, which also serve as points of recognition by the antibodies that distinguish blood groups.

16. Intracellular Messengers from Phosphatidylinositols When the hormone vasopressin stimulates cleavage of phosphatidylinositol 4,5-bisphosphate by hormone-sensitive phospholipase C, two products are formed. What are they? Compare their properties and their solubilities in water, and predict whether either would diffuse readily through the cytosol.

Answer Phosphatidylinositol 4,5-bisphosphate is a membrane lipid. The two products of cleavage are a diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). Diacylglycerol is not water-soluble and remains in the membrane, acting as a second messenger. The IP_3 is highly polar and very soluble in water; it readily diffuses in the cytosol, acting as a soluble second messenger.

17. Storage of Fat-Soluble Vitamins In contrast to water-soluble vitamins, which must be part of our daily diet, fat-soluble vitamins can be stored in the body in amounts sufficient for many months. Suggest an explanation for this difference.

Answer Unlike water-soluble compounds, lipid-soluble compounds are not readily mobilized—that is, they do not readily pass into aqueous solution. The body's lipids provide a reservoir for storage of lipid-soluble vitamins. Water-soluble vitamins cannot be stored and are rapidly removed from the blood by the kidneys.

18. Hydrolysis of Lipids Name the products of mild hydrolysis with dilute NaOH of **(a)** 1-stearoyl-2, 3-dipalmitoylglycerol; **(b)** 1-palmitoyl-2-oleoylphosphatidylcholine.

Answer Mild hydrolysis cleaves the ester linkages between glycerol and fatty acids, forming **(a)** glycerol and the sodium salts of palmitic and stearic acids; **(b)** D-glycerol 3-phosphocholine and the sodium salts of palmitic and oleic acids.

19. Effect of Polarity on Solubility Rank the following in order of increasing solubility in water: a triacylglycerol, a diacylglycerol, and a monoacylglycerol, all containing only palmitic acid.

Answer Solubilities: monoacylglycerol > diacylglycerol > triacylglycerol. Increasing the number of palmitic acid moieties increases the proportion of the molecule that is hydrophobic.

20. Chromatographic Separation of Lipids A mixture of lipids is applied to a silica gel column, and the column is then washed with increasingly polar solvents. The mixture consists of phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, cholesteryl palmitate (a sterol ester), sphingomyelin, palmitate, *n*-tetradecanol, triacylglycerol, and cholesterol. In what order will the lipids elute from the column? Explain your reasoning.

Answer Because silica gel is polar, the most hydrophobic lipids elute first, the most hydrophilic last. The neutral lipids elute first: cholesteryl palmitate and triacylglycerol. Cholesterol and *n*-tetradecanol, neutral but somewhat more polar, elute next. The neutral phospholipids phosphatidylcholine and phosphatidylethanolamine follow. Sphingomyelin, neutral but slightly more polar, elutes after the neutral phospholipids. The negatively charged phosphatidylserine and palmitate elute last—phosphatidylserine first because it is larger and has a lower charge-to-mass ratio.

21. Identification of Unknown Lipids Johann Thudichum, who practiced medicine in London about 100 years ago, also dabbled in lipid chemistry in his spare time. He isolated a variety of lipids from neural tissue, and characterized and named many of them. His carefully sealed and labeled vials of isolated lipids were rediscovered many years later.

(a) How would you confirm, using techniques not available to Thudichum, that the vials labeled "sphingomyelin" and "cerebroside" actually contain these compounds?

(b) How would you distinguish sphingomyelin from phosphatidylcholine by chemical, physical, or enzymatic tests?

Answer

(a) First, create an acid hydrolysate of each compound. Sphingomyelin yields sphingosine, fatty acids, phosphocholine, choline, and phosphate. Cerebroside yields sphingosine, fatty acids, and sugars, but no phosphate. Subject each hydrolysate to chromatography (gas-liquid or silica gel thin-layer chromatography) and compare the result with known standards.

(b) On strong alkaline hydrolysis, sphingomyelin yields sphingosine, fatty acids, and phosphocholine; phosphatidylcholine yields glycerol, fatty acids, and phosphocholine. The distinguishing features are the presence of *sphingosine* in sphingomyelin and *glycerol* in phosphatidylcholine, which can be detected on thin-layer chromatograms of each hydrolysate compared against known standards. The hydrolysates could also be distinguished by their reaction with the Sanger reagent (1-fluoro-2,4-dinitrobenzene, FDNB); only the sphingosine in the sphingomyelin hydrolysate has a primary amine that would react with FDNB to form a colored product. Alternatively, enzymatic treatment of the two samples with phospholipase A_1 or A_2 would release free fatty acids from phosphatidylcholine, but not from sphingomyelin.

22. Ninhydrin to Detect Lipids on TLC Plates Ninhydrin reacts specifically with primary amines to form a purplish-blue product. A thin-layer chromatogram of rat liver phospholipids is sprayed with ninhydrin, and the color is allowed to develop. Which phospholipids can be detected in this way?

Answer Phosphatidylethanolamine and phosphatidylserine; they are the only phospholipids that have primary amine groups that can react with ninhydrin.

Data Analysis Problem

23. Determining the Structure of the Abnormal Lipid in Tay-Sachs Disease Box 10–2, Figure 1, shows the pathway of breakdown of gangliosides in healthy (normal) individuals and individuals with certain genetic diseases. Some of the data on which the figure is based were presented in a paper by Lars Svennerholm (1962). Note that the sugar Neu5Ac, *N*-acetylneuraminic acid, represented in the Box 10–2 figure as a purple ◆, is a sialic acid.

Svennerholm reported that "about 90% of the monosialiogangliosides isolated from normal human brain" consisted of a compound with ceramide, hexose, *N*-acetylgalactosamine, and *N*-acetylneuraminic acid in the molar ratio 1:3:1:1.

(a) Which of the gangliosides (GM1 through GM3 and globoside) in Box 10–2, Figure 1, fits this description? Explain your reasoning.

(b) Svennerholm reported that 90% of the gangliosides from a patient with Tay-Sachs had a molar ratio (of the same four components given above) of 1:2:1:1. Is this consistent with the Box 10–2 figure? Explain your reasoning.

To determine the structure in more detail, Svennerholm treated the gangliosides with neuraminidase to remove the *N*-acetylneuraminic acid. This resulted in an asialoganglioside that was much easier to analyze. He hydrolyzed it with acid, collected the ceramide-containing products, and determined the molar ratio of the sugars in each product. He did this for both the normal and the Tay-Sachs gangliosides. His results are shown below.

Ganglioside	Ceramide	Glucose	Galactose	Galactosamine
Normal				
Fragment 1	1	1	0	0
Fragment 2	1	1	1	0
Fragment 3	1	1	1	1
Fragment 4	1	1	2	1
Tay-Sachs				
Fragment 1	1	1	0	0
Fragment 2	1	1	1	0
Fragment 3	1	1	1	1

(c) Based on these data, what can you conclude about the structure of the normal ganglioside? Is this consistent with the structure in Box 10–2? Explain your reasoning.

(d) What can you conclude about the structure of the Tay-Sachs ganglioside? Is this consistent with the structure in Box 10–2? Explain your reasoning.

Svennerholm also reported the work of other researchers who "permethylated" the normal asialoganglioside. Permethylation is the same as exhaustive methylation: a methyl group is added to every free hydroxyl group on a sugar. They found the following permethylated sugars: 2,3,6-trimethylglycopyranose; 2,3,4,6-tetramethylgalactopyranose; 2,4,6-trimethylgalactopyranose; and 4,6-dimethyl-2-deoxy-2-aminogalactopyranose.

(e) To which sugar of GM1 does each of the permethylated sugars correspond? Explain your reasoning.

(f) Based on all the data presented so far, what pieces of information about normal ganglioside structure are missing?

Answer

(a) GM1 and globoside. Both glucose and galactose are hexoses, so "hexose" in the molar ratio refers to glucose + galactose. The ratios for the four gangliosides are: GM1, 1:3:1:1; GM2, 1:2:1:1; GM3, 1:2:0:1; globoside, 1:3:1:0.

(b) Yes. The ratio matches GM2, the ganglioside expected to build up in Tay-Sachs disease (see Box 10–2, Fig. 1).

(c) This analysis is similar to that used by Sanger to determine the amino acid sequence of insulin. The analysis of each fragment reveals only its *composition,* not its *sequence,* but because each fragment is formed by sequential removal of one sugar, we can draw conclusions about sequence. The structure of the normal asialoganglioside is ceramide–glucose–galactose–galactosamine–galactose, consistent with Box 10–2 (excluding Neu5Ac, removed before hydrolysis).

(d) The Tay-Sachs asialoganglioside is ceramide–glucose–galactose–galactosamine, consistent with Box 10–2.

(e) The structure of the normal asialoganglioside, GM1, is: *ceramide–glucose* (2 —OH involved in glycosidic links; 1 —OH involved in ring structure; 3 —OH (2,3,6) free for methylation)– *galactose* (2 —OH in links; 1 —OH in ring; 3 —OH (2,4,6) free for methylation)–*galactosamine* (2 —OH in links; 1 —OH in ring; 1 —NH_2 instead of an —OH; 2 —OH (4,6) free for methylation)–*galactose* (1 —OH in link; 1 —OH in ring; 4 —OH (2,3,4,6) free for methylation).

(f) Two key pieces of information are missing: What are the linkages between the sugars? Where is Neu5Ac attached?

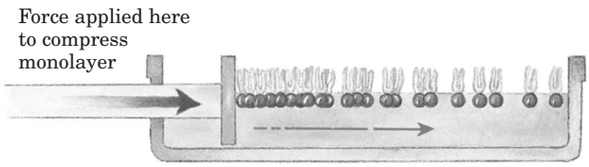
Reference

Svennerholm, L. (1962) The chemical structure of normal human brain and Tay-Sachs gangliosides. *Biochem. Biophys. Res. Comm.* **9,** 436–441.

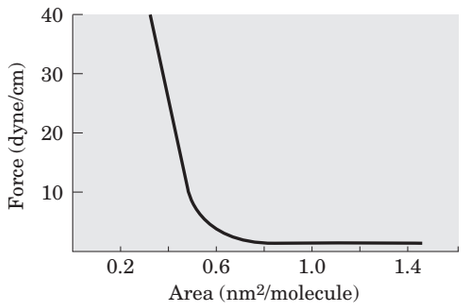
chapter

11 Biological Membranes and Transport

1. **Determining the Cross-Sectional Area of a Lipid Molecule** When phospholipids are layered gently onto the surface of water, they orient at the air-water interface with their head groups in the water and their hydrophobic tails in the air. An experimental apparatus **(a)** has been devised that reduces the surface area available to a layer of lipids. By measuring the force necessary to push the lipids together, it is possible to determine when the molecules are packed tightly in a continuous monolayer; as that area is approached, the force needed to further reduce the surface area increases sharply **(b).** How would you use this apparatus to determine the average area occupied by a single lipid molecule in the monolayer?



(a)



(b)

Answer Determine the surface area of the water at which the pressure increases sharply. Divide this surface area by the number of lipid molecules on the surface, which is calculated by multiplying the number of moles (calculated from the concentration and the molecular weight) by Avogadro's number.

2. **Evidence for a Lipid Bilayer** In 1925, E. Gorter and F. Grendel used an apparatus like that described in Problem 1 to determine the surface area of a lipid monolayer formed by lipids extracted from erythrocytes of several animal species. They used a microscope to measure the dimensions of individual cells, from which they calculated the average surface area of one erythrocyte. They obtained the data shown in the following table. Were these investigators justified in concluding that "chromocytes [erythrocytes] are covered by a layer of fatty substances that is two molecules thick" (i.e., a lipid bilayer)?

Animal	Volume of packed cells (mL)	Number of cells (per mm^3)	Total surface area of lipid monolayer from cells (m^2)	Total surface area of one cell (μm^2)
Dog	40	8,000,000	62	98
Sheep	10	9,900,000	6.0	29.8
Human	1	4,740,000	0.92	99.4

Source: Data from Gorter, E. & Grendel, F. (1925) On bimolecular layers of lipoids on the chromocytes of the blood. *J. Exp. Med.* **41,** 439–443.

Answer The conclusions are justified for dog erythrocytes but not for sheep or human erythrocytes. The table provides the total surface area of the lipid monolayer. To determine the monolayer surface area per cell, first calculate the total number of cells. For example, for dog erythrocytes, the number of cells is 8×10^6 per $mm^3 = 8 \times 10^9$ per cm^3 (or per mL). In 40 mL, there is a total of (40 mL)$(8 \times 10^9$ cells/mL$) = 3 \times 10^{11}$ cells. From the table, this number of cells yielded a monolayer surface area of 62 $m^2 = 6.2 \times 10^5$ cm^2. Dividing the surface area by the number of cells gives

$$\frac{6.2 \times 10^5 \text{ cm}^2}{3 \times 10^{11} \text{ cells}} = 2 \times 10^{-6} \text{ cm}^2\text{/cell}$$

Comparing this number to the total surface area of one erythrocyte ($98\ \mu m^2 = 0.98 \times 10^{-6}$ cm^2), we find a 2-to-1 relationship. This result justifies the investigators' conclusion of a lipid bilayer in dog erythrocytes. Similar calculations for the sheep and human erythrocytes reveal a 1-to-1 relationship, suggesting a lipid monolayer. However, there were significant experimental errors in these early experiments; recent, more accurate measurements support a bilayer in all cases.

3. Number of Detergent Molecules per Micelle When a small amount of the detergent sodium dodecyl sulfate (SDS; $Na^+CH_3(CH_2)_{11}OSO_3^-$) is dissolved in water, the detergent ions enter the solution as monomeric species. As more detergent is added, a concentration is reached (the critical micelle concentration) at which the monomers associate to form micelles. The critical micelle concentration of SDS is 8.2 mM. The micelles have an average particle weight (the sum of the molecular weights of the constituent monomers) of 18,000. Calculate the number of detergent molecules in the average micelle.

Answer The molecular weight of sodium dodecyl sulfate is 288. Given an average micelle particle weight of 18,000, there are 18,000/288 = 63 SDS molecules per micelle.

4. Properties of Lipids and Lipid Bilayers Lipid bilayers formed between two aqueous phases have this important property: they form two-dimensional sheets, the edges of which close upon each other and undergo self-sealing to form liposomes.

(a) What properties of lipids are responsible for this property of bilayers? Explain.

(b) What are the consequences of this property for the structure of biological membranes?

Answer

(a) Lipids that form bilayers are amphipathic molecules: they contain hydrophilic and hydrophobic regions. In order to minimize the hydrophobic area exposed to the water surface, these lipids form two-dimensional sheets, with the hydrophilic regions exposed to water and the hydrophobic regions buried in the interior of the sheet. Furthermore, to avoid exposing the hydrophobic edges of the sheet to water, lipid bilayers close upon themselves. Similarly, if the sheet is perforated, the hole will seal because the membrane is semifluid.

(b) These sheets form the closed membrane surfaces that envelop cells and compartments within cells (organelles).

5. **Length of a Fatty Acid Molecule** The carbon–carbon bond distance for single-bonded carbons such as those in a saturated fatty acyl chain is about 1.5 Å. Estimate the length of a single molecule of palmitate in its fully extended form. If two molecules of palmitate were placed end to end, how would their total length compare with the thickness of the lipid bilayer in a biological membrane?

   Answer Given that the C–C bond length is 0.15 nm and that the bond angle of tetrahedral carbon is 109°, the distance between the first and third carbons in an acyl chain (calculated by trigonometry) is about 0.24 nm. For palmitate (16:0), the length of the extended chain is about 8×0.24 nm = 2 nm. Two palmitate chains end to end (as in a bilayer) would extend 4 nm. This is about the thickness of a lipid bilayer.

6. **Temperature Dependence of Lateral Diffusion** The experiment described in Figure 11–17 was performed at 37 °C. If the experiment were carried out at 10 °C, what effect would you expect on the rate of diffusion? Why?

   Answer When the temperature drops, the fluidity of a membrane decreases. This is caused by a decrease in the rate of diffusion of lipids. Consequently, all processes depending on diffusion, such as the lateral diffusion experiment shown in Figure 11–17, would slow down.

7. **Synthesis of Gastric Juice: Energetics** Gastric juice (pH 1.5) is produced by pumping HCl from blood plasma (pH 7.4) into the stomach. Calculate the amount of free energy required to concentrate the H^+ in 1 L of gastric juice at 37 °C. Under cellular conditions, how many moles of ATP must be hydrolyzed to provide this amount of free energy? The free-energy change for ATP hydrolysis under cellular conditions is about −58 kJ/mol (as explained in Chapter 13). Ignore the effects of the transmembrane electrical potential.

   Answer Given that $\text{pH} = -\log [H^+]$, then $[H^+] = 10^{-\text{pH}}$.

   At pH 1.5, $[H^+] = 10^{-1.5} = 3.2 \times 10^{-2}$ M.

   At pH 7.4, $[H^+] = 10^{-7.4} = 4.0 \times 10^{-8}$ M.

   Because $\Delta G_t = RT \ln (C_2/C_1)$, and at 37 °C, $RT = 2.58$ kJ/mol,

   $$\Delta G_t = (2.58 \text{ kJ/mol}) \ln \left(\frac{3.2 \times 10^{-2}}{4.0 \times 10^{-8}} \right) = 35 \text{ kJ/mol}.$$

   The amount of ATP required to provide 35 kJ is

   $$\frac{35 \text{ kJ}}{58 \text{ kJ/mol}} = 0.60 \text{ mol}$$

8. **Energetics of the Na^+K^+ ATPase** For a typical vertebrate cell with a membrane potential of −0.070 V (inside negative), what is the free-energy change for transporting 1 mol of Na^+ out of the cell and into the blood at 37 °C? Assume the concentration of Na^+ inside the cell is 12 mM, and that in blood plasma is 145 mM.

   Answer

   $$\Delta G_t = RT \ln (C_2/C_1) + Z\mathcal{F}\Delta\psi$$

   $$= (2.58 \text{ kJ/mol}) \ln \left(\frac{145}{12} \right) + (1)(96{,}480 \text{ J/V} \cdot \text{mol})(0.070 \text{ V})$$

   $$= 6.4 \text{ kJ/mol} + 6.8 \text{ kJ/mol} = 13 \text{ kJ/mol}$$

   Note that 6.8 kJ/mol is the membrane potential portion.

9. **Action of Ouabain on Kidney Tissue** Ouabain specifically inhibits the Na^+K^+ ATPase activity of animal tissues but is not known to inhibit any other enzyme. When ouabain is added to thin slices

of living kidney tissue, it inhibits oxygen consumption by 66%. Why? What does this observation tell us about the use of respiratory energy by kidney tissue?

Answer Oxidative phosphorylation to supply the cell with ATP accounts for the vast majority of oxygen consumption. A decrease in oxygen consumption by 66% on addition of ouabain indicates that consumption of ATP by the Na^+K^+ ATPase in kidney cells accounts for about two-thirds of the tissue's ATP requirements, and thus of its use of respiratory energy.

10. Energetics of Symport Suppose that you determined experimentally that a cellular transport system for glucose, driven by symport of Na^+, could accumulate glucose to concentrations 25 times greater than in the external medium, while the external [Na^+] was only 10 times greater than the intracellular [Na^+]. Would this violate the laws of thermodynamics? If not, how could you explain this observation?

Answer No; the symport may be able to transport more than one equivalent of glucose per Na^+.

11. Location of a Membrane Protein The following observations are made on an unknown membrane protein, X. It can be extracted from disrupted erythrocyte membranes into a concentrated salt solution, and it can be cleaved into fragments by proteolytic enzymes. Treatment of erythrocytes with proteolytic enzymes followed by disruption and extraction of membrane components yields intact X. However, treatment of erythrocyte "ghosts" (which consist of just plasma membranes, produced by disrupting the cells and washing out the hemoglobin) with proteolytic enzymes followed by disruption and extraction yields extensively fragmented X. What do these observations indicate about the location of X in the plasma membrane? Do the properties of X resemble those of an integral or peripheral membrane protein?

Answer Because protein X can be removed by salt treatment, it must be a peripheral membrane protein. Inability to digest the protein with proteases unless the membrane has been disrupted indicates that protein X is located internally, bound to the inner surface of the erythrocyte plasma membrane.

12. Membrane Self-sealing Cellular membranes are self-sealing—if they are punctured or disrupted mechanically, they quickly and automatically reseal. What properties of membranes are responsible for this important feature?

Answer Hydrophobic interactions are the driving force for membrane formation. Because these forces are noncovalent and reversible, membranes can easily anneal after disruption.

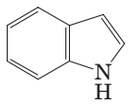
13. Lipid Melting Temperatures Membrane lipids in tissue samples obtained from different parts of the leg of a reindeer have different fatty acid compositions. Membrane lipids from tissue near the hooves contain a larger proportion of unsaturated fatty acids than those from tissue in the upper leg. What is the significance of this observation?

Answer The temperature of body tissues at the extremities, such as near the hooves, is generally lower than that of tissues closer to the center of the body. To maintain fluidity, as required by the fluid-mosaic model, membranes at lower temperatures must contain a higher percentage of polyunsaturated fatty acids: a higher content of unsaturated fatty acids lowers the melting point of lipid mixtures.

14. Flip-Flop Diffusion The inner leaflet (monolayer) of the human erythrocyte membrane consists predominantly of phosphatidylethanolamine and phosphatidylserine. The outer leaflet consists predominantly of phosphatidylcholine and sphingomyelin. Although the phospholipid components of the membrane can diffuse in the fluid bilayer, this sidedness is preserved at all times. How?

Answer The energy required to flip a charged polar head group through a hydrophobic lipid bilayer is prohibitively high.

15. Membrane Permeability At pH 7, tryptophan crosses a lipid bilayer at about one-thousandth the rate of indole, a closely related compound:



Suggest an explanation for this observation.

Answer At pH 7, tryptophan exists as a zwitterion (having a positive and negative charge), whereas indole is uncharged. The movement of the less polar indole through the hydrophobic core of the bilayer is more energetically favorable.

16. Water Flow through an Aquaporin A human erythrocyte has about 2×10^5 AQP-1 monomers. If water molecules flow through the plasma membrane at a rate of 5×10^8 per AQP-1 tetramer per second, and the volume of an erythrocyte is 5×10^{-11} mL, how rapidly could an erythrocyte halve its volume as it encountered the high osmolarity (1 M) in the interstitial fluid of the renal medulla? Assume that the erythrocyte consists entirely of water.

Answer First, calculate the number of water molecules that must leave the erythrocyte to halve its volume. The volume of the cell is 5×10^{-11} mL. For $[H_2O] = 55$ M, the number of water molecules in the cell is

$$(5 \times 10^{-11}\text{mL/cell})(6.02 \times 10^{20}\text{ molecules/mmol})(55\text{ mmol } H_2O\text{/mL}) = 1.7 \times 10^{12}$$

Half of these molecules (8.5×10^{11}) must leave to halve the cell volume.

Next, calculate how fast the cell can lose water molecules. The cell has 2×10^5 aquaporin monomers, or 5×10^4 tetramers. Each tetramer allows passage of 5×10^8 H_2O molecules per second, so the flux of water molecules through the plasma membrane is

$$(5 \times 10^8\ H_2O\text{ molecules/s/aquaporin tetramer})(5 \times 10^4\text{ aquaporin tetramers/cell})$$

$$= 2.5 \times 10^{13}\ H_2O\text{ molecules/s}$$

Removal of half the volume of water would take

$$(8.5 \times 10^{11}\ H_2O\text{ molecules})/(2.5 \times 10^{13}\ H_2O\text{ molecules/s}) = 3 \times 10^{-2}\text{ s}$$

17. Labeling the Lactose Transporter A bacterial lactose transporter, which is highly specific for lactose, contains a Cys residue that is essential to its transport activity. Covalent reaction of *N*-ethylmaleimide (NEM) with this Cys residue irreversibly inactivates the transporter. A high concentration of lactose in the medium prevents inactivation by NEM, presumably by sterically protecting the Cys residue, which is in or near the lactose-binding site. You know nothing else about the transporter protein. Suggest an experiment that might allow you to determine the M_r of the Cys-containing transporter polypeptide.

Answer Treat a suspension of the bacteria as follows: Add lactose at a concentration well above the K_t, so that virtually every molecule of galactoside transporter binds lactose. Next, add nonradiolabeled NEM and allow it to react with all available —SH groups on the cell surface. Remove excess lactose by centrifuging and resuspending the cells, then add radiolabeled NEM. The only Cys residues now available to react with NEM are those in the transporter protein. Dissolve the membrane proteins in sodium dodecylsulfate (SDS), and separate them on the basis of size by SDS gel electrophoresis. The M_r of the labeled band should represent that of the galactoside transporter.

18. Predicting Membrane Protein Topology from Sequence You have cloned the gene for a human erythrocyte protein, which you suspect is a membrane protein. From the nucleotide sequence of the gene, you know the amino acid sequence. From this sequence alone, how would you evaluate the possibility that

the protein is an integral protein? Suppose the protein proves to be an integral protein, either type I or type II. Suggest biochemical or chemical experiments that might allow you to determine which type it is.

Answer Construct and analyze a hydropathy plot for the protein. You can assume that any hydrophobic regions of more than 20 consecutive residues are transmembrane segments of an integral protein. To determine whether the external domain is carboxyl- or amino-terminal, treat intact erythrocytes with a membrane-impermeant reagent known to react with primary amines and determine whether the protein reacts. If it does, the amino terminus is on the external surface of the erythrocyte membrane and this is a type I protein (see Fig. 11–8). If it does not, a type II protein is indicated.

19. Intestinal Uptake of Leucine You are studying the uptake of L-leucine by epithelial cells of the mouse intestine. Measurements of the rate of uptake of L-leucine and several of its analogs, with and without Na^+ in the assay buffer, yield the results given in the table. What can you conclude about the properties and mechanism of the leucine transporter? Would you expect L-leucine uptake to be inhibited by ouabain?

	Uptake in presence of Na^+		Uptake in absence of Na^+	
Substrate	V_{max}	K_t (mM)	V_{max}	K_t (mM)
L-Leucine	420	0.24	23	0.2
D-Leucine	310	4.7	5	4.7
L-Valine	225	0.31	19	0.31

Answer The similar K_t values for L-leucine and L-valine indicate that the transporter binding site can accommodate the side chains of both amino acids equally well; it is probably a hydrophobic pocket of suitable size for either R group. The 20-fold higher K_t for D- than for L-leucine indicates that the binding site recognizes differences of configuration about the α carbon. Based on the lower V_{max} in the absence of Na^+ for all three substrates, we know that Na^+ entry is essential for amino acid uptake; the transporter acts by symport of leucine (or valine) and Na^+.

20. Effect of an Ionophore on Active Transport Consider the leucine transporter described in Problem 19. Would V_{max} and/or K_t change if you added a Na^+ ionophore to the assay solution containing Na^+? Explain.

Answer By dissipating the transmembrane Na^+ gradient, the ionophore would prevent symport of L-leucine and reduce the rate of uptake, measured as V_{max}. The value of K_t, a measure of the transporter's affinity for the substrate (L-leucine), should not change. Valinomycin (the likely ionophore here) does not resemble L-leucine in structure and almost certainly would not bind the transporter to affect K_t.

21. Surface Density of a Membrane Protein *E. coli* can be induced to make about 10,000 copies of the lactose transporter (M_r 31,000) per cell. Assume that *E. coli* is a cylinder 1 μm in diameter and 2 μm long. What fraction of the plasma membrane surface is occupied by the lactose transporter molecules? Explain how you arrived at this conclusion.

Answer The surface area of a cylinder is $2\pi r^2 + \pi dh$, where r = radius, d = diameter, and h = height. For a cylinder 2 μm high and 1 μm in diameter, the surface area is $2\pi(0.5\ \mu m)^2 + \pi(1\ \mu m)(2\ \mu m) = 2.5\pi\ \mu m^2 = 8\ \mu m^2$. This is the *E. coli* surface area.

To estimate the cross-sectional area of a globular protein of M_r 31,000, we can use the dimensions for hemoglobin (M_r = 64,500; diameter = 5.5 nm; see text p. 159), thus a protein of M_r 31,000 has a diameter of about 3 nm, assuming the proteins have the same density. The cross-sectional area of a sphere of diameter 3 nm (0.003 μm)—or of a single transporter molecule—is

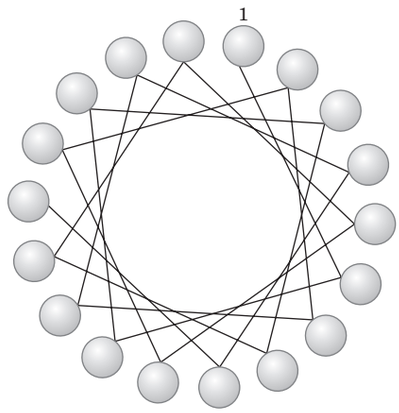
$$\pi r^2 = 3.14(1.5 \times 10^{-3}\ \mu m)^2 = 7 \times 10^{-6}\ \mu m^2$$

and the total cross-sectional area of 10,000 transporter molecules is $7 \times 10^{-2}\ \mu m^2$. Thus, the fraction of an *E. coli* cell surface covered by transporter molecules is

$$(7 \times 10^{-2}\ \mu m^2)/(8\ \mu m^2) = 0.009, \text{ or about } 1\%.$$

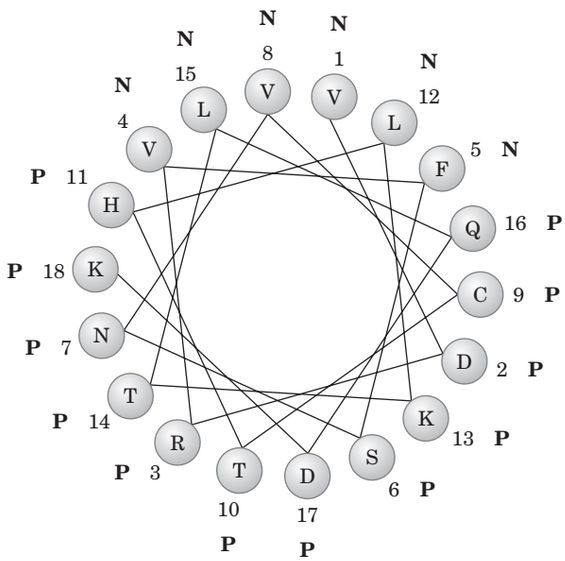
This answer is clearly an approximation, given the method of estimating the diameter of the transporter molecule, but it is certainly of the right order of magnitude.

22. Use of the Helical Wheel Diagram A helical wheel is a two-dimensional representation of a helix, a view along its central axis (see Fig. 11–29b; see also Fig. 4–4d). Use the helical wheel diagram below to determine the distribution of amino acid residues in a helical segment with the sequence –Val–Asp–Arg–Val–Phe–Ser–Asn–Val–Cys–Thr–His–Leu–Lys–Thr–Leu–Gln–Asp–Lys–



What can you say about the surface properties of this helix? How would you expect the helix to be oriented in the tertiary structure of an integral membrane protein?

Answer A helical wheel is a two-dimensional representation of a helix obtained by projecting the helix down its central axis. An α helix contains 3.6 residues per turn, so each amino acid in the helix lies 100° around the axis from the previous residue: (360°/turn)/(3.6 residues/turn) = 100° per residue. For the 18 amino acid helix considered here, the 18 vertices are separated by 20° increments. If there were a 19th residue, it would lie under the first residue on the projection, but five turns down the helix: 5 turns × 0.54 nm/turn (pitch for an α helix) = 2.70 nm "behind" residue 1. To complete the diagram, follow the lines from residue 1 to residue 2, and so on, numbering the residues. Then, using the sequence given, label each residue with its one-letter abbreviation and a characterization of its R group properties—**P** for polar, and **N** for nonpolar.



The "top" side of the helix contains only hydrophobic side chains, while the other surfaces are polar or charged; this is an amphipathic helix. As an integral membrane protein, it is likely to dip its hydrophobic surface into the lipid bilayer but expose the other surfaces to the aqueous phase. An alternative arrangement might be to cluster, say, 10 helices, one from each of 10 subunits, around a central hydrophilic core, while exposing only the hydrophobic surface to the lipid bilayer.

23. Molecular Species in the *E. coli* Membrane The plasma membrane of *E. coli* is about 75% protein and 25% phospholipid by weight. How many molecules of membrane lipid are present for each molecule of membrane protein? Assume an average protein M_r of 50,000 and an average phospholipid M_r of 750. What more would you need to know to estimate the fraction of the membrane surface that is covered by lipids?

Answer Consider a sample that contains 1 g of membrane, of which 0.75 g is protein (M_r = 50,000) and 0.25 g is phospholipid (M_r = 750).

$$(0.75 \text{ g protein})(1 \text{ mol}/5 \times 10^4 \text{ g}) = 1.5 \times 10^{-5} \text{ mol protein in 1 g of membrane}$$

$$(0.25 \text{ g phospholipid})(1 \text{ mol}/750 \text{ g}) = 3.3 \times 10^{-4} \text{ mol phospholipid in 1 g of membrane}$$

$$3.3 \times 10^{-4} \text{ mol phospholipid}/1.5 \times 10^{-5} \text{ mol protein} = 22 \text{ mol phospholipid/mol protein}$$

To estimate the percentage of the surface covered by phospholipid, you would need to know (or estimate) the average cross-sectional area of a phospholipid in a bilayer (which you might learn from an experiment such as that diagrammed in Problem 1, above) and the average cross-sectional area of a 50 kDa protein.

Biochemistry on the Internet

24. Membrane Protein Topology The receptor for the hormone epinephrine in animal cells is an integral membrane protein (M_r 64,000) that is believed to have seven membrane-spanning regions.

(a) Show that a protein of this size is capable of spanning the membrane seven times.

(b) Given the amino acid sequence of this protein, how would you predict which regions of the protein form the membrane-spanning helices?

(c) Go to the Protein Data Bank (www.rcsb.org). Use the PDB identifier 1DEP to retrieve the data page for a portion of the β-adrenergic receptor (one type of epinephrine receptor) from a turkey. Using Jmol to explore the structure, predict where this portion of the receptor is located: within the membrane or at the membrane surface. Explain.

(d) Retrieve the data for a portion of another receptor, the acetylcholine receptor of neurons and myocytes, using the PDB identifier 1A11. As in **(c),** predict where this portion of the receptor is located and explain your answer.

If you have not used the PDB, see Box 4–4 (p. 129) for more information.

Answer

(a) Assume that the transmembrane portion of the peptide is an α helix. The rise per amino acid (AA) residue of an α helix is 1.5Å/AA = 1.5 nm/AA. Assume the lipid bilayer is 4.0 nm thick; thus (4.0 nm)/(0.15 nm/AA) = 27 AA are needed to span the bilayer, and seven spans require 7 × 27 = 189 residues. For an average AA residue M_r of 110, a protein of M_r 64,000 has approximately 64,000/110 = 580 AA residues.

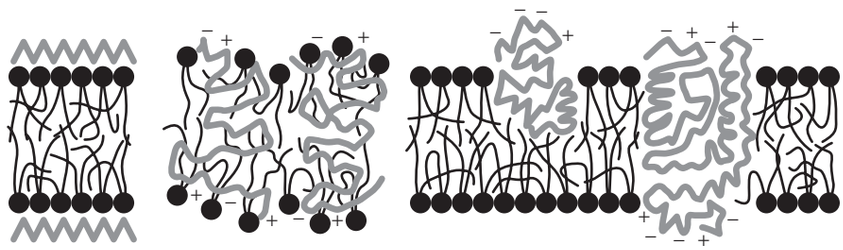
(b) Hydropathy plots are used to identify potential transmembrane regions. The most hydrophobic (hydropathic) stretches are those most likely to pass through the apolar lipid bilayer.

(c) This portion of the epinephrine receptor is an intracellular loop that connects adjacent membrane-spanning regions of the protein. You can predict that this α helix is *not* located in the membrane on the basis of the properties of its amino acids. At the PDB, click on the icon to display the PDB file. The helix has only 15 amino acid residues—RSPDFRKAFKRLLCF—too few to span the bilayer. About half of these residues are charged. Look at the structure in Jmol, using the console controls to display the side chains as ball-and-stick structures. Rotate the molecule so that you look directly down the axis of the helix, with the side chains spiraling around the outside. Note that most of the nonpolar residues fall along one side of this amphipathic helix.

(d) This portion of the acetylcholine receptor is one of the membrane-spanning regions of the protein. You can predict that this α helix is membrane-spanning on the basis of the properties of its amino acid residues. In the PDB file you can see that the amino acid sequence is GSEKMSTAISVLLAQAVFLLLTSQR. In Jmol, display the side chains in space-filling mode. Notice that the charged residues occur primarily at the ends. The long run of hydrophobic or uncharged residues between Lys at position 4 and Arg at position 25 is enough to span a lipid bilayer.

Data Analysis Problem

25. The Fluid Mosaic Model of Biological Membrane Structure Figure 11–3 shows the currently accepted fluid mosaic model of biological membrane structure. This model was presented in detail in a review article by S. J. Singer in 1971. In the article, Singer presented the three models of membrane structure that had been proposed by that time:



A B C

A. The Davson-Danielli-Robertson Model. This was the most widely accepted model in 1971, when Singer's review was published. In this model, the phospholipids are arranged as a bilayer. Proteins are found on both surfaces of the bilayer, attached to it by ionic interactions between the charged head groups of the phospholipids and charged groups in the proteins. Crucially, there is no protein in the interior of the bilayer.

B. The Benson Lipoprotein Subunit Model. Here, the proteins are globular and the membrane is a protein-lipid mixture. The hydrophobic tails of the lipids are embedded in the hydrophobic parts of the proteins. The lipid head groups are exposed to the solvent. There is no lipid bilayer.

C. The Lipid-Globular Protein Mosaic Model. This is the model shown in Figure 11–3. The lipids form a bilayer and proteins are embedded in it, some extending through the bilayer and others not. Proteins are anchored in the bilayer by hydrophobic interactions between the hydrophobic tails of the lipids and hydrophobic portions of the protein.

For the data given below, consider how each piece of information aligns with each of the three models of membrane structure. Which model(s) are supported, which are not supported, and what reservations do you have about the data or their interpretation? Explain your reasoning.

(a) When cells were fixed, stained with osmium tetroxide, and examined in the electron microscope, they gave images like that in Figure 11–1: the membranes showed a "railroad track" appearance, with two dark-staining lines separated by a light space.

(b) The thickness of membranes in cells fixed and stained in the same way was found to be 5 to 9 nm. The thickness of a "naked" phospholipid bilayer, without proteins, was 4 to 4.5 nm. The thickness of a single monolayer of proteins was about 1 nm.

(c) In Singer's words: "The average amino acid composition of membrane proteins is not distinguishable from that of soluble proteins. In particular, a substantial fraction of the residues is hydrophobic" (p. 165).

(d) As described in Problems 1 and 2 of this chapter, researchers had extracted membranes from cells, extracted the lipids, and compared the area of the lipid monolayer with the area of the original cell membrane. The interpretation of the results was complicated by the issue illustrated in the graph of Problem 1: the area of the monolayer depended on how hard it was pushed. With very light pressures, the ratio of monolayer area to cell membrane area was about 2.0. At higher pressures—thought to be more like those found in cells—the ratio was substantially lower.

(e) Circular dichroism spectroscopy uses changes in polarization of UV light to make inferences about protein secondary structure (see Fig. 4–9). On average, this technique showed that membrane proteins have a large amount of α helix and little or no β sheet. This finding was consistent with most membrane proteins having a globular structure.

(f) Phospholipase C is an enzyme that removes the polar head group (including the phosphate) from phospholipids. In several studies, treatment of intact membranes with phospholipase C removed about 70% of the head groups without disrupting the "railroad track" structure of the membrane.

(g) Singer described a study in which "a glycoprotein of molecular weight about 31,000 in human red blood cell membranes is cleaved by tryptic treatment of the membranes into soluble glycopeptides of about 10,000 molecular weight, while the remaining portions are quite hydrophobic" (p. 199). Trypsin treatment did not cause gross changes in the membranes, which remained intact.

Singer's review also included many more studies in this area. In the end, though, the data available in 1971 did not conclusively prove Model C was correct. As more data have accumulated, this model of membrane structure has been accepted by the scientific community.

Answer

(a) *Model A*: supported. The two dark lines are either the protein layers or the phospholipid heads, and the clear space is either the bilayer or the hydrophobic core, respectively. *Model B*: not supported. This model requires a more-or-less uniformly stained band surrounding the cell. *Model C*: supported, with one reservation. The two dark lines are the phospholipid heads; the clear zone is the tails. This assumes that the membrane proteins are not visible, because they do not stain with osmium or do not happen to be in the sections viewed.

(b) *Model A*: supported. A "naked" bilayer (4.5 nm) + two layers of protein (2 nm) sums to 6.5 nm, which is within the observed range of thickness. *Model B*: neither. This model makes no predictions about membrane thickness. *Model C*: unclear. The result is hard to reconcile with this model, which predicts a membrane as thick as, or slightly thicker than (due to the projecting ends of embedded proteins), a "naked" bilayer. The model is supported only if the smallest values for membrane thickness are correct or if a substantial amount of protein projects from the bilayer.

(c) *Model A*: unclear. The result is hard to reconcile with this model. If the proteins are bound to the membrane by ionic interactions, the model predicts that the proteins contain a high proportion of charged amino acids, in contrast to what was observed. Also, because the protein layer must be very thin (see **(b)**), there would not be much room for a hydrophobic protein core, so hydrophobic residues would be exposed to the solvent. *Model B*: supported. The proteins have a mixture of hydrophobic residues (interacting with lipids) and charged residues (interacting with water). *Model C*: supported. The proteins have a mixture of hydrophobic residues (anchoring in the membrane) and charged residues (interacting with water).

(d) *Model A*: unclear. The result is hard to reconcile with this model, which predicts a ratio of exactly 2.0; this would be hard to achieve under physiologically relevant pressures. *Model B*: neither. This model makes no predictions about amount of lipid in the membrane. *Model C*: supported. Some membrane surface area is taken up with proteins, so the ratio would be less than 2.0, as was observed under more physiologically relevant conditions.

(e) *Model A*: unclear. The model predicts proteins in extended conformations rather than globular, so supported only if one assumes that proteins layered on the surfaces include helical segments. *Model B*: supported. The model predicts mostly globular proteins (containing some helical segments). *Model C*: supported. The model predicts mostly globular proteins.

(f) *Model A*: unclear. The phosphorylamine head groups are protected by the protein layer, but only if the proteins completely cover the surface will the phospholipids be completely protected from phospholipase. *Model B*: supported. Most head groups are accessible to phospholipase. *Model C*: supported. All head groups are accessible to phospholipase.

(g) *Model A*: not supported. Proteins are entirely accessible to trypsin digestion and virtually all will undergo multiple cleavage, with no protected hydrophobic segments. *Model B*: not supported. Virtually all proteins are in the bilayer and inaccessible to trypsin. *Model C*: supported. Segments of protein that penetrate or span the bilayer are protected from trypsin; those exposed at the surfaces will be cleaved. The trypsin-resistant portions have a high proportion of hydrophobic residues.

Reference

Singer, S.J. (1971) The molecular organization of biological membranes. In *Structure and Function of Biological Membranes* (Rothfield, L.I., ed.), pp. 145–222, Academic Press, Inc., New York.

chapter

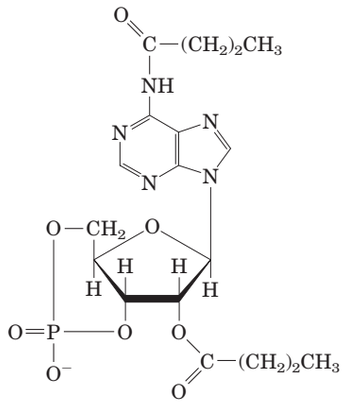
12

Biosignaling

1. **Hormone Experiments in Cell-Free Systems** In the 1950s, Earl W. Sutherland, Jr., and his colleagues carried out pioneering experiments to elucidate the mechanism of action of epinephrine and glucagon. Given what you have learned in this chapter about hormone action, interpret each of the experiments described below. Identify substance X and indicate the significance of the results.
   (a) Addition of epinephrine to a homogenate of normal liver resulted in an increase in the activity of glycogen phosphorylase. However, if the homogenate was first centrifuged at a high speed and epinephrine or glucagon was added to the clear supernatant fraction that contains phosphorylase, no increase in the phosphorylase activity occurred.
   (b) When the particulate fraction from the centrifugation in **(a)** was treated with epinephrine, substance X was produced. The substance was isolated and purified. Unlike epinephrine, substance X activated glycogen phosphorylase when added to the clear supernatant fraction of the centrifuged homogenate.
   (c) Substance X was heat-stable; that is, heat treatment did not affect its capacity to activate phosphorylase. (Hint: Would this be the case if substance X were a protein?) Substance X was nearly identical to a compound obtained when pure ATP was treated with barium hydroxide. (Fig. 8–6 will be helpful.)

   Answer Substance X is cyclic AMP. Epinephrine stimulates glycogen phosphorylase by activating the enzyme adenylyl cyclase, which catalyzes formation of cAMP, the second messenger.
   (a) Adenylyl cyclase is a membrane-bound protein; centrifugation sediments it into the particulate fraction.
   (b) Cyclic AMP directly stimulates glycogen phosphorylase.
   (c) Cyclic AMP is heat-stable; it can be prepared by treating ATP with barium hydroxide.

2. **Effect of Dibutyryl cAMP versus cAMP on Intact Cells** The physiological effects of epinephrine should in principle be mimicked by addition of cAMP to the target cells. In practice, addition of cAMP to intact target cells elicits only a minimal physiological response. Why? When the structurally related derivative dibutyryl cAMP (shown below) is added to intact cells, the expected physiological response is readily apparent. Explain the basis for the difference in cellular response to these two substances. Dibutyryl cAMP is widely used in studies of cAMP function.



Dibutyryl cAMP
(N^6,$O^{2'}$-Dibutyryl adenosine 3′,5′-cyclic monophosphate)

Answer Unlike cAMP, dibutyryl cAMP passes readily through the plasma membrane.

3. Effect of Cholera Toxin on Adenylyl Cyclase The gram-negative bacterium *Vibrio cholerae* produces a protein, cholera toxin (M_r 90,000), that is responsible for the characteristic symptoms of cholera: extensive loss of body water and Na^+ through continuous, debilitating diarrhea. If body fluids and Na^+ are not replaced, severe dehydration results; untreated, the disease is often fatal. When the cholera toxin gains access to the human intestinal tract it binds tightly to specific sites in the plasma membrane of the epithelial cells lining the small intestine, causing adenylyl cyclase to undergo prolonged activation (hours or days).

(a) What is the effect of cholera toxin on [cAMP] in the intestinal cells?

(b) Based on the information above, suggest how cAMP normally functions in intestinal epithelial cells.

(c) Suggest a possible treatment for cholera.

Answer (a) It increases [cAMP]. **(b)** The observations suggest that cAMP regulates Na^+ permeability. **(c)** Replace lost body fluids and electrolytes.

4. Mutations in PKA Explain how mutations in the R or C subunit of cAMP-dependent protein kinase (PKA) might lead to **(a)** a constantly active PKA or **(b)** a constantly inactive PKA.

Answer

(a) If a mutation in the R subunit makes it unable to bind to the C subunit, the C subunit is never inhibited; it is constantly active.

(b) If a mutation prevents the binding of cAMP to the R subunit but still allows normal R-C interaction, the inhibition of C by R cannot be relieved by elevated [cAMP], so the enzyme is constantly inactive.

5. Therapeutic Effects of Albuterol The respiratory symptoms of asthma result from constriction of the bronchi and bronchioles of the lungs caused by contraction of the smooth muscle of their walls. This constriction can be reversed by raising the [cAMP] in the smooth muscle. Explain the therapeutic effects of albuterol, a β-adrenergic agonist taken (by inhalation) for asthma. Would you expect this drug to have any side effects? How might one design a better drug that did not have these effects?

Answer By mimicking the actions of epinephrine on smooth muscle, albuterol raises [cAMP], leading to relaxation and enlargement (dilation) of the bronchi and bronchioles. Because β-adrenergic receptors also control many other processes, drugs that act as β-adrenergic

agonists generally have other, undesirable effects. To minimize such side effects, the goal is to find an agonist that is specific for the subtype of β-adrenergic receptors found in bronchial smooth muscle.

6. **Termination of Hormonal Signals** Signals carried by hormones must eventually be terminated. Describe several different mechanisms for signal termination.

   Answer A hormone can be degraded by extracellular enzymes (such as acetylcholinesterase). The GTP bound to a G protein can be hydrolyzed to GDP. A second messenger can be degraded (cAMP, cGMP), further metabolized (IP_3), or resequestered (Ca^{2+}, in the endoplasmic reticulum). A receptor can be desensitized (acetylcholine receptor/channel), phosphorylated/inactivated, bound to an arrestin, or removed from the surface (β-adrenergic receptor, rhodopsin).

7. **Using FRET to Explore Protein-Protein Interactions in Vivo** Figure 12–8 shows the interaction between β-arrestin and the β-adrenergic receptor. How would you use FRET (see Box 12–3) to demonstrate this interaction in living cells? Which proteins would you fuse? Which wavelengths would you use to illuminate the cells, and which would you monitor? What would you expect to observe if the interaction occurred? If it did not occur? How might you explain the failure of this approach to demonstrate this interaction?

   Answer Fuse CFP to β-arrestin and YFP to the cytoplasmic domain of the β-adrenergic receptor, or vice versa. In either case, illuminate at 433 nm and observe at both 476 and 527 nm. If the interaction occurs, emitted light intensity will decrease at 476 nm and increase at 527 nm on addition of epinephrine to cells expressing the fusion proteins. If the interaction does not occur, the wavelength of the emitted light will remain at 476 nm. There are several reasons why this might fail; for example, the fusion proteins (1) are inactive or otherwise unable to interact, (2) are not translocated to their normal subcellular location, or (3) are not stable to proteolytic breakdown.

8. **EGTA Injection** EGTA (ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid) is a chelating agent with high affinity and specificity for Ca^{2+}. By microinjecting a cell with an appropriate Ca^{2+}-EGTA solution, an experimenter can prevent cytosolic [Ca^{2+}] from rising above 10^{-7} M. How would EGTA microinjection affect a cell's response to vasopressin (see Table 12–4)? To glucagon?

   Answer Vasopressin acts through a PLC-coupled GPCR. The IP_3 released by PLC normally elevates cytosolic [Ca^{2+}] to 10^{-6} M, activating (with diacylglycerol) protein kinase C. Preventing this elevation of [Ca^{2+}] by using EGTA to "buffer" the internal [Ca^{2+}] would block vasopressin action, but should not directly affect the response to glucagon, which uses cAMP, *not* Ca^{2+}, as its intracellular second messenger.

9. **Amplification of Hormonal Signals** Describe all the sources of amplification in the insulin receptor system.

   Answer The amplification results from catalysts activating catalysts—including protein kinases that act in enzyme cascades. Two molecules of insulin activate an *insulin receptor* dimer for a finite period, during which the receptor phosphorylates many molecules of *IRS-1.* Through a series of interactions with other proteins (including Grb2, Sos, Ras), IRS-1 activates *Raf,* which phosphorylates and activates many molecules of *MEK,* each of which phosphorylates and activates many molecules of *ERK.* Each activated ERK phosphorylates and activates several molecules of a *transcription factor,* and each of these stimulates the transcription of multiple copies of *mRNA* for specific genes. Each mRNA can direct the synthesis of many copies of the protein it encodes. (See Fig. 12–15.)

10. Mutations in *ras* How does a mutation in *ras* that leads to formation of a Ras protein with no GTPase activity affect a cell's response to insulin?

Answer When active, Ras activates the protein kinase Raf, which initiates the MAPK cascade that leads to the phosphorylation of nuclear proteins. A Ras protein without GTPase activity would, once activated by the binding of GTP, remain active, continuing to produce an insulin response.

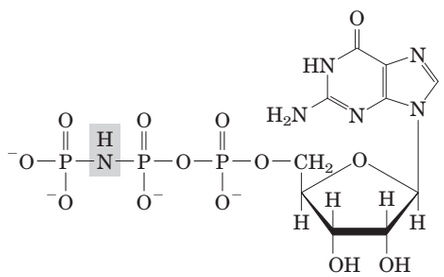
11. Differences among G Proteins Compare the G proteins G_s, which acts in transducing the signal from β-adrenergic receptors, and Ras. What properties do they share? How do they differ? What is the functional difference between G_s and G_i?

Answer *Shared properties of Ras and G_s*: both can bind either GDP or GTP; both are activated by GTP; both can, when active, activate a downstream enzyme; both have intrinsic GTPase activity that shuts them off after a short period of activation. *Differences between Ras and G_s*: Ras is a small, monomeric protein; G_s is heterotrimeric. *Functional differences between G_s and G_i*: G_s activates adenylyl cyclase, G_i inhibits it.

12. Mechanisms for Regulating Protein Kinases Identify eight general types of protein kinases found in eukaryotic cells, and explain what factor is *directly* responsible for activating each type.

Answer Kinase (factor(s)): PKA (cAMP); PKG (cGMP); PKC (Ca^{2+}, DAG); Ca^{2+}/CaM kinase (Ca^{2+}, CaM); cyclin-dependent kinase (cyclin); protein Tyr kinase (ligand for the receptor, such as insulin); MAPK (Raf); Raf (Ras); glycogen phosphorylase kinase (PKA).

13. Nonhydrolyzable GTP Analogs Many enzymes can hydrolyze GTP between the β and γ phosphates. The GTP analog β,γ-imidoguanosine 5′-triphosphate Gpp(NH)p, shown below, cannot be hydrolyzed between the β and γ phosphates. Predict the effect of microinjection of Gpp(NH)p into a myocyte on the cell's response to β-adrenergic stimulation.



Gpp(NH)p
(β,γ-imidoguanosine 5′-triphosphate)

Answer Nonhydrolyzable analogs of GTP have the effect of keeping the stimulatory G protein (G_s) in its activated form once it has encountered the receptor-hormone complex; it cannot shut itself off by converting the bound GTP (analog) to GDP. Injection of the analog would therefore be expected to prolong the effect of epinephrine on the injected cell.

14. Use of Toxin Binding to Purify a Channel Protein α-Bungarotoxin is a powerful neurotoxin found in the venom of a poisonous snake (*Bungarus multicinctus*). It binds with high specificity to the nicotinic acetylcholine receptor (AChR) protein and prevents the ion channel from opening. This interaction was used to purify AChR from the electric organ of torpedo fish.

(a) Outline a strategy for using α-bungarotoxin covalently bound to chromatography beads to purify the AChR protein. (Hint: See Fig. 3–17c.)

(b) Outline a strategy for the use of [^{125}I]α-bungarotoxin to purify the AChR protein.

Answer

(a) Use the (α-bungarotoxin–bound beads for affinity purification (see Fig. 3–17c, p. 87) of AChR. Extract proteins from the electric organs and pass the mixture through the chromatography column; the AChR binds selectively to the beads. Elute the AChR with a solution of NaCl or a solvent of lower pH, which weakens its interaction with α-bungarotoxin.

(b) Use binding of [^{125}I]α-bungarotoxin as a *quantitative assay* for AChR during purification by various techniques. At each step, assay AChR by measuring [^{125}I]α-bungarotoxin binding to the proteins in the sample. Optimize purification for the highest specific activity of AChR (counts/min of bound [^{125}I]α-bungarotoxin per mg of protein) in the final material.

15. Resting Membrane Potential A variety of unusual invertebrates, including giant clams, mussels, and polychaete worms, live on the fringes of deep-sea hydrothermal vents, where the temperature is 60 °C.

(a) The adductor muscle of a giant clam has a resting membrane potential of −95 mV. Given the intracellular and extracellular ionic compositions shown below, would you have predicted this membrane potential? Why or why not?

	Concentration (mM)	
Ion	**Intracellular**	**Extracellular**
Na^+	50	440
K^+	400	20
Cl^-	21	560
Ca^{2+}	0.4	10

(b) Assume that the adductor muscle membrane is permeable to only one of the ions listed above. Which ion could determine the V_m?

Answer

(a) In most myocytes at rest, the plasma membrane is permeable primarily to K^+ ions. V_m is a function of the distribution of K^+ ions across the membrane. If V_m in the clam adductor muscle is determined primarily by K^+, then V_m at rest would be predicted by the Nernst equation

$$E_{ion} = (RT/Z\mathcal{F}) \ln (C_{out}/C_{in})$$

using the values for [K^+] given in the table.

$$E_{K^+} = [(8.315 \text{ J/mol} \cdot \text{K})(333 \text{ K})/(1)(96{,}480 \text{ J/V} \cdot \text{mol})] \ln (20/400)$$

$$= 0.0287 \text{ V} \times (-3.0) = -0.09 \text{ V, or } -90 \text{ mV}$$

Because the experimentally observed V_m is −95 mV, the plasma membrane in the adductor muscle must be permeable to some other ion or combination of ions.

(b) Use the Nernst equation to calculate E for each ion. The ion with an E value closest to the membrane potential is the permeant ion that influences V_m.

E_{K^+} = −90 mV (see above)

E_{Na^+} = 0.0287 V × ln (440/50) = 0.06 V, or 60 mV

E_{Cl^-} = (0.0287 V/−1)× ln (560/21) = −0.094 V, or −94 mV (note that Z for Cl^- is −1)

$E_{Ca^{2+}}$ = (0.0287 V/2) × ln (10/0.4) = 0.05 V, or 50 mV (Z for Ca^{2+} is 2)

Thus, because $E_{Cl^-} = -94$ mV is very close to the resting V_m of -95 mV, it is likely that the membrane is permeable only to Cl^- ions at rest. You could verify this experimentally by changing the extracellular [Cl^-], then measuring the effect on resting membrane potential. If this potential does depend only on Cl^- ions, the Nernst equation should predict how the membrane potential will change.

16. Membrane Potentials in Frog Eggs Fertilization of a frog oocyte by a sperm cell triggers ionic changes similar to those observed in neurons (during movement of the action potential) and initiates the events that result in cell division and development of the embryo. Oocytes can be stimulated to divide without fertilization by suspending them in 80 mM KCl (normal pond water contains 9 mM KCl).

(a) Calculate how much the change in extracellular [KCl] changes the resting membrane potential of the oocyte. (Hint: Assume the oocyte contains 120 mM K^+ and is permeable *only* to K^+.) Assume a temperature of 20 °C.

(b) When the experiment is repeated in Ca^{2+}-free water, elevated [KCl] has no effect. What does this suggest about the mechanism of the KCl effect?

Answer

(a) $$V_m = \frac{RT}{Z\mathcal{F}} \ln\left(\frac{[K^+]_{out}}{[K^+]_{in}}\right)$$

$$= [(8.315 \text{ J/mol} \cdot \text{K})(293 \text{ K})/(1)(96{,}480 \text{ J/V} \cdot \text{mol})] \ln ([K^+]_{out}/[K^+]_{in})$$

$$= (0.025 \text{ V}) \ln ([K^+]_{out}/[K^+]_{in})$$

V_m in pond water = 0.025 V ln (9/120) = -0.06 V, or -60 mV

V_m in 80 mM KCl = 0.025 V ln (80/120) = -0.01 V, or -10 mV

The membrane of the oocyte has been *depolarized*—the resting membrane potential has become less negative—by exposure to elevated extracellular [K^+].

(b) This observation suggests that the effect of increased [KCl] depends on an influx of Ca^{2+} from the extracellular medium, which is required to stimulate cell division. High [KCl] treatment must depolarize the oocyte sufficiently to open voltage-dependent Ca^{2+} channels in the plasma membrane.

17. Excitation Triggered by Hyperpolarization In most neurons, membrane *depolarization* leads to the opening of voltage-dependent ion channels, generation of an action potential, and ultimately an influx of Ca^{2+}, which causes release of neurotransmitter at the axon terminus. Devise a cellular strategy by which *hyperpolarization* in rod cells could produce excitation of the visual pathway and passage of visual signals to the brain. (Hint: The neuronal signaling pathway in higher organisms consists of a *series* of neurons that relay information to the brain (see Fig. 12–35). The signal released by one neuron can be either excitatory or inhibitory to the following, postsynaptic neuron.)

Answer Hyperpolarization of rod cells in the retina occurs when the membrane potential, V_m, becomes more negative. This results in the closing of voltage-dependent Ca^{2+} channels in the presynaptic region of the rod cell. The resulting decrease in intracellular [Ca^{2+}] causes a corresponding decrease in the release of neurotransmitter by exocytosis. The neurotransmitter released by rod cells is actually an inhibitory neurotransmitter, which leads to suppression of activity in the next neuron of the visual circuit. When this inhibition is removed in response to a light stimulus, the circuit becomes active and visual centers in the brain are excited.

18. Genetic "Channelopathies" There are many genetic diseases that result from defects in ion channels. For each of the following, explain how the molecular defect might lead to the symptoms described.

(a) A loss-of-function mutation in the gene encoding the α subunit of the cGMP-gated cation channel of retinal cone cells leads to a complete inability to distinguish colors.

(b) Loss-of-function alleles of the gene encoding the α subunit of the ATP-gated K^+ channel shown in Figure 23–29 lead to a condition known as congenital hyperinsulinism—persistently high levels of insulin in the blood.

(c) Mutations affecting the β subunit of the ATP-gated K^+ channel that prevent ATP binding lead to neonatal diabetes—persistently low levels of insulin in the blood in newborn babies.

Answer

(a) Loss of function of the cGMP-gated channel prevents influx of Na^+ and Ca^{2+} into cone cells in response to light; consequently, the cells fail to signal the brain that light had been received. Because rod cells are unaffected, the individual can see but does not have color vision.

(b) A loss-of-function mutation in the ATP-gated cation channel prevents efflux of K^+ through these channels, leading to continuous depolarization of the β-cell membrane and constitutive release of insulin into the blood.

(c) ATP is responsible for closing this channel, so in an individual with the mutant protein, the channels will remain open, preventing depolarization of the β-cell membrane and thereby preventing release of insulin, resulting in diabetes.

19. Visual Desensitization Oguchi's disease is an inherited form of night blindness. Affected individuals are slow to recover vision after a flash of bright light against a dark background, such as the headlights of a car on the freeway. Suggest what the molecular defect(s) might be in Oguchi's disease. Explain in molecular terms how this defect would account for night blindness.

Answer Some individuals with Oguchi's disease have a defective rhodopsin kinase that slows the recycling of rhodopsin after its conversion to the all-trans form on illumination. This defect leaves retinal rod and cone cells insensitive for some time after a bright flash. Other individuals have genetic defects in arrestin that prevent it from interacting with phosphorylated rhodopsin to trigger the process that leads to replacement of all-*trans*-retinal with 11-*cis*-retinal.

20. Effect of a Permeant cGMP Analog on Rod Cells An analog of cGMP, 8-Br-cGMP, will permeate cellular membranes, is only slowly degraded by a rod cell's PDE activity, and is as effective as cGMP in opening the gated channel in the cell's outer segment. If you suspended rod cells in a buffer containing a relatively high [8-Br-cGMP], then illuminated the cells while measuring their membrane potential, what would you observe?

Answer Rod cells would no longer show any change in membrane potential in response to light. This experiment has been done. Illumination did activate PDE, but the enzyme could not significantly reduce the 8-Br-cGMP level, which remained well above that needed to keep the gated ion channels open. Thus, light had no impact on membrane potential.

21. Hot and Cool Taste Sensations The sensations of heat and cold are transduced by a group of temperature-gated cation channels. For example, TRPV1, TRPV3, and TRPM8 are usually closed, but open under the following conditions: TRPV1 at $\geq$43 °C; TRPV3 at $\geq$33 °C; and TRPM8 at $<$25 °C. These channels are expressed in sensory neurons known to be responsible for temperature sensation.

(a) Propose a reasonable model to explain how exposing a sensory neuron containing TRPV1 to high temperature leads to a sensation of heat.

(b) Capsaicin, one of the active ingredients in "hot" peppers, is an agonist of TRPV1. Capsaicin shows 50% activation of the TRPV1 response at a concentration (i.e., it has an EC_{50}) of 32 nM. Explain why even a very few drops of hot pepper sauce can taste very "hot" without actually burning you.

(c) Menthol, one of the active ingredients in mint, is an agonist of TRPM8 (EC_{50} = 30 μM) and TRPV3 (EC_{50} = 20 μM). What sensation would you expect from contact with low levels of menthol? With high levels?

Answer

(a) On exposure to heat, TRPV1 channels open, causing an influx of Na^+ and Ca^{2+} into the sensory neuron. This depolarizes the neuron, triggering an action potential. When the action potential reaches the axon terminus, neurotransmitter is released, signaling the nervous system that heat has been sensed.

(b) Capsaicin mimics the effects of heat by binding to and opening the TRPV1 channel at low temperature, leading to the false sensation of heat. The extremely low EC_{50} indicates that even very small amounts of capsaicin will have dramatic sensory effects.

(c) At low levels, menthol should open the TRPM8 channel, leading to a sensation of cool; at high levels, both TRPM8 and TRPV3 will open, leading to a mixed sensation of cool and heat, such as you may have experienced with very strong peppermints.

22. Oncogenes, Tumor-Suppressor Genes, and Tumors For each of the following situations, provide a plausible explanation for how it could lead to unrestricted cell division.

(a) Colon cancer cells often contain mutations in the gene encoding the prostaglandin E_2 receptor. PGE_2 is a growth factor required for the division of cells in the gastrointestinal tract.

(b) Kaposi sarcoma, a common tumor in people with untreated AIDS, is caused by a virus carrying a gene for a protein similar to the chemokine receptors CXCR1 and CXCR2. Chemokines are cell-specific growth factors.

(c) Adenovirus, a tumor virus, carries a gene for the protein E1A, which binds to the retinoblastoma protein, pRb. (Hint: See Fig. 12–48.)

(d) An important feature of many oncogenes and tumor suppressor genes is their cell-type specificity. For example, mutations in the PGE_2 receptor are not typically found in lung tumors. Explain this observation. (Note that PGE_2 acts through a GPCR in the plasma membrane.)

Answer

(a) These mutations might lead to permanent activation of the PGE_2 receptor. The mutant cells would behave as though stimulatory levels of PGE_2 were always present, leading to unregulated cell division and tumor formation.

(b) The viral gene might encode a constitutively active form of the receptor, such that the cells send a constant signal for cell division. This unrestrained division would lead to tumor formation.

(c) E1A protein might bind to pRb and prevent E2F from binding, so E2F is constantly active as a transcription factor. It constantly activates genes that trigger cell division, so cells divide uncontrollably.

(d) Lung cells do not normally respond to PGE_2 because they do not express the PGE_2 receptor; mutations resulting in a constitutively active PGE_2 receptor do not affect lung cells.

23. Mutations in Tumor Suppressor Genes and Oncogenes Explain why mutations in tumor suppressor genes are recessive (both copies of the gene must be defective for the regulation of cell division to be defective), whereas mutations in oncogenes are dominant.

Answer A *tumor suppressor gene* in its normal cellular form encodes a protein that restrains cell division. Mutant forms of the protein fail to suppress cell division, but if either of the two alleles of the gene present in the individual encodes a normal protein, normal function will continue. Only if both alleles are defective will the suppression of cell division fail, leading to unregulated division. An *oncogene* in its normal form encodes a regulatory protein that signals the cell to divide, but only when other, external or internal factors (such as growth factors) signal cell division. If a defective oncogene product is formed by either of the two alleles, unregulated cell growth and division will occur: the mutant protein sends the signal for cell division, whether or not growth factors are present.

24. Retinoblastoma in Children Explain why some children with retinoblastoma develop multiple tumors of the retina in both eyes, whereas others have a single tumor in only one eye.

Answer Children who develop multiple tumors in both eyes were born with a defective copy of the *Rb* gene, occurring in every cell of the retina. Early in their lives, as retinal cells divided, one or several cells independently underwent a second mutation that damaged the remaining good copy of the *Rb* gene. Each cell with two defective *Rb* alleles develops into a tumor. In the later onset, single-tumor form of the disease, children were born with two good copies of the *Rb* gene. A tumor develops when mutation in a single retinal cell damages one allele of the *Rb* gene, then a second mutation damages the second allele in the same cell. Two mutations in the same gene in the same cell are extremely rare, and when this does happen, it occurs in only one cell and develops into a single tumor.

25. Specificity of a Signal for a Single Cell Type Discuss the validity of the following proposition. A signaling molecule (hormone, growth factor, or neurotransmitter) elicits identical responses in different types of target cells if they contain identical receptors.

Answer The proposition is invalid. Two cells expressing the same surface receptor for a given hormone may have different complements of target proteins for phosphorylation by protein kinases, resulting in different physiological and biochemical responses in different cells.

Data Analysis Problem

26. Exploring Taste Sensation in Mice Figure 12–41 shows the signal-transduction pathway for sweet taste in mammals. Pleasing tastes are an evolutionary adaptation to encourage animals to consume nutritious foods. Zhao and coauthors (2003) examined the two major pleasurable taste sensations: sweet and umami. Umami is a "distinct savory taste" triggered by amino acids, especially aspartate and glutamate, and probably encourages animals to consume protein-rich foods. Monosodium glutamate (MSG) is a flavor enhancer that exploits this sensitivity.

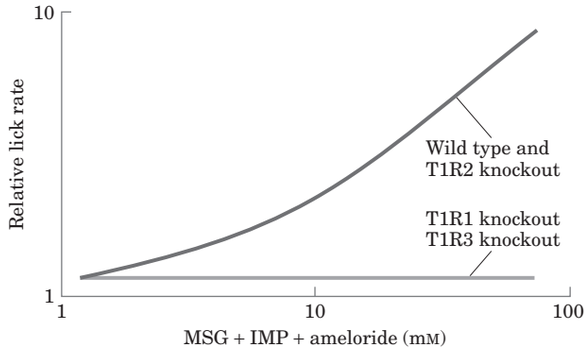
At the time the article was published, specific taste receptor proteins (labeled SR in Fig. 12–41) for sweet and umami had been tentatively characterized. Three such proteins were known—T1R1, T1R2, and T1R3—which function as heterodimeric receptor complexes: T1R1-T1R3 was tentatively identified as the umami receptor, and T1R2-T1R3 as the sweet receptor. It was not clear how taste sensation was encoded and sent to the brain, and two possible models had been suggested. In the cell-based model, individual taste-sensing cells express only one kind of receptor; that is, there are "sweet cells," "bitter cells," "umami cells," and so on, and each type of cell sends its information to the brain via a different nerve. The brain "knows" which taste is detected by the identity of the nerve fiber that transmits the message. In the receptor-based model, individual taste-sensing cells have several kinds of receptors and send different messages along the same nerve fiber to the brain, the message depending on which receptor is activated. Also unclear at the time was whether there was any interaction between the different taste sensations, or whether parts of one taste-sensing system were required for other taste sensations.

(a) Previous work had shown that different taste receptor proteins are expressed in nonoverlapping sets of taste receptor cells. Which model does this support? Explain your reasoning.

Zhao and colleagues constructed a set of "knockout mice"—mice homozygous for loss-of-function alleles for one of the three receptor proteins, T1R1, T1R2, or T1R3—and double-knockout mice with nonfunctioning T1R2 and T1R3. The researchers measured the taste perception of these mice by measuring their "lick rate" of solutions containing different taste molecules. Mice will lick the spout of a feeding bottle with a pleasant-tasting solution more often than one with an unpleasant-tasting solution. The researchers measured relative lick rates: how often the mice licked a sample solution compared with water. A relative lick rate of 1 indicated no preference; <1, an aversion; and >1, a preference.

(b) All four types of knockout strains had the same responses to salt and bitter tastes as did wild-type mice. Which of the above issues did this experiment address? What do you conclude from these results?

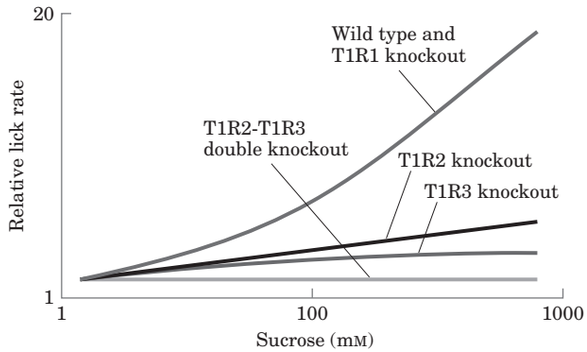
The researchers then studied umami taste reception by measuring the relative lick rates of the different mouse strains with different quantities of MSG in the feeding solution. Note that the solutions also contained inosine monophosphate (IMP), a strong potentiator of umami taste reception (and a common ingredient in ramen soups, along with MSG), and ameloride, which suppresses the pleasant salty taste imparted by the sodium of MSG. The results are shown in the graph.



(c) Are these data consistent with the umami taste receptor consisting of a heterodimer of T1R1 and T1R3? Why or why not?

(d) Which model(s) of taste encoding does this result support? Explain your reasoning.

Zhao and coworkers then performed a series of similar experiments using sucrose as a sweet taste. These results are shown below.



(e) Are these data consistent with the sweet taste receptor consisting of a heterodimer of T1R2 and T1R3? Why or why not?

(f) There were some unexpected responses at very high sucrose concentrations. How do these complicate the idea of a heterodimeric system as presented above?

In addition to sugars, humans also taste other compounds (e.g., the peptides monellin and aspartame) as sweet; mice do not taste these as sweet. Zhao and coworkers inserted into T1R2 knockout mice a copy of the human T1R2 gene under the control of the mouse T1R2 promoter. These modified mice now tasted monellin and saccharin as sweet. The researchers then went further, adding to T1R1 knockout mice the RASSL protein—a G protein–linked receptor for the synthetic opiate spiradoline; the RASSL gene was under the control of a promoter that could be induced by feeding the mice tetracycline. These mice did not prefer spiradoline in the absence of tetracycline; in the presence of tetracycline, they showed a strong preference for nanomolar concentrations of spiradoline.

(g) How do these results strengthen Zhao and coauthors' conclusions about the mechanism of taste sensation?

Answer

(a) The cell-based model, which predicts different receptors present on different cells.

(b) This experiment addresses the issue of the independence of different taste sensations. Even though the receptors for sweet and/or umami are missing, the animals' other taste sensations are normal; thus, pleasant and unpleasant taste sensations are independent.

(c) Yes. Loss of either T1R1 or T1R3 subunits abolishes umami taste sensation.

(d) Both models. With either model, removing one receptor would abolish that taste sensation.

(e) Yes. Loss of either the T1R2 or T1R3 subunits almost completely abolishes the sweet taste sensation; complete elimination of sweet taste requires deletion of both subunits.

(f) At very high sucrose concentrations, T1R2 and, to a lesser extent, T1R3 receptors, as homodimers, can detect sweet taste.

(g) The results are consistent with either model of taste encoding, but do strengthen the researchers' conclusions. Ligand binding can be completely separated from taste sensation. If the ligand for the receptor in "sweet-tasting cells" binds a molecule, mice prefer that molecule as a sweet compound.

Reference

Zhao, G.Q., Zhang, Y., Hoon, M.A., Chandrashekar, J., Erlenbach, I., Ryba, N.J.P., & Zuker, C. (2003) The receptors for mammalian sweet and umami taste. *Cell* **115,** 255–266.

chapter

13 Bioenergetics and Biochemical Reaction Types

1. **Entropy Changes during Egg Development** Consider a system consisting of an egg in an incubator. The white and yolk of the egg contain proteins, carbohydrates, and lipids. If fertilized, the egg is transformed from a single cell to a complex organism. Discuss this irreversible process in terms of the entropy changes in the system, surroundings, and universe. Be sure that you first clearly define the system and surroundings.

   Answer Consider the developing chick as the system. The nutrients, egg shell, and outside world are the surroundings. Transformation of the single cell into a chick drastically reduces the entropy of the system (increases the order). Initially, the parts of the egg outside the embryo (within the surroundings) contain complex fuel molecules (a low-entropy condition). During incubation, some of these complex molecules are converted to large numbers of CO_2 and H_2O molecules (high entropy). This increase in entropy of the surroundings is larger than the decrease in entropy of the chick (the system). Thus, the entropy of the universe (the system + surroundings) increases.

2. **Calculation of $\Delta G'^\circ$ from an Equilibrium Constant** Calculate the standard free-energy change for each of the following metabolically important enzyme-catalyzed reactions, using the equilibrium constants given for the reactions at 25 °C and pH 7.0.

   (a) Glutamate + oxaloacetate $\xrightleftharpoons{\text{aspartate aminotransferase}}$ aspartate + α-ketoglutarate $\quad K'_{eq} = 6.8$

   (b) Dihydroxyacetone phosphate $\xrightleftharpoons{\text{triose phosphate isomerase}}$ glyceraldehyde 3-phosphate $\quad K'_{eq} = 0.0475$

   (c) Fructose 6-phosphate + ATP $\xrightleftharpoons{\text{phosphofructokinase}}$ fructose 1,6-bisphosphate + ADP $\quad K'_{eq} = 254$

   Answer

   $$\Delta G = \Delta G'^\circ + RT \ln [\text{products}]/[\text{reactants}]$$

   and [products]/[reactants] is the mass-action ratio, Q. At equilibrium, $\Delta G = 0$ and $Q = K'_{eq}$, so

   $$\Delta G'^\circ = -RT \ln K'_{eq}$$

   where $R = 8.315$ J/mol · K and $T = 25$ °C = 298 K. Using the value $RT = 2.48$ kJ/mol, we can calculate the $\Delta G'^\circ$ values from the K'_{eq} for each reaction.

   (a) $\Delta G'^\circ = -(2.48 \text{ kJ/mol}) \ln 6.8 = -4.8$ kJ/mol

   (b) $\Delta G'^\circ = -(2.48 \text{ kJ/mol}) \ln 0.0475 = 7.56$ kJ/mol

   (c) $\Delta G'^\circ = -(2.48 \text{ kJ/mol}) \ln 254 = -13.7$ kJ/mol

3. Calculation of the Equilibrium Constant from $\Delta G'^\circ$ Calculate the equilibrium constant K'_{eq} for each of the following reactions at pH 7.0 and 25 °C, using the $\Delta G'^\circ$ values in Table 13–4.

(a) Glucose 6-phosphate + $H_2O \xrightleftharpoons[\text{6-phosphatase}]{\text{glucose}}$ glucose + P_i

(b) Lactose + $H_2O \xrightleftharpoons{\beta\text{-galactosidase}}$ glucose + galactose

(c) Malate $\xrightleftharpoons{\text{fumarase}}$ fumarate + H_2O

Answer

As noted in Problem 2, $\Delta G = \Delta G'^\circ + RT \ln Q$, and at equilibrium, $Q = K'_{eq}$, $\Delta G = 0$, and

$$\Delta G'^\circ = -RT \ln K'_{eq}$$

So, at equilibrium, $\ln K'_{eq} = -\Delta G'^\circ/RT$, or $K'_{eq} = e^{-(\Delta G'^\circ/RT)}$; at 25 °C, $RT = 2.48$ kJ/mol.

From these relationships, we can calculate K'_{eq} for each reaction using the values of $\Delta G'^\circ$ in Table 13–4.

(a) For glucose 6-phosphatase:

$\Delta G'^\circ = -13.8$ kJ/mol

$\ln K'_{eq} = -(-13.8 \text{ kJ/mol})/(2.48 \text{ kJ/mol}) = 5.57$

$K'_{eq} = e^{5.57} = 262$

(b) For β-galactosidase:

$\Delta G'^\circ = -15.9$ kJ/mol

$\ln K'_{eq} = -(-15.9 \text{ kJ/mol})/(2.48 \text{ kJ/mol}) = 6.41$

$K'_{eq} = e^{6.41} = 608$

(c) For fumarase:

$\Delta G'^\circ = 3.1$ kJ/mol

$\ln K'_{eq} = -(3.1 \text{ kJ/mol})/(2.48 \text{ kJ/mol}) = -1.2$

$K'_{eq} = e^{-1.2} = 0.30$

4. Experimental Determination of K'_{eq} and $\Delta G'^\circ$ If a 0.1 M solution of glucose 1-phosphate at 25 °C is incubated with a catalytic amount of phosphoglucomutase, the glucose 1-phosphate is transformed to glucose 6-phosphate. At equilibrium, the concentrations of the reaction components are

$$\underset{4.5 \times 10^{-3}\text{ M}}{\text{Glucose 1-phosphate}} \rightleftharpoons \underset{9.6 \times 10^{-2}\text{ M}}{\text{glucose 6-phosphate}}$$

Calculate K'_{eq} and $\Delta G'^\circ$ for this reaction.

Answer

$K'_{eq} = [\text{G6P}]/[\text{G1P}] = (9.6 \times 10^{-2} \text{ M})/(4.5 \times 10^{-3} \text{ M})$

$= 21$

$\Delta G'^\circ = -RT \ln K'_{eq}$

$= -(2.48 \text{ kJ/mol})(\ln 21) = -7.6$ kJ/mol

5. Experimental Determination of $\Delta G'^\circ$ for ATP Hydrolysis A direct measurement of the standard free-energy change associated with the hydrolysis of ATP is technically demanding because the minute amount of ATP remaining at equilibrium is difficult to measure accurately. The value of $\Delta G'^\circ$ can be calculated indirectly, however, from the equilibrium constants of two other enzymatic reactions having less favorable equilibrium constants:

$$\text{Glucose 6-phosphate} + H_2O \longrightarrow \text{glucose} + P_i \qquad K'_{eq} = 270$$

$$\text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose 6-phosphate} \qquad K'_{eq} = 890$$

Using this information for equilibrium constants determined at 25 °C, calculate the standard free energy of hydrolysis of ATP.

Answer The reactions, if coupled together, constitute a "futile cycle" that results in the net hydrolysis of ATP:

(1) $\text{G6P} + H_2O \longrightarrow \text{glucose} + P_i$
(2) $\text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{G6P}$

Sum: $\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i$

Calculating from $\Delta G'^\circ = -RT \ln K'_{eq}$:

$\Delta G'^\circ_1 = (-2.48 \text{ kJ/mol})(\ln 270) = -14 \text{ kJ/mol}$
$\Delta G'^\circ_2 = (-2.48 \text{ kJ/mol})(\ln 890) = -17 \text{ kJ/mol}$
$\Delta G'^\circ_{sum} = \Delta G'^\circ_1 + \Delta G'^\circ_2 = -31 \text{ kJ/mol}$

6. Difference between $\Delta G'^\circ$ and ΔG Consider the following interconversion, which occurs in glycolysis (Chapter 14):

$$\text{Fructose 6-phosphate} \rightleftharpoons \text{glucose 6-phosphate} \qquad K'_{eq} = 1.97$$

(a) What is $\Delta G'^\circ$ for the reaction (K'_{eq} measured at 25 °C)?
(b) If the concentration of fructose 6-phosphate is adjusted to 1.5 M and that of glucose 6-phosphate is adjusted to 0.50 M, what is ΔG?
(c) Why are $\Delta G'^\circ$ and ΔG different?

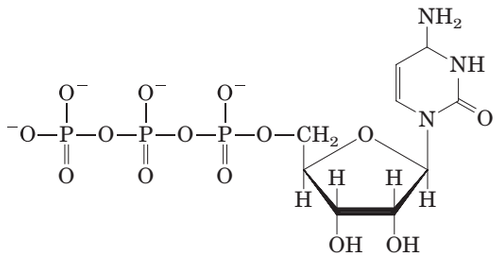
Answer

(a) At equilibrium, $\Delta G'^\circ = -RT \ln K'_{eq}$
$= -(2.48 \text{ kJ/mol}) \ln 1.97$
$= -1.68 \text{ kJ/mol}$

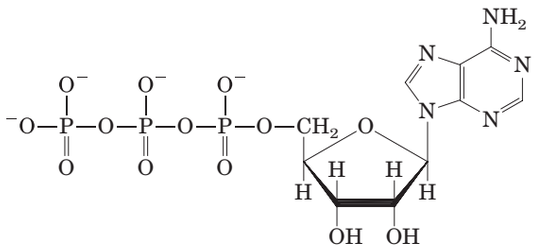
(b) $\Delta G = \Delta G'^\circ + RT \ln Q$
$Q = [\text{G6P}]/[\text{F6P}] = 0.5 \text{ M}/1.5 \text{ M} = 0.33$
$\Delta G = -1.68 \text{ kJ/mol} + (2.48 \text{ kJ/mol}) \ln 0.33$
$= -4.4 \text{ kJ/mol}$

(c) $\Delta G'^\circ$ for any reaction is a fixed parameter because it is defined for standard conditions of temperature (25 °C = 298 K) and concentration (both F6P and G6P = 1 M). In contrast, ΔG is a variable and can be calculated for any set of product and reactant concentrations. ΔG is defined as $\Delta G'^\circ$ (standard conditions) plus whatever difference occurs in ΔG on moving to nonstandard conditions.

7. Free Energy of Hydrolysis of CTP Compare the structure of the nucleoside triphosphate CTP with the structure of ATP.



Cytidine triphosphate (CTP)



Adenosine triphosphate (ATP)

Now predict the K'_{eq} and $\Delta G'^{\circ}$ for the following reaction:

$$\text{ATP} + \text{CDP} \longrightarrow \text{ADP} + \text{CTP}$$

Answer $\Delta G'^{\circ}$ near 0; K'_{eq} near 1. The high $\Delta G'^{\circ}$ of ATP is related to structural features not of the base or the sugar, but primarily of the anhydride linkages between phosphate groups. In this structural feature, CTP is equivalent to ATP, and thus it most likely has about the same $\Delta G'^{\circ}$ as ATP. If this is the case, the reaction ATP + CDP $\longrightarrow$ ADP + CTP has a $\Delta G'^{\circ}$ very close to zero, and a K'_{eq} close to 1 (see Table 13–3).

8. Dependence of ΔG on pH The free energy released by the hydrolysis of ATP under standard conditions at pH 7.0 is −30.5 kJ/mol. If ATP is hydrolyzed under standard conditions except at pH 5.0, is more or less free energy released? Explain. Use the Living Graph to explore this relationship.

Answer Less; the overall equation for ATP hydrolysis can be approximated as

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+$$

(This is only an approximation, because the ionized species shown here are the major, but not the only, forms present.) Under standard conditions (i.e., [ATP] = [ADP] = [P_i] = 1 M), the concentration of water is 55 M and does not change during the reaction. Because H^+ ions are produced in the reaction, the lower the pH at which the reaction proceeds—that is, the higher the [H^+]—the more the equilibrium shifts toward reactants. As a result, at lower pH the reaction does not proceed as far toward products, and less free energy is released.

9. The $\Delta G'^{\circ}$ for Coupled Reactions Glucose 1-phosphate is converted into fructose 6-phosphate in two successive reactions:

$$\text{Glucose 1-phosphate} \longrightarrow \text{glucose 6-phosphate}$$

$$\text{Glucose 6-phosphate} \longrightarrow \text{fructose 6-phosphate}$$

Using the $\Delta G'^{\circ}$ values in Table 13–4, calculate the equilibrium constant, K'_{eq}, for the sum of the two reactions:

$$\text{Glucose 1-phosphate} \longrightarrow \text{fructose 6-phosphate}$$

Answer

(1) G1P $\longrightarrow$ G6P $\quad \Delta G_1'^{\circ} = -7.3$ kJ/mol
(2) G6P $\longrightarrow$ F6P $\quad \Delta G_2'^{\circ} = 1.7$ kJ/mol

Sum: G1P $\longrightarrow$ F6P $\quad \Delta G'^{\circ}_{sum} = -5.6$ kJ/mol

$$\ln K'_{eq} = -\Delta G'^{\circ}/RT$$
$$= -(-5.6 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$$
$$= 2.3$$
$$K'_{eq} = 10$$

10. Effect of [ATP]/[ADP] Ratio on Free Energy of Hydrolysis of ATP Using Equation 13–4, plot ΔG against ln Q (mass-action ratio) at 25 °C for the concentrations of ATP, ADP, and P_i in the table below. $\Delta G'^{\circ}$ for the reaction is –30.5 kJ/mol. Use the resulting plot to explain why metabolism is regulated to keep the ratio [ATP]/[ADP] high.

	Concentration (mM)				
ATP	5	3	1	0.2	5
ADP	0.2	2.2	4.2	5.0	25
P_i	10	12.1	14.1	14.9	10

Answer The reaction is ATP $\longrightarrow$ ADP + P_i. From Equation 13–4, with Q (the mass action ratio) = [ADP][P_i]/[ATP], expressed as molar concentrations, the free-energy change for this reaction is:

$$\Delta G = \Delta G'^{\circ} + RT \ln ([\text{ADP}][P_i]/[\text{ATP}])$$

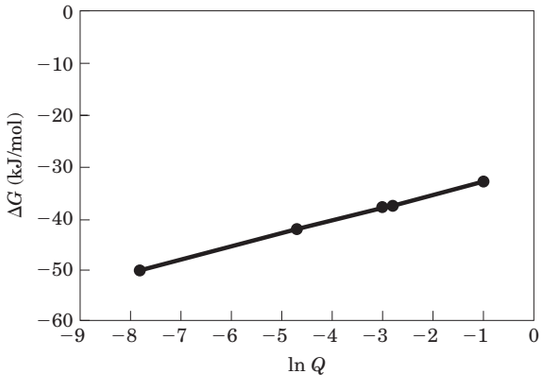
Calculate ln Q for each of the five cases:

$$\ln Q_1 = \ln [(2 \times 10^{-4})(1.0 \times 10^{-2})/(5 \times 10^{-3})] = -7.8$$
$$\ln Q_2 = \ln [(2.2 \times 10^{-3})(1.21 \times 10^{-2})/(3 \times 10^{-3})] = -4.7$$
$$\ln Q_3 = \ln [(4.2 \times 10^{-3})(1.41 \times 10^{-2})/(1 \times 10^{-3})] = -2.8$$
$$\ln Q_4 = \ln [(5.0 \times 10^{-3})(1.49 \times 10^{-2})/(2 \times 10^{-4})] = -1.0$$
$$\ln Q_5 = \ln [(2.5 \times 10^{-2})(1.0 \times 10^{-2})/(5 \times 10^{-3})] = -3.0$$

Substitute each of these values for ln Q, −30.5 kJ/mol for $\Delta G'^{\circ}$, and 2.48 kJ/mol for RT in Equation 13–4:

$$\Delta G_1 = -30.5 \text{ kJ/mol} + (2.48 \text{ kJ/mol})(-7.8) = -50 \text{ kJ/mol}$$
$$\Delta G_2 = -30.5 \text{ kJ/mol} + (2.48 \text{ kJ/mol})(-4.7) = -42 \text{ kJ/mol}$$
$$\Delta G_3 = -30.5 \text{ kJ/mol} + (2.48 \text{ kJ/mol})(-2.8) = -38 \text{ kJ/mol}$$
$$\Delta G_4 = -30.5 \text{ kJ/mol} + (2.48 \text{ kJ/mol})(-1.0) = -33 \text{ kJ/mol}$$
$$\Delta G_5 = -30.5 \text{ kJ/mol} + (2.48 \text{ kJ/mol})(-3.0) = -38 \text{ kJ/mol}$$

Now plot ΔG versus ln Q for each case:



The ΔG for ATP hydrolysis is smaller when [ATP]/[ADP] is low (<<1) than when [ATP]/[ADP] is high. The energy available to a cell from a given amount of ATP is smaller when [ATP]/[ADP] falls and greater when this ratio rises.

11. Strategy for Overcoming an Unfavorable Reaction: ATP-Dependent Chemical Coupling The phosphorylation of glucose to glucose 6-phosphate is the initial step in the catabolism of glucose. The direct phosphorylation of glucose by P_i is described by the equation

$$\text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \qquad \Delta G'^\circ = 13.8 \text{ kJ/mol}$$

(a) Calculate the equilibrium constant for the above reaction at 37 °C. In the rat hepatocyte the physiological concentrations of glucose and P_i are maintained at approximately 4.8 mM. What is the equilibrium concentration of glucose 6-phosphate obtained by the direct phosphorylation of glucose by P_i? Does this reaction represent a reasonable metabolic step for the catabolism of glucose? Explain.

(b) In principle, at least, one way to increase the concentration of glucose 6-phosphate is to drive the equilibrium reaction to the right by increasing the intracellular concentrations of glucose and P_i. Assuming a fixed concentration of P_i at 4.8 mM, how high would the intracellular concentration of glucose have to be to give an equilibrium concentration of glucose 6-phosphate of 250 μM (the normal physiological concentration)? Would this route be physiologically reasonable, given that the maximum solubility of glucose is less than 1 M?

(c) The phosphorylation of glucose in the cell is coupled to the hydrolysis of ATP; that is, part of the free energy of ATP hydrolysis is used to phosphorylate glucose:

(1) $\text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \qquad \Delta G'^\circ = 13.8 \text{ kJ/mol}$

(2) $\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i \qquad \Delta G'^\circ = -30.5 \text{ kJ/mol}$

Sum: $\text{Glucose} + \text{ATP} \longrightarrow \text{glucose 6-phosphate} + \text{ADP}$

Calculate K'_{eq} at 37 °C for the overall reaction. For the ATP-dependent phosphorylation of glucose, what concentration of glucose is needed to achieve a 250 μM intracellular concentration of glucose 6-phosphate when the concentrations of ATP and ADP are 3.38 mM and 1.32 mM, respectively? Does this coupling process provide a feasible route, at least in principle, for the phosphorylation of glucose in the cell? Explain.

(d) Although coupling ATP hydrolysis to glucose phosphorylation makes thermodynamic sense, we have not yet specified how this coupling is to take place. Given that coupling requires a common intermediate, one conceivable route is to use ATP hydrolysis to raise the intracellular concentration of P_i and thus drive the unfavorable phosphorylation of glucose by P_i. Is this a reasonable route? (Think about the solubility products of metabolic intermediates.)

(e) The ATP-coupled phosphorylation of glucose is catalyzed in hepatocytes by the enzyme glucokinase. This enzyme binds ATP and glucose to form a glucose-ATP-enzyme complex, and the phosphoryl group is transferred directly from ATP to glucose. Explain the advantages of this route.

Answer

(a) $\Delta G'^\circ = -RT \ln K'_{eq}$

$\ln K'_{eq} = -\Delta G'^\circ/RT$

$= -(13.8 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$

$K'_{eq} = e^{-5.56}$

$= 3.85 \times 10^{-3} \text{ M}^{-1}$

(Note: this value has units M^{-1} because the expression for K'_{eq} from the chemical equilibrium includes H_2O; see below.)

$$K'_{eq} = \frac{[\text{G6P}]}{[\text{Glc}][\text{P}_i]}$$

$[\text{G6P}] = K'_{eq}[\text{Glc}][\text{P}_i]$

$= (3.85 \times 10^{-3} \text{ M}^{-1})(4.8 \times 10^{-3} \text{ M})(4.8 \times 10^{-3} \text{ M})$

$= 8.9 \times 10^{-8} \text{ M}$

This would not be a reasonable route for glucose catabolism because the cellular [G6P] is likely to be much higher than 8.9×10^{-8} M, and the reaction would be unfavorable.

(b) Because $K'_{eq} = \dfrac{[\text{G6P}]}{[\text{Glc}][\text{P}_i]}$

$$\text{then } [\text{Glc}] = \frac{[\text{G6P}]}{K'_{eq}[\text{P}_i]}$$

$$= \frac{250 \times 10^{-6} \text{ M}}{(3.85 \times 10^{-3} \text{ M}^{-1})(4.8 \times 10^{-3} \text{ M})} = 14 \text{ M}$$

This would not be a reasonable route because the maximum solubility of glucose is less than 1 M.

(c) (1) $\text{Glc} + \text{P}_i \longrightarrow \text{G6P} + \text{H}_2\text{O}$ $\quad \Delta G'^\circ_1 = 13.8 \text{ kJ/mol}$

(2) $\text{ATP} + \text{H}_2\text{O} \longrightarrow \text{ADP} + \text{P}_i$ $\quad \Delta G'^\circ_2 = -30.5 \text{ kJ/mol}$

Sum: $\text{Glc} + \text{ATP} \longrightarrow \text{G6P} + \text{ADP}$ $\quad \Delta G'^\circ_{sum} = -16.7 \text{ kJ/mol}$

$\ln K'_{eq} = -\Delta G'^\circ/RT$

$= -(-16.7 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$

$= 6.73$

$K'_{eq} = 837$

$$\text{Because } K'_{eq} = \frac{[\text{G6P}][\text{ADP}]}{[\text{Glc}][\text{ATP}]}$$

$$\text{then } [\text{Glc}] = \frac{[\text{G6P}][\text{ADP}]}{K'_{eq}[\text{ATP}]}$$

$$= \frac{(250 \times 10^{-6} \text{ M})(1.32 \times 10^{-3} \text{ M})}{(837)(3.38 \times 10^{-3} \text{ M})}$$

$$= 1.2 \times 10^{-7} \text{ M}$$

This route is feasible because the glucose concentration is reasonable.

(d) No; this is not reasonable. When glucose is at its physiological level, the required P_i concentration would be so high that phosphate salts of divalent cations would precipitate out.

(e) Direct transfer of the phosphoryl group from ATP to glucose takes advantage of the high phosphoryl group transfer potential of ATP and does not demand that the concentration of intermediates be very high, unlike the mechanism proposed in **(d).** In addition, the usual benefits of enzymatic catalysis apply, including binding interactions between the enzyme and its substrates; induced fit leading to the exclusion of water from the active site, so that only glucose is phosphorylated; and stabilization of the transition state.

12. Calculations of $\Delta G'^\circ$ for ATP-Coupled Reactions From data in Table 13–6 calculate the $\Delta G'^\circ$ value for the reactions.

(a) Phosphocreatine + ADP $\longrightarrow$ creatine + ATP

(b) ATP + fructose $\longrightarrow$ ADP + fructose 6-phosphate

Answer

(a) The $\Delta G'^\circ$ value for the overall reaction is calculated from the sum of the $\Delta G'^\circ$ values for the coupled reactions.

(1)	Phosphocreatine + $H_2O \longrightarrow$ creatine + P_i	$\Delta G_1'^\circ = -43.0$ kJ/mol
(2)	ADP + $P_i \longrightarrow$ ATP + H_2O	$\Delta G_2'^\circ = 30.5$ kJ/mol
Sum:	Phosphocreatine + ADP $\longrightarrow$ creatine + ATP	$\Delta G'^\circ_{sum} = -12.5$ kJ/mol

(b)

(1)	ATP + $H_2O \longrightarrow$ ADP + P_i	$\Delta G_1'^\circ = -30.5$ kJ/mol
(2)	Fructose + $P_i \longrightarrow$ F6P + H_2O	$\Delta G_2'^\circ = 15.9$ kJ/mol
Sum:	ATP + fructose $\longrightarrow$ ADP + F6P	$\Delta G'^\circ_{sum} = -14.6$ kJ/mol

13. Coupling ATP Cleavage to an Unfavorable Reaction To explore the consequences of coupling ATP hydrolysis under physiological conditions to a thermodynamically unfavorable biochemical reaction, consider the hypothetical transformation X $\rightarrow$ Y, for which $\Delta G'^\circ = 20$ kJ/mol.

(a) What is the ratio [Y]/[X] at equilibrium?

(b) Suppose X and Y participate in a sequence of reactions during which ATP is hydrolyzed to ADP and P_i. The overall reaction is

$$X + ATP + H_2O \longrightarrow Y + ADP + P_i$$

Calculate [Y]/[X] for this reaction at equilibrium. Assume that the temperature is 25 °C and the equilibrium concentrations of ATP, ADP, and P_i are all 1 M.

(c) We know that [ATP], [ADP], and [P_i] are *not* 1 M under physiological conditions. Calculate [Y]/[X] for the ATP-coupled reaction when the values of [ATP], [ADP], and [P_i] are those found in rat myocytes (Table 13–5).

Answer

(a) The ratio $[Y]_{eq}/[X]_{eq}$ is equal to the equilibrium constant, K'_{eq}.

$$\begin{aligned} \ln K'_{eq} &= -\Delta G'^\circ/RT \\ &= -(20 \text{ kJ/mol})/(2.48 \text{ kJ/mol}) \\ &= -8 \\ K'_{eq} &= e^{-8} = 3 \times 10^{-4} = [Y]_{eq}/[X]_{eq} \end{aligned}$$

This is a very small value of K'_{eq}; consequently, $\Delta G'^\circ$ is large and positive, making the reaction energetically unfavorable as written.

(b) First, we need to calculate $\Delta G'^\circ$ for the overall reaction.

(1)	X $\longrightarrow$ Y	$\Delta G_1'^\circ = 20$ kJ/mol
(2)	ATP + $H_2O \longrightarrow$ ADP + P_i	$\Delta G_2'^\circ = -30.5$ kJ/mol
Sum:	X + ATP + $H_2O \longrightarrow$ ADP + P_i + Y	$\Delta G'^\circ_{sum} = -10.5$ kJ/mol

$$K'_{eq} = \frac{[Y]_{eq}[P_i]_{eq}[ADP]_{eq}}{[X]_{eq}[ATP]_{eq}}; \text{ note: water is omitted.}$$

Because [ADP], [ATP], and [P_i] are 1 M, this simplifies to K'_{eq} = [Y]/[X] in units of M.

$$\ln K'_{eq} = -\Delta G'^{\circ}/RT$$
$$= -(-10.5 \text{ kJ/mol})/(2.48 \text{ kJ/mol}) = 4.23$$
$$K'_{eq} = e^{4.23} = 68.7 = [Y]/[X]$$

$\Delta G'^{\circ}$ is fairly large and negative; the coupled reaction is favorable as written.

(c) Here we are dealing with the nonstandard conditions of the cell. Under physiological conditions, a favorable reaction (under standard conditions) becomes even more favorable.

$$K'_{eq} = \frac{[Y]_{eq}[P_i]_{eq}[ADP]_{eq}}{[X]_{eq}[ATP]_{eq}}$$

If we hold the values of [P_i], [ADP], and [ATP] at the values known to exist in the cell, we can calculate the values of [X] and [Y] that meet the equilibrium expression above, giving the equilibrium constant we calculated in **(b).**

$$[Y]/[X] = \frac{K'_{eq}[ATP]}{[P_i][ADP]}$$
$$= \frac{(68.7 \text{ M})(8.05 \times 10^{-3} \text{ M})}{(8.05 \times 10^{-3} \text{ M})(0.93 \times 10^{-3} \text{ M})}$$
$$= 7.4 \times 10^{4}$$

So by coupling the conversion X → Y to ATP hydrolysis, and by holding [ATP], [ADP], and [P_i] far from their equilibrium levels, the cell can greatly increase the ratio [product]/[reactant]; the reaction goes essentially to completion.

14. Calculations of Δ*G* at Physiological Concentrations Calculate the actual, physiological ΔG for the reaction

$$\text{Phosphocreatine} + \text{ADP} \longrightarrow \text{creatine} + \text{ATP}$$

at 37 °C, as it occurs in the cytosol of neurons, with phosphocreatine at 4.7 mM, creatine at 1.0 mM, ADP at 0.73 mM, and ATP at 2.6 mM.

Answer

Using $\Delta G'^{\circ}$ values from Table 13–6:

(1)	Phosphocreatine + $H_2O \longrightarrow$ creatine + P_i	$\Delta G'^{\circ}_1 = -43.0$ kJ/mol
(2)	ADP + $P_i \longrightarrow$ ATP + H_2O	$\Delta G'^{\circ}_2 = 30.5$ kJ/mol
Sum:	Phosphocreatine = ADP $\longrightarrow$ creatine + ATP	$\Delta G'^{\circ}_{sum} = -12.5$ kJ/mol

$$\text{Mass-action ratio, } Q = \frac{[\text{products}]}{[\text{reactants}]} = \frac{[\text{creatine}][\text{ATP}]}{[\text{phosphocreatine}][\text{ADP}]}$$
$$= \frac{(1 \times 10^{-3} \text{ M})(2.6 \times 10^{-3} \text{ M})}{(4.7 \times 10^{-3} \text{ M})(7.3 \times 10^{-4} \text{ M})}$$
$$= 0.75$$
$$\Delta G = \Delta G'^{\circ} + RT \ln Q$$
$$= -12.5 \text{ kJ/mol} + (8.315 \text{ J/mol} \cdot \text{K})(310 \text{ K}) \ln 0.75$$
$$= -13 \text{ kJ/mol}$$

15. Free Energy Required for ATP Synthesis under Physiological Conditions In the cytosol of rat hepatocytes, the temperature is 37 °C and the mass-action ratio, Q, is

$$\frac{[\text{ATP}]}{[\text{ADP}][\text{P}_\text{i}]} = 5.33 \times 10^2\ \text{M}^{-1}$$

Calculate the free energy required to synthesize ATP in a rat hepatocyte.

Answer The reaction for the synthesis of ATP is

$$\text{ADP} + \text{P}_\text{i} \longrightarrow \text{ATP} + \text{H}_2\text{O} \qquad \Delta G'^\circ = 30.5\ \text{kJ/mol}$$

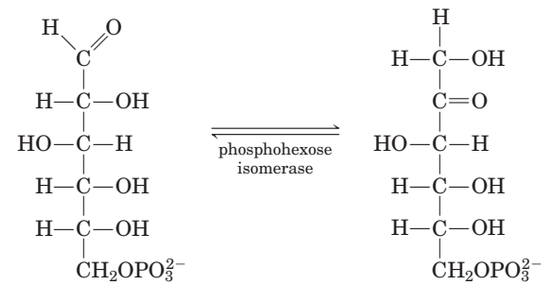
The mass-action ratio is

$$\frac{[\text{products}]}{[\text{reactants}]} = \frac{[\text{ATP}]}{[\text{P}_\text{i}][\text{ADP}]} = 5.33 \times 10^2\ \text{M}^{-1}$$

Because $\Delta G = \Delta G'^\circ + RT \ln [\text{products}]/[\text{reactants}]$,

$$\begin{aligned}\Delta G &= 30.5\ \text{kJ/mol} + (8.315\ \text{J/mol} \cdot \text{K})(310\ \text{K}) \ln 5.33 \times 10^2\ \text{M}^{-1} \\ &= 46.7\ \text{kJ/mol}\end{aligned}$$

16. Chemical Logic In the glycolytic pathway, a six-carbon sugar (fructose 1,6-bisphosphate) is cleaved to form two three-carbon sugars, which undergo further metabolism (see Fig. 14–5). In this pathway, an isomerization of glucose 6-phosphate to fructose 6-phosphate (shown below) occurs two steps before the cleavage reaction (the intervening step is phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate (p. 532)).

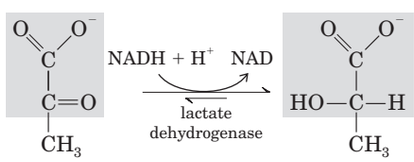


Glucose 6-phosphate — Fructose 6-phosphate

What does the isomerization step accomplish from a chemical perspective? (Hint: Consider what might happen if the C—C bond cleavage were to proceed without the preceding isomerization.)

Answer C—C bond cleavage is facilitated by the presence of a carbonyl group one carbon removed from the bond being cleaved. Isomerization moves the carbonyl group from C-1 to C-2, setting up a carbon–carbon bond cleavage between C-3 and C-4. Without isomerization, bond cleavage would occur between C-2 and C-3, generating one two-carbon and one four-carbon compound.

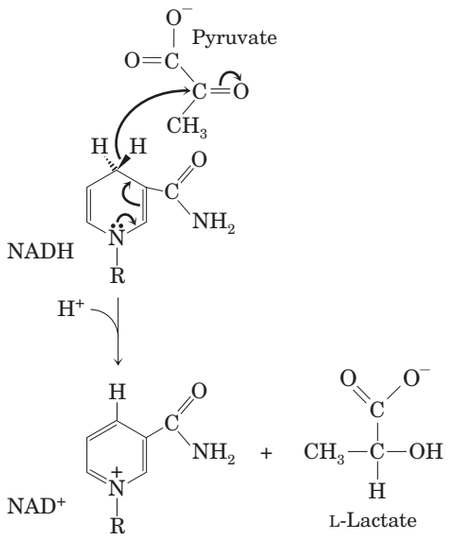
17. Enzymatic Reaction Mechanisms I Lactate dehydrogenase is one of the many enzymes that require NADH as coenzyme. It catalyzes the conversion of pyruvate to lactate:



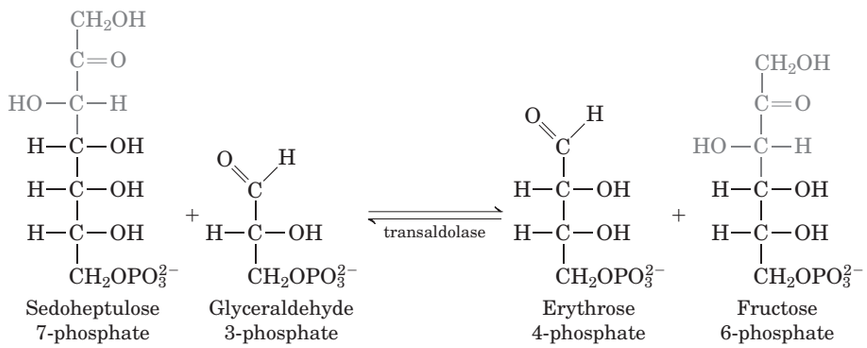
Pyruvate — L-Lactate

Draw the mechanism of this reaction (show electron-pushing arrows). (Hint: This is a common reaction throughout metabolism; the mechanism is similar to that catalyzed by other dehydrogenases that use NADH, such as alcohol dehydrogenase.)

Answer The mechanism is the same as that of the alcohol dehydrogenase reaction (Fig. 14–13, p. 547).

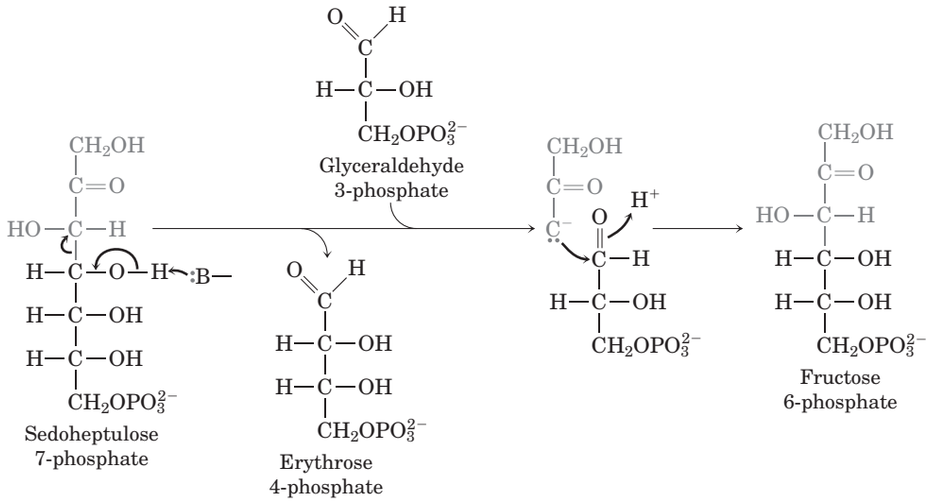


18. Enzymatic Reaction Mechanisms II Biochemical reactions often look more complex than they really are. In the pentose phosphate pathway (Chapter 14), sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate react to form erythrose 4-phosphate and fructose 6-phosphate in a reaction catalyzed by transaldolase.



Draw a mechanism for this reaction (show electron-pushing arrows). (Hint: Take another look at aldol condensations, then consider the name of this enzyme.)

Answer The first step is the reverse of an aldol condensation (see the aldolase mechanism, Fig. 14–5, p. 534); the second step is an aldol condensation (see Fig. 13–4, p. 497).



19. Daily ATP Utilization by Human Adults

(a) A total of 30.5 kJ/mol of free energy is needed to synthesize ATP from ADP and P_i when the reactants and products are at 1 M concentrations and the temperature is 25 °C (standard state). Because the actual physiological concentrations of ATP, ADP, and P_i are not 1 M, and the temperature is 37 °C, the free energy required to synthesize ATP under physiological conditions is different from $\Delta G'°$. Calculate the free energy required to synthesize ATP in the human hepatocyte when the physiological concentrations of ATP, ADP, and P_i are 3.5, 1.50, and 5.0 mM, respectively.

(b) A 68 kg (150 lb) adult requires a caloric intake of 2,000 kcal (8,360 kJ) of food per day (24 hours). The food is metabolized and the free energy is used to synthesize ATP, which then provides energy for the body's daily chemical and mechanical work. Assuming that the efficiency of converting food energy into ATP is 50%, calculate the weight of ATP used by a human adult in 24 hours. What percentage of the body weight does this represent?

(c) Although adults synthesize large amounts of ATP daily, their body weight, structure, and composition do not change significantly during this period. Explain this apparent contradiction.

Answer

(a) $ADP + P_i \longrightarrow ATP + H_2O \qquad \Delta G'° = 30.5 \text{ kJ/mol}$

$$\text{Mass action ratio, } Q = \frac{[ATP]}{[P_i][ADP]} = \frac{[3.5 \times 10^{-3}\ \text{M}]}{[1.5 \times 10^{-3}\ \text{M}]\,[5.0 \times 10^{-3}\ \text{M}]} = 4.7 \times 10^{2}\ \text{M}^{-1}$$

$$\begin{aligned}\Delta G &= \Delta G'° + RT \ln Q \\ &= 30.5 \text{ kJ/mol} + (2.58 \text{ kJ/mol}) \ln (4.7 \times 10^{2}\ \text{M}^{-1}) \\ &= 46 \text{ kJ/mol}\end{aligned}$$

(b) The energy going into ATP synthesis in 24 hr is 8,360 kJ × 50% = 4,180 kJ. Using the value of ΔG from **(a),** the amount of ATP synthesized is

$$(4{,}180 \text{ kJ})/(46 \text{ kJ/mol}) = 91 \text{ mol}$$

The molecular weight of ATP is 503 (calculated by summing atomic weights). Thus, the weight of ATP synthesized is

$$(91 \text{ mol ATP})(503 \text{ g/mol}) = 46 \text{ kg}$$

As a percentage of body weight:

$$100\%\,(46 \text{ kg ATP})/(68 \text{ kg body weight}) = 68\%$$

(c) The concentration of ATP in a healthy body is maintained in a steady state; this is an example of homeostasis, a condition in which the body synthesizes and breaks down ATP as needed.

20. Rates of Turnover of γ and β Phosphates of ATP If a small amount of ATP labeled with radioactive phosphorus in the terminal position, [γ-^{32}P]ATP, is added to a yeast extract, about half of the ^{32}P activity is found in P_i within a few minutes, but the concentration of ATP remains unchanged. Explain. If the same experiment is carried out using ATP labeled with ^{32}P in the central position, [β-^{32}P]ATP, the ^{32}P does not appear in P_i within such a short time. Why?

Answer We can represent ATP as A-P-P-P (the P farthest from A is the γ P) and a radiolabeled phosphate group as *P. One possible reaction for γ-labeled ATP would be phosphorylation of glucose:

$$\text{A-P-P-*P + Glc} \longrightarrow \text{A-P-P + G6*P} \rightarrow \rightarrow \rightarrow \text{*P}_i$$

or, more generally:

$$\text{A-P-P-*P + H}_2\text{O} \longrightarrow \text{A-P-P + *P}_i$$

The ATP system is in a dynamic steady state; [ATP] remains constant because the rate of ATP consumption, as depicted above, equals its rate of synthesis. ATP consumption involves release of the terminal (γ) phosphoryl group; synthesis of ATP from ADP involves replacement of this phosphoryl group. Hence, the terminal phosphate undergoes rapid turnover.

The reaction

$$\text{A-P-*P-P + H}_2\text{O} \longrightarrow \text{A-P + *P}_i + \text{P}_i$$

occurs more slowly: the central (β) phosphate undergoes only relatively slow turnover.

21. Cleavage of ATP to AMP and PP_i during Metabolism Synthesis of the activated form of acetate (acetyl-CoA) is carried out in an ATP-dependent process:

$$\text{Acetate + CoA + ATP} \longrightarrow \text{acetyl-CoA + AMP + PP}_i$$

(a) The $\Delta G'^\circ$ for the hydrolysis of acetyl-CoA to acetate and CoA is -32.2 kJ/mol and that for hydrolysis of ATP to AMP and PP_i is -30.5 kJ/mol. Calculate $\Delta G'^\circ$ for the ATP-dependent synthesis of acetyl-CoA.

(b) Almost all cells contain the enzyme inorganic pyrophosphatase, which catalyzes the hydrolysis of PP_i to P_i. What effect does the presence of this enzyme have on the synthesis of acetyl-CoA? Explain.

Answer

(a) The $\Delta G'^\circ$ can be determined for the coupled reactions:

(1)	$\text{Acetate + CoA} \longrightarrow \text{acetyl-CoA + H}_2\text{O}$	$\Delta G_1'^\circ = 32.2$ kJ/mol
(2)	$\text{ATP + H}_2\text{O} \longrightarrow \text{AMP + PP}_i$	$\Delta G_2'^\circ = -30.5$ kJ/mol
Sum:	$\text{Acetate + CoA + ATP} \longrightarrow \text{acetyl-CoA + AMP + PP}_i$	$\Delta G'^\circ_{\text{sum}} = 1.7$ kJ/mol

(b) Hydrolysis of PP_i would drive the reaction forward, favoring the synthesis of acetyl-CoA.

22. Energy for H^+ Pumping The parietal cells of the stomach lining contain membrane "pumps" that transport hydrogen ions from the cytosol (pH 7.0) into the stomach, contributing to the acidity of gastric juice (pH 1.0). Calculate the free energy required to transport 1 mol of hydrogen ions through these pumps. (Hint: see Chapter 11.) Assume a temperature of 37 °C.

Answer The free energy required to transport 1 mol of H^+ from the interior of the cell, where $[H^+]$ is 10^{-7} M, across the membrane to where $[H^+]$ is 10^{-1} M is

$$\begin{aligned}\Delta G_t &= RT \ln (C_2/C_1)\\ &= RT \ln (10^{-1}/10^{-7})\\ &= (8.315 \text{ J/mol} \cdot \text{K})(310 \text{ K}) \ln 10^6\\ &= 36 \text{ kJ/mol}\end{aligned}$$

23. Standard Reduction Potentials The standard reduction potential, $E'°$, of any redox pair is defined for the half-cell reaction:

$$\text{Oxidizing agent} + n \text{ electrons} \longrightarrow \text{reducing agent}$$

The $E'°$ values for the NAD^+/NADH and pyruvate/lactate conjugate redox pairs are -0.32 V and -0.19 V, respectively.

(a) Which redox pair has the greater tendency to lose electrons? Explain.

(b) Which pair is the stronger oxidizing agent? Explain.

(c) Beginning with 1 M concentrations of each reactant and product at pH 7 and 25 °C, in which direction will the following reaction proceed?

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+$$

(d) What is the standard free-energy change ($\Delta G'°$) for the conversion of pyruvate to lactate?

(e) What is the equilibrium constant (K'_{eq}) for this reaction?

Answer

(a) The NAD^+/NADH pair is more likely to lose electrons. The equations in Table 13–7 are written in the direction of reduction (gain of electrons). $E'°$ is positive if the oxidized member of a conjugate pair has a tendency to accept electrons. $E'°$ is negative if the oxidized member of a conjugate pair does *not* have a tendency to accept electrons. Both NAD^+/NADH and pyruvate/lactate have negative $E'°$ values. The $E'°$ of NAD^+/NADH (-0.0320 V) is more negative than that for pyruvate/lactate (-0.185 V), so this pair has the greater tendency to accept electrons and is thus the stronger oxidizing system.

(b) The pyruvate/lactate pair is the more likely to accept electrons and thus is the stronger oxidizing agent. For the same reason that NADH tends to donate electrons to pyruvate, pyruvate tends to accept electrons from NADH. Pyruvate is reduced to lactate; NADH is oxidized to NAD^+. Pyruvate is the oxidizing agent; NADH is the reducing agent.

(c) From the answers to **(a)** and **(b),** it is evident that the reaction will tend to go in the direction of lactate formation.

(d) The first step is to calculate $\Delta E'°$ for the reaction, using the $E'°$ values in Table 13–7. Recall that, by convention, $\Delta E'° = (E'° \text{ of electron acceptor}) - (E'° \text{ of electron donor})$. For

$$\text{NADH} + \text{pyruvate} \longrightarrow NAD^+ + \text{lactate}$$

$$\begin{aligned}\Delta E'° &= (E'° \text{ for pyruvate/lactate}) - (E'° \text{ for } NAD^+/\text{NADH})\\ &= -0.185 \text{ V} - (-0.320 \text{ V}) = 0.135 \text{ V}\\ \Delta G'° &= -n\mathcal{F}\,\Delta E'°\\ &= -2(96.5 \text{ kJ/V} \cdot \text{mol})(0.135 \text{ V})\\ &= -26.1 \text{ kJ/mol}\end{aligned}$$

(e) $\ln K'_{eq} = -\Delta G'^{\circ}/RT$

$= -(-26.1 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$

$= -10.5$

$K'_{eq} = e^{10.5} = 3.63 \times 10^4$

24. Energy Span of the Respiratory Chain Electron transfer in the mitochondrial respiratory chain may be represented by the net reaction equation

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons H_2O + NAD^+$$

(a) Calculate $\Delta E'^{\circ}$ for the net reaction of mitochondrial electron transfer. Use E'° values from Table 13–7.

(b) Calculate $\Delta G'^{\circ}$ for this reaction.

(c) How many ATP molecules can *theoretically* be generated by this reaction if the free energy of ATP synthesis under cellular conditions is 52 kJ/mol?

Answer

(a) Using E'° values from Table 13–7:

For

$$NADH + H^+ + \tfrac{1}{2}O_2 \longrightarrow H_2O + NAD^+$$

$\Delta E'^{\circ} = (E'^{\circ} \text{ for } \frac{1}{2}O_2/H_2O) - (E'^{\circ} \text{ for } NAD^+/NADH)$

$= 0.816 \text{ V} - (-0.320 \text{ V}) = 1.14 \text{ V}$

(b) $\Delta G'^{\circ} = -n\mathcal{F}\,\Delta E'^{\circ}$

$= -2(96.5 \text{ kJ/V} \cdot \text{mol})(1.14 \text{ V})$

$= -220 \text{ kJ/mol}$

(c) For ATP synthesis, the reaction is

$$ADP + P_i \longrightarrow ATP$$

The free energy required for this reaction in the cell is 52 kJ/mol. Thus, the number of ATP molecules that could, in theory, be generated is

$$\frac{220 \text{ kJ/mol}}{52 \text{ kJ/mol}} = 4.2 \approx 4$$

25. Dependence of Electromotive Force on Concentrations Calculate the electromotive force (in volts) registered by an electrode immersed in a solution containing the following mixtures of NAD^+ and NADH at pH 7.0 and 25 °C, with reference to a half-cell of E'° 0.00 V.

(a) 1.0 mM NAD^+ and 10 mM NADH

(b) 1.0 mM NAD^+ and 1.0 mM NADH

(c) 10 mM NAD^+ and 1.0 mM NADH

Answer The relevant equation for calculating E for this system is

$$E = E'^{\circ} + \frac{RT}{n\mathcal{F}} \ln \frac{[NAD^+]}{[NADH]}$$

At 25 °C, the $RT/n\mathcal{F}$ term simplifies to 0.026 V/n.

(a) From Table 13–7, E'° for the NAD^+/NADH redox pair is −0.320 V. Because two electrons are transferred, $n = 2$. Thus,

$E = (-0.320 \text{ V}) + (0.026 \text{ V}/2) \ln (1 \times 10^{-3})/(10 \times 10^{-3})$

$= -0.320 \text{ V} + (-0.03 \text{ V}) = -0.35 \text{ V}$

(b) The conditions specified here are "standard conditions," so we expect that $E = E'°$. As proof, we know that $\ln 1 = 0$, so under standard conditions the term $[(0.026\ \text{V}/n) \ln 1] = 0$, and $E = E'° = -0.320$ V.

(c) Here the concentration of NAD^+ (the electron acceptor) is 10 times that of NADH (the electron donor). This affects the value of E:

$$E = (-0.320\ \text{V}) + (0.026/2\ \text{V}) \ln (10 \times 10^{-3})/(1 \times 10^{-3})$$
$$= -0.320\ \text{V} + 0.03\ \text{V} = -0.29\ \text{V}$$

26. Electron Affinity of Compounds List the following in order of increasing tendency to accept electrons: (a), α-ketoglutarate + CO_2 (yielding isocitrate); (b), oxaloacetate; (c), O_2; (d), $NADP^+$.

Answer To solve this problem, first write the half-reactions as in Table 13–7, and then find the value for $E'°$ for each. Pay attention to the sign!

Half-reaction	$E'°$ (V)
(a) α-Ketoglutarate + CO_2 + $2H^+$ + $2e^-$ ⟶ isocitrate	−0.38
(b) Oxaloacetate + $2H^+$ + $2e^-$ ⟶ malate	−0.166
(c) $\frac{1}{2}O_2$ + $2H^+$ + $2e^-$ ⟶ H_2O	+0.816
(d) $NADP^+$ + H^+ + $2e^-$ ⟶ NADPH	−0.324

The more positive the $E'°$, the more likely the substance will accept electrons; thus, we can list the substances in order of increasing tendency to accept electrons: (a), (d), (b), (c).

27. Direction of Oxidation-Reduction Reactions Which of the following reactions would you expect to proceed in the direction shown, under standard conditions, assuming that the appropriate enzymes are present to catalyze them?

(a) Malate + NAD^+ ⟶ oxaloacetate + NADH + H^+

(b) Acetoacetate + NADH + H^+ ⟶ β-hydroxybutyrate + NAD^+

(c) Pyruvate + NADH + H^+ ⟶ lactate + NAD^+

(d) Pyruvate + β-hydroxybutyrate ⟶ lactate + acetoacetate

(e) Malate + pyruvate ⟶ oxaloacetate + lactate

(f) Acetaldehyde + succinate ⟶ ethanol + fumarate

Answer It is important to note that standard conditions do not exist in the cell. The value of $\Delta E'°$, as calculated in this problem, gives an indication of whether a reaction would or would not occur in a cell without additional energy being added (usually from ATP); but $\Delta E'°$ does not tell the entire story. The actual cellular concentrations of the electron donors and electron acceptors contribute significantly to the value of $E'°$ (e.g., see Problem 25). Under nonstandard conditions, the potential can either add to an already favorable $\Delta E'°$ or be such a large positive number as to "overwhelm" an unfavorable $\Delta E'°$, making ΔE favorable.

To solve this problem, calculate the $\Delta E'°$ for each reaction. $\Delta E'° = (E'°$ of electron acceptor in the reaction$) - (E'°$ of electron donor in the reaction). Use $E'°$ values in Table 13–7.

(a) Not favorable.

$$\Delta E'° = (E'° \text{ for oxaloacetate/malate}) - (E'° \text{ for } NAD^+/\text{NADH})$$
$$= -0.320\ \text{V} - (-0.166\ \text{V})$$
$$= -0.154\ \text{V}$$

(b) Not favorable.

$$\begin{aligned}\Delta E'^\circ &= (E'^\circ \text{ for acetoacetate/}\beta\text{-hydroxybutyrate}) - (E'^\circ \text{ for } NAD^+/NADH)\\ &= (-0.346\ \text{V}) - (-0.320\ \text{V})\\ &= -0.026\ \text{V}\end{aligned}$$

(c) Favorable.

$$\begin{aligned}\Delta E'^\circ &= (E'^\circ \text{ for pyruvate/lactate}) - (E'^\circ \text{ for } NAD^+/NADH)\\ &= -0.185\ \text{V} - (-0.320\ \text{V})\\ &= 0.135\ \text{V}\end{aligned}$$

(d) Favorable.

$$\begin{aligned}\Delta E'^\circ &= (E'^\circ \text{ for pyruvate/lactate}) - (E'^\circ \text{ for acetoacetate/}\beta\text{-hydroxybutyrate})\\ &= -0.185\ \text{V} - (-0.346\ \text{V})\\ &= 0.161\ \text{V}\end{aligned}$$

(e) Not favorable.

$$\begin{aligned}\Delta E'^\circ &= (E'^\circ \text{ for pyruvate/lactate}) - (E'^\circ \text{ for oxaloacetate/malate})\\ &= -0.185\ \text{V} - (-0.166\ \text{V})\\ &= -0.019\ \text{V}\end{aligned}$$

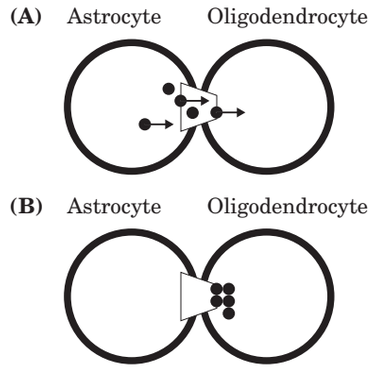
(f) Not favorable.

$$\begin{aligned}\Delta E'^\circ &= (E'^\circ \text{ for acetaldehyde/ethanol}) - (E'^\circ \text{for fumarate/succinate})\\ &= -0.197\ \text{V} - (+0.031\ \text{V})\\ &= -0.228\ \text{V}\end{aligned}$$

Data Analysis Problem

28. Thermodynamics Can Be Tricky Thermodynamics is a challenging area of study and one with many opportunities for confusion. An interesting example is found in an article by Robinson, Hampson, Munro, and Vaney, published in *Science* in 1993. Robinson and colleagues studied the movement of small molecules between neighboring cells of the nervous system through cell-to-cell channels (gap junctions). They found that the dyes Lucifer yellow (a small, negatively charged molecule) and biocytin (a small zwitterionic molecule) moved in only one direction between two particular types of glia (nonneuronal cells of the nervous system). Dye injected into astrocytes would rapidly pass into adjacent astrocytes, oligodendrocytes, or Müller cells, but dye injected into oligodendrocytes or Müller cells passed slowly if at all into astrocytes. All of these cell types are connected by gap junctions.

Although it was not a central point of their article, the authors presented a molecular model for how this unidirectional transport might occur, as shown in their Figure 3:



The figure legend reads: "Model of the unidirectional diffusion of dye between coupled oligodendrocytes and astrocytes, based on differences in connection pore diameter. Like a fish in a fish trap, dye molecules (black circles) can pass from an astrocyte to an oligodendrocyte (A) but not back in the other direction (B)."

Although this article clearly passed review at a well-respected journal, several letters to the editor (1994) followed, showing that Robinson and coauthors' model violated the second law of thermodynamics.

(a) Explain how the model violates the second law. Hint: Consider what would happen to the entropy of the system if one started with equal concentrations of dye in the astrocyte and oligodendrocyte connected by the "fish trap" type of gap junctions.

(b) Explain why this model cannot work for small molecules, although it may allow one to catch fish.

(c) Explain why a fish trap *does* work for fish.

(d) Provide two plausible mechanisms for the unidirectional transport of dye molecules between the cells that do not violate the second law of thermodynamics.

Answer

(a) The lowest-energy, highest-entropy state occurs when the dye concentration is the same in both cells. If a "fish trap" gap junction allowed unidirectional transport, more of the dye would end up in the oligodendrocyte and less in the astrocyte. This would be a higher-energy, lower-entropy state than the starting state, violating the second law of thermodynamics. Robinson et al.'s model requires an impossible spontaneous decrease in entropy. In terms of energy, the model entails a spontaneous change from a lower-energy to a higher-energy state without an energy input—again, thermodynamically impossible.

(b) Molecules, unlike fish, do not exhibit *directed behavior*; they move randomly by Brownian motion. Diffusion results in *net* movement of molecules from a region of higher concentration to a region of lower concentration simply because it is more likely that a molecule on the high-concentration side will enter the connecting channel. Look at this as a pathway with a rate-limiting step: the narrow end of the channel. The narrower end limits the rate at which molecules pass through because random motion of the molecules is less likely to move them through the smaller cross section. The wide end of the channel does *not* act like a funnel for molecules, although it may for fish, because molecules are not "crowded" by the sides of the narrowing funnel as fish would be. The narrow end limits the rate of movement equally in both directions. When the concentrations on both sides are equal, the rates of movement in both directions are equal and there will be no change in concentration.

(c) Fish exhibit *nonrandom behavior*, adjusting their actions in response to the environment. Fish that enter the large opening of the channel tend to move forward because fish have behavior that tends to make them prefer forward movement, and they experience "crowding" as they move through the narrowing channel. It is easy for fish to enter the large opening, but they don't move out of the trap as readily because they are less likely to enter the small opening.

(d) There are many possible explanations, some of which were proposed by the letter-writers who criticized the article. Here are two. (1) *The dye could bind to a molecule in the oligodendrocyte*. Binding effectively removes the dye from the bulk solvent, so it doesn't "count" as a solute for thermodynamic considerations yet remains visible in the fluorescence microscope. (2) *The dye could be sequestered in a subcellular organelle of the oligodendrocyte*, either actively pumped at the expense of ATP or drawn in by its attraction to other molecules in that organelle.

References

Letters to the editor. (1994) *Science* **265,** 1017–1019.

Robinson, S.R., Hampson, E.C.G.M., Munro, M.N., & Vaney, D.I. (1993) Unidirectional coupling of gap junctions between neuroglia. *Science* **262,** 1072–1074.

chapter

14 Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway

1. Equation for the Preparatory Phase of Glycolysis Write balanced biochemical equations for all the reactions in the catabolism of glucose to two molecules of glyceraldehyde 3-phosphate (the preparatory phase of glycolysis), including the standard free-energy change for each reaction. Then write the overall or net equation for the preparatory phase of glycolysis, with the net standard free-energy change.

Answer The initial phase of glycolysis requires ATP; it is endergonic. There are five reactions in this phase:

1. Glucose + ATP ⟶ glucose 6-phosphate + ADP $\Delta G'^\circ = -16.7$ kJ/mol

2. Glucose 6-phosphate ⟶ fructose 6-phosphate $\Delta G'^\circ = 1.7$ kJ/mol

3. Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate $\Delta G'^\circ = 14.2$ kJ/mol

4. Fructose 1,6-bisphosphate ⟶ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate $\Delta G'^\circ = 23.8$ kJ/mol

5. Dihydroxyacetone phosphate ⟶ glyceraldehyde 3-phosphate $\Delta G'^\circ = 7.5$ kJ/mol

The net equation for this phase is

$$\text{Glucose} + 2\text{ATP} \longrightarrow 2 \text{ glyceraldehyde 3-phosphate} + 2\text{ADP} + 2\text{H}^+$$

The overall standard free-energy change can be calculated by summing the individual reactions: $\Delta G'^\circ = 2.1$ kJ/mol (endergonic).

2. The Payoff Phase of Glycolysis in Skeletal Muscle In working skeletal muscle under anaerobic conditions, glyceraldehyde 3-phosphate is converted to pyruvate (the payoff phase of glycolysis), and the pyruvate is reduced to lactate. Write balanced biochemical equations for all the reactions in this process, with the standard free-energy change for each reaction. Then write the overall or net equation for the payoff phase of glycolysis (with lactate as the end product), including the net standard free-energy change.

Answer The payoff phase of glycolysis produces ATP, and thus is exergonic. This phase consists of five reactions, designated 6 to 10 in the text:

6. Glyceraldehyde 3-phosphate + P_i + NAD^+ ⟶ 1,3-bisphosphoglycerate + NADH + H^+ $\Delta G'^\circ = 6.3$ kJ/mol

7. 1,3-Bisphosphoglycerate + ADP ⟶ 3-phosphoglycerate + ATP $\Delta G'^\circ = -185$ kJ/mol

8. 3-Phosphoglycerate ⟶ 2-phosphoglycerate $\Delta G'^\circ = 4.4$ kJ/mol

9. 2-Phosphoglycerate ⟶ phosphoenolpyruvate $\Delta G'^\circ = 7.5$ kJ/mol

10. Phosphoenolpyruvate + ADP ⟶ pyruvate + ATP $\Delta G'^\circ = -31.4$ kJ/mol

The pyruvate is then converted to lactate:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \longrightarrow \text{lactate} + \text{NAD}^+ \qquad \Delta G'^\circ = -25.1 \text{ kJ/mol}$$

The net equation is

$$\text{Glyceraldehyde 3-phosphate} + 2\text{ADP} + \text{P}_\text{i} \longrightarrow \text{lactate} + \text{NAD}^+ \qquad \Delta G'^\circ = -57 \text{ kJ/mol}$$

Because the payoff phase uses two glyceraldehyde 3-phosphate molecules from each glucose entering glycolysis, the net equation is

$$2 \text{ Glyceraldehyde 3-phosphate} + 4\text{ADP} + 2\text{P}_\text{i} \longrightarrow 2 \text{ lactate} + 2\text{NAD}^+$$

and the energetic payoff for the net reaction is $\Delta G'^\circ = -114$ kJ/mol.

3. GLUT Transporters Compare the localization of GLUT4 with that of GLUT2 and GLUT3, and explain why these localizations are important in the response of muscle, adipose tissue, brain, and liver to insulin.

Answer GLUT2 (and GLUT1) is found in liver and is always present in the plasma membrane of hepatocytes. GLUT3 is always present in the plasma membrane of certain brain cells. GLUT4 is normally sequestered in vesicles in cells of muscle and adipose tissue and enters the plasma membrane only in response to insulin. Thus, liver and brain can take up glucose from blood regardless of insulin level, but muscle and adipose tissue take up glucose only when insulin levels are elevated in response to high blood glucose.

4. Ethanol Production in Yeast When grown anaerobically on glucose, yeast (*S. cerevisiae*) converts pyruvate to acetaldehyde, then reduces acetaldehyde to ethanol using electrons from NADH. Write the equation for the second reaction, and calculate its equilibrium constant at 25 °C, given the standard reduction potentials in Table 13–7.

Answer

$$\underset{\text{Acetaldehyde}}{\text{CH}_3\text{CHO}} + \text{NADH} + \text{H}^+ \rightleftharpoons \underset{\text{Ethanol}}{\text{CH}_3\text{CH}_2\text{OH}} + \text{NAD}^+$$

Solve for K'_{eq} using the E'° values in Table 13–7 and Equations 13–3 and 13–7.

$$\Delta G'^\circ = -RT\ln K'_{eq}$$

$$\Delta G'^\circ = -n\mathcal{F}\Delta E'^\circ$$

$$RT\ln K'_{eq} = n\mathcal{F}\Delta E'^\circ$$

$$\ln K'_{eq} = \frac{n\mathcal{F}\Delta E'^\circ}{RT}$$

In this reaction, $n = 2$, and $\Delta E'^\circ = 0.123$ V (calculated from values in Table 13–7 as shown in Worked Example 13–3). Substitute the standard values for the faraday and R, and 298 K for the temperature:

$$\ln K'_{eq} = \frac{2(96{,}480 \text{ J/V} \cdot \text{mol})(0.123 \text{ V})}{(8.315 \text{ J/mol} \cdot \text{K})(298 \text{ K})} = 9.58$$

$$K'_{eq} = e^{9.58} = 1.45 \times 10^4$$

5. Energetics of the Aldolase Reaction Aldolase catalyzes the glycolytic reaction

Fructose 1,6-bisphosphate $\longrightarrow$ glyceraldehyde 3-phosphate + dihydroxyacetone phosphate

The standard free-energy change for this reaction in the direction written is +23.8 kJ/mol. The concentrations of the three intermediates in the hepatocyte of a mammal are: fructose 1,6-bisphosphate, 1.4×10^{-5} M; glyceraldehyde 3-phosphate, 3×10^{-6} M; and dihydroxyacetone phosphate, 1.6×10^{-5} M. At body temperature (37 °C), what is the actual free-energy change for the reaction?

Answer For this reaction, $\Delta G' = \Delta G'^{\circ} + RT \ln$ [glyceraldehyde 3-phosphate][dihydroxyacetone phosphate]/[fructose 1,6-bisphosphate]:

$$\Delta G' = 23.8 \text{ kJ/mol} + (8.315 \times 10^{-3} \text{ kJ/mol} \cdot \text{K})\ (310 \text{ K}) \ln [(3 \times 10^{-6})(1.6 \times 10^{-5})/(1.4 \times 10^{-5})]$$
$$= 23.8 \text{ kJ/mol} + (2.578 \text{ kJ/mol}) \ln (3.43 \times 10^{-6})$$
$$= 23.8 \text{ kJ/mol} + (2.578 \text{ kJ/mol})\ (-12.58)$$
$$= 23.8 \text{ kJ/mol} + (-32.4 \text{ kJ/mol}) = -\ 8.6 \text{ kJ/mol}$$

6. Pathway of Atoms in Fermentation A "pulse-chase" experiment using ^{14}C-labeled carbon sources is carried out on a yeast extract maintained under strictly anaerobic conditions to produce ethanol. The experiment consists of incubating a small amount of ^{14}C-labeled substrate (the pulse) with the yeast extract just long enough for each intermediate in the fermentation pathway to become labeled. The label is then "chased" through the pathway by the addition of excess unlabeled glucose. The chase effectively prevents any further entry of labeled glucose into the pathway.

(a) If [1-^{14}C]glucose (glucose labeled at C-1 with ^{14}C) is used as a substrate, what is the location of ^{14}C in the product ethanol? Explain.

(b) Where would ^{14}C have to be located in the starting glucose to ensure that all the ^{14}C activity is liberated as $^{14}CO_2$ during fermentation to ethanol? Explain.

Answer Anaerobiosis requires the regeneration of NAD^+ from NADH in order to allow glycolysis to continue.

(a) Figure 14–6 illustrates the fate of the carbon atoms of glucose. C-1 (or C-6) becomes C-3 of glyceraldehyde 3-phosphate and subsequently pyruvate. When pyruvate is decarboxylated and reduced to ethanol, C-3 of pyruvate becomes the C-2 of ethanol ($^{14}CH_3$—CH_2—OH).

(b) If all the labeled carbon from glucose is converted to $^{14}CO_2$ during ethanol fermentation, the original label must have been on C-3 and/or C-4 of glucose, because these are converted to the carboxyl group of pyruvate.

7. Heat from Fermentations Large-scale industrial fermenters generally require constant, vigorous cooling. Why?

Answer Fermentation releases energy, some conserved in the form of ATP but much of it dissipated as heat. Unless the fermenter contents are cooled to counterbalance this heat production, the temperature would become high enough to kill the microorganisms.

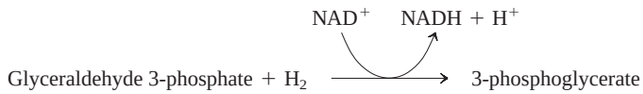
8. Fermentation to Produce Soy Sauce Soy sauce is prepared by fermenting a salted mixture of soybeans and wheat with several microorganisms, including yeast, over a period of 8 to 12 months. The resulting sauce (after solids are removed) is rich in lactate and ethanol. How are these two compounds produced? To prevent the soy sauce from having a strong vinegar taste (vinegar is dilute acetic acid), oxygen must be kept out of the fermentation tank. Why?

Answer Soybeans and wheat contain starch, a polymer of glucose, which is broken down to glucose by the microorganisms. The glucose is then degraded to pyruvate via glycolysis. Because the process is carried out in the absence of oxygen (i.e., it is a fermentation), pyruvate is reduced to lactic acid and ethanol. If oxygen were present, pyruvate would be oxidized to acetyl-CoA and then to CO_2 and H_2O. Some of the acetyl-CoA, however, would also be hydrolyzed to acetic acid (vinegar) in the presence of oxygen.

9. Equivalence of Triose Phosphates ^{14}C-Labeled glyceraldehyde 3-phosphate was added to a yeast extract. After a short time, fructose 1,6-bisphosphate labeled with ^{14}C at C-3 and C-4 was isolated. What was the location of the ^{14}C label in the starting glyceraldehyde 3-phosphate? Where did the second ^{14}C label in fructose 1,6-bisphosphate come from? Explain.

Answer Problem 1 outlines the steps in glycolysis involving fructose 1,6-bisphosphate, glyceraldehyde 3-phosphate, and dihydroxyacetone phosphate. Keep in mind that the aldolase reaction is readily reversible and the triose phosphate isomerase reaction catalyzes extremely rapid interconversion of its substrates. Thus, the label at C-1 of glyceraldehyde 3-phosphate would equilibrate with C-1 of dihydroxyacetone phosphate ($\Delta G'^\circ = 7.5$ kJ/mol). Because the aldolase reaction has $\Delta G'^\circ = -23.8$ kJ/mol in the direction of hexose formation, fructose 1,6-bisphosphate would be readily formed, and labeled in C-3 and C-4 (see Fig. 14–6).

10. Glycolysis Shortcut Suppose you discovered a mutant yeast whose glycolytic pathway was shorter because of the presence of a new enzyme catalyzing the reaction



Would shortening the glycolytic pathway in this way benefit the cell? Explain.

Answer Under anaerobic conditions, the phosphoglycerate kinase and pyruvate kinase reactions are essential. The shortcut in the mutant yeast would bypass the formation of an acyl phosphate by glyceraldehyde 3-phosphate dehydrogenase and therefore would not allow the formation of 1,3-bisphosphoglycerate. Without the formation of a substrate for 3-phosphoglycerate kinase, no ATP would be formed. Under anaerobic conditions, the net reaction for glycolysis normally produces 2 ATP per glucose. In the mutant yeast, net production of ATP would be zero and growth could not occur. Under aerobic conditions, however, because the majority of ATP formation occurs via oxidative phosphorylation, the mutation would have no observable effect.

11. Role of Lactate Dehydrogenase During strenuous activity, the demand for ATP in muscle tissue is vastly increased. In rabbit leg muscle or turkey flight muscle, the ATP is produced almost exclusively by lactic acid fermentation. ATP is formed in the payoff phase of glycolysis by two reactions, promoted by phosphoglycerate kinase and pyruvate kinase. Suppose skeletal muscle were devoid of lactate dehydrogenase. Could it carry out strenuous physical activity; that is, could it generate ATP at a high rate by glycolysis? Explain.

Answer The key point here is that NAD^+ must be regenerated from NADH in order for glycolysis to continue. Some tissues, such as skeletal muscle, obtain almost all their ATP through the glycolytic pathway and are capable of short-term exercise only (see Box 14–2). In order to generate ATP at a high rate, the NADH formed during glycolysis must be oxidized. In the absence of significant amounts of O_2 in the tissues, lactate dehydrogenase converts pyruvate and NADH to lactate and NAD^+. In the absence of this enzyme, NAD^+ could not be regenerated and glycolytic production of ATP would stop—and as a consequence, muscle activity could not be maintained.

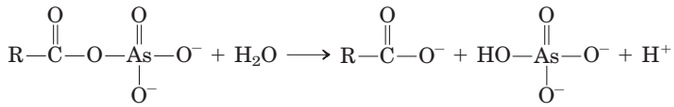
12. Efficiency of ATP Production in Muscle The transformation of glucose to lactate in myocytes releases only about 7% of the free energy released when glucose is completely oxidized to CO_2 and H_2O. Does this mean that anaerobic glycolysis in muscle is a wasteful use of glucose? Explain.

Answer The transformation of glucose to lactate occurs when myocytes are low in oxygen, and it provides a means of generating ATP under oxygen-deficient conditions. Because lactate can be transformed to pyruvate, glucose is not wasted: the pyruvate can be oxidized by aerobic reactions when oxygen becomes plentiful. This metabolic flexibility gives the organism a greater capacity to adapt to its environment.

13. Free-Energy Change for Triose Phosphate Oxidation The oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, catalyzed by glyceraldehyde 3-phosphate dehydrogenase, proceeds with an unfavorable equilibrium constant ($K'_{eq} = 0.08$; $\Delta G'^{\circ} = 6.3$ kJ/mol), yet the flow through this point in the glycolytic pathway proceeds smoothly. How does the cell overcome the unfavorable equilibrium?

Answer In organisms, where directional flow in a pathway is required, exergonic reactions are coupled to endergonic reactions to overcome unfavorable free-energy changes. The endergonic glyceraldehyde 3-phosphate dehydrogenase reaction is followed by the phosphoglycerate kinase reaction, which rapidly removes the product of the former reaction. Consequently, the dehydrogenase reaction does not reach equilibrium and its unfavorable free-energy change is thus circumvented. The net $\Delta G'^{\circ}$ of the two reactions, when coupled, is -18.5 kJ/mol + 6.3 kJ/mol = -12.2 kJ/mol.

14. Arsenate Poisoning Arsenate is structurally and chemically similar to inorganic phosphate (P_i), and many enzymes that require phosphate will also use arsenate. Organic compounds of arsenate are less stable than analogous phosphate compounds, however. For example, acyl *arsenates* decompose rapidly by hydrolysis:



On the other hand, acyl *phosphates,* such as 1,3-bisphosphoglycerate, are more stable and undergo further enzyme-catalyzed transformation in cells.

(a) Predict the effect on the net reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase if phosphate were replaced by arsenate.

(b) What would be the consequence to an organism if arsenate were substituted for phosphate? Arsenate is very toxic to most organisms. Explain why.

Answer

(a) In the presence of arsenate, the product of the glyceraldehyde 3-phosphate dehydrogenase reaction is 1-arseno-3-phosphoglycerate, which nonenzymatically decomposes to 3-phosphoglycerate and arsenate; the substrate for the phosphoglycerate kinase is therefore bypassed.

(b) No ATP can be formed in the presence of arsenate because 1,3-bisphosphoglycerate is not formed. Under anaerobic conditions, this would result in no net glycolytic synthesis of ATP. Arsenate poisoning can be used as a test for the presence of an acyl phosphate intermediate in a reaction pathway.

15. Requirement for Phosphate in Ethanol Fermentation In 1906 Harden and Young, in a series of classic studies on the fermentation of glucose to ethanol and CO_2 by extracts of brewer's yeast, made

the following observations. (1) Inorganic phosphate was essential to fermentation; when the supply of phosphate was exhausted, fermentation ceased before all the glucose was used. (2) During fermentation under these conditions, ethanol, CO_2, and a hexose bisphosphate accumulated. (3) When arsenate was substituted for phosphate, no hexose bisphosphate accumulated, but the fermentation proceeded until all the glucose was converted to ethanol and CO_2.

(a) Why did fermentation cease when the supply of phosphate was exhausted?

(b) Why did ethanol and CO_2 accumulate? Was the conversion of pyruvate to ethanol and CO_2 essential? Why? Identify the hexose bisphosphate that accumulated. Why did it accumulate?

(c) Why did the substitution of arsenate for phosphate prevent the accumulation of the hexose bisphosphate yet allow fermentation to ethanol and CO_2 to go to completion? (See Problem 14.)

Answer Ethanol fermentation in yeast has the following overall equation

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_\text{i} \longrightarrow 2\ \text{ethanol} + 2\text{CO}_2 + 2\text{ATP} + 2\text{H}_2\text{O}$$

It is clear that phosphate is required for the continued operation of glycolysis and ethanol formation. In extracts to which glucose is added, fermentation proceeds until ADP and P_i (present in the extracts) are exhausted.

(a) Phosphate is required in the glyceraldehyde 3-phosphate dehydrogenase reaction, and glycolysis will stop at this step when P_i is exhausted. Because glucose remains, it will be phosphorylated by ATP, but P_i will not be released.

(b) Fermentation in yeast cells produces ethanol and CO_2 rather than lactate (see Box 14–3). Without these reactions (in the absence of oxygen), NADH would accumulate and no new NAD^+ would be available for further glycolysis (see Problem 11). The hexose bisphosphate that accumulates is fructose 1,6-bisphosphate; in terms of energetics, this intermediate lies at a "low point" or valley in the pathway, between the energy-input reactions that precede it and the energy-payoff reactions that follow.

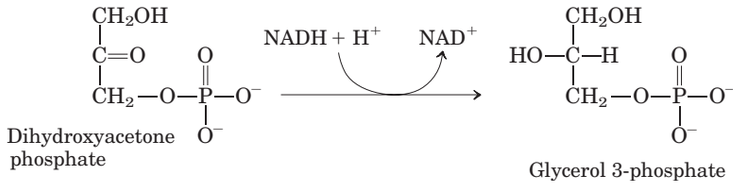
(c) Arsenate replaces P_i in the glyceraldehyde 3-phosphate dehydrogenase reaction to yield an acyl arsenate, which spontaneously hydrolyzes. This prevents formation of fructose 1,6-bisphosphate and ATP but allows formation of 3-phosphoglycerate, which continues through the pathway.

16. Role of the Vitamin Niacin Adults engaged in strenuous physical activity require an intake of about 160 g of carbohydrate daily but only about 20 mg of niacin for optimal nutrition. Given the role of niacin in glycolysis, how do you explain the observation?

Answer Dietary niacin is used to synthesize NAD^+. Oxidations carried out by NAD^+ are part of cyclic oxidation-reduction processes, with NAD^+/NADH as an electron carrier. Because of this cycling, one molecule of NAD^+ can oxidize many thousands of molecules of glucose, and thus the dietary requirement for the precursor vitamin (niacin) is relatively small.

17. Synthesis of Glycerol Phosphate The glycerol 3-phosphate required for the synthesis of glycerophospholipids can be synthesized from a glycolytic intermediate. Propose a reaction sequence for this conversion.

Answer Glycerol 3-phosphate and dihydroxyacetone 3-phosphate differ only at C-2. A dehydrogenase with the cofactor NADH acting on dihydroxyacetone 3-phosphate would form glycerol 3-phospate.



In fact, the enzyme glycerol 3-phosphate dehydrogenase catalyzes this reaction (see Fig. 21–17).

18. Severity of Clinical Symptoms Due to Enzyme Deficiency The clinical symptoms of two forms of galactosemia—deficiency of galactokinase or of UDP-glucose:galactose 1-phosphate uridylyltransferase—show radically different severity. Although both types produce gastric discomfort after milk ingestion, deficiency of the transferase also leads to liver, kidney, spleen, and brain dysfunction and eventual death. What products accumulate in the blood and tissues with each type of enzyme deficiency? Estimate the relative toxicities of these products from the above information.

Answer In galactokinase deficiency, galactose accumulates; in UDP-glucose:galactose 1-phosphate uridylyltransferase deficiency, galactose 1-phosphate accumulates (see Fig. 14–12). The latter metabolite is clearly more toxic.

19. Muscle-Wasting in Starvation One consequence of starvation is a reduction in muscle mass. What happens to the muscle proteins?

Answer Muscle proteins are selectively degraded by proteases in myocytes, and the resulting amino acids move, in the bloodstream, from muscle to liver. In the liver, glucogenic amino acids are the starting materials for gluconeogenesis, to provide glucose for export to the brain (which cannot use fatty acids as fuel).

20. Pathway of Atoms in Gluconeogenesis A liver extract capable of carrying out all the normal metabolic reactions of the liver is briefly incubated in separate experiments with the following ^{14}C-labeled precursors:

(a) [^{14}C]Bicarbonate, $HO—{}^{14}C(=O)O^-$

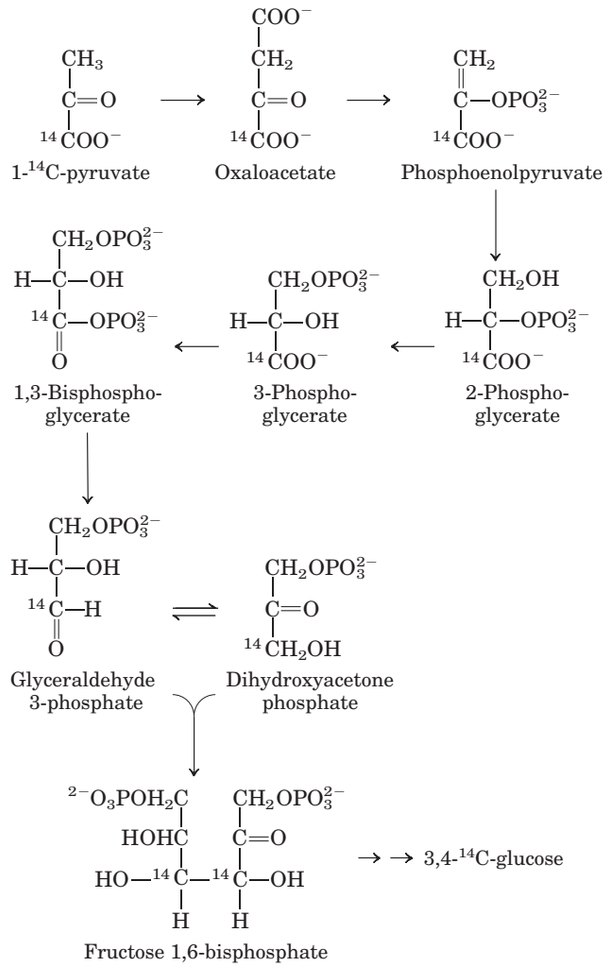
(b) [1-^{14}C]Pyruvate, $CH_3—C(=O)—{}^{14}COO^-$

Trace the pathway of each precursor through gluconeogenesis. Indicate the location of ^{14}C in all intermediates and in the product, glucose.

Answer

(a) In the pyruvate carboxylase reaction, $^{14}CO_2$ is added to pyruvate to form [4-^{14}C]oxaloacetate, but the phosphoenolpyruvate carboxykinase reaction removes the *same* CO_2 in the next step. Thus, ^{14}C is not (initially) incorporated into glucose.

(b)



21. Energy Cost of a Cycle of Glycolysis and Gluconeogenesis What is the cost (in ATP equivalents) of transforming glucose to pyruvate via glycolysis and back again to glucose via gluconeogenesis?

Answer The overall reaction of glycolysis is

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_\text{i} + 2\text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}$$

The overall reaction of gluconeogenesis is

$$2\ \text{Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2\text{NADH} + 2\text{H}^+ + 4\text{H}_2\text{O} \longrightarrow \text{glucose} + 2\text{NAD}^+ + 4\text{ADP} + 2\text{GDP} + 6\text{P}_\text{i}$$

The cost of transforming glucose to pyruvate and back to glucose is given by the difference between these two equations:

$$2\text{ATP} + 2\text{GTP} + 2\text{H}_2\text{O} \longrightarrow 2\text{ADP} + 2\text{GDP} + 4\text{P}_\text{i}$$

The energy cost is four ATP equivalents per glucose molecule.

22. Relationship between Gluconeogenesis and Glycolysis Why is it important that gluconeogenesis is not the exact reversal of glycolysis?

Answer If gluconeogenesis were simply the reactions of glycolysis in reverse, the process would be energetically unfeasible (highly endergonic), because of the three reactions with large, negative standard free-energy changes in the catabolic (glycolytic) direction. Furthermore, if the same

enzymes were used for all reactions in the two pathways, it would be impossible to regulate the two processes separately; anything that stimulated (or inhibited) the forward reaction for a given enzyme would stimulate (or inhibit) the reverse reaction to the same extent.

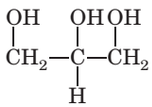
23. Energetics of the Pyruvate Kinase Reaction Explain in bioenergetic terms how the conversion of pyruvate to phosphoenolpyruvate in gluconeogenesis overcomes the large, negative standard free-energy change of the pyruvate kinase reaction in glycolysis.

Answer In converting pyruvate to PEP, the cell invests two ATP equivalents: ATP in the pyruvate carboxylase reaction, then GTP (equivalent to ATP) in the PEP carboxykinase reaction. By coupling the expenditure of two ATP equivalents to the conversion of pyruvate to PEP, the gluconeogenic process is made exergonic.

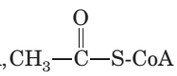
24. Glucogenic Substrates A common procedure for determining the effectiveness of compounds as precursors of glucose in mammals is to starve the animal until the liver glycogen stores are depleted and then administer the compound in question. A substrate that leads to a *net* increase in liver glycogen is termed glucogenic because it must first be converted to glucose 6-phosphate. Show by means of known enzymatic reactions which of the following substances are glucogenic:

(a) Succinate, $^{-}OOC-CH_2-CH_2-COO^{-}$

(b) Glycerol,



(c) Acetyl-CoA,



(d) Pyruvate,

(e) Butyrate, $CH_3-CH_2-CH_2-COO^{-}$

Answer

(a) Glucogenic. In the citric acid cycle, succinate is converted to fumarate by succinate dehydrogenase, then to malate by fumarase, then to oxaloacetate by malate dehydrogenase. OAA can then leave the mitochondrion via the malate-aspartate shuttle, and in the cytosol is converted to PEP, which is glucogenic.

(b) Glucogenic. Glycerol kinase converts glycerol to glycerol 1-phosphate, which is then converted by a dehydrogenase (using NAD^+) to dihydroxyacetone phosphate, which is glucogenic.

(c) Not glucogenic. Higher animals do not have the enzymes to convert acetyl-CoA to pyruvate.

(d) Glucogenic. Pyruvate carboxylase converts pyruvate to oxaloacetate, which is used for gluconeogenesis as in **(a).**

(e) Not glucogenic. Butyrate is converted to butyryl-CoA by an acyl-CoA synthetase, and a single turn of the β-oxidation pathway converts butyryl-CoA to two molecules of acetyl-CoA, which is not glucogenic.

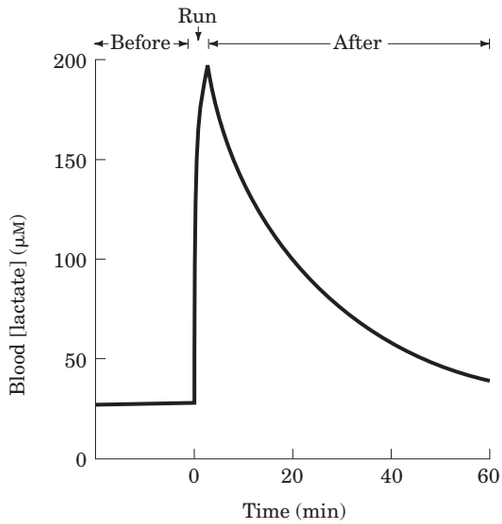
25. Ethanol Affects Blood Glucose Levels The consumption of alcohol (ethanol), especially after periods of strenuous activity or after not eating for several hours, results in a deficiency of glucose in the blood, a condition known as hypoglycemia. The first step in the metabolism of ethanol by the liver is oxidation to acetaldehyde, catalyzed by liver alcohol dehydrogenase:

$$CH_3CH_2OH + NAD^+ \longrightarrow CH_3CHO + NADH + H^+$$

Explain how this reaction inhibits the transformation of lactate to pyruvate. Why does this lead to hypoglycemia?

Answer The first step in the synthesis of glucose from lactate in the liver is oxidation of the lactate to pyruvate; like the oxidation of ethanol to acetaldehyde, this requires NAD^+. Consumption of alcohol forces a competition for NAD^+ between ethanol metabolism and gluconeogenesis, reducing the conversion of lactate to glucose and resulting in hypoglycemia. The problem is compounded by strenuous exercise and lack of food because at these times the level of blood glucose is already low.

26. Blood Lactate Levels during Vigorous Exercise The concentrations of lactate in blood plasma before, during, and after a 400 m sprint are shown in the graph.



(a) What causes the rapid rise in lactate concentration?

(b) What causes the decline in lactate concentration after completion of the sprint? Why does the decline occur more slowly than the increase?

(c) Why is the concentration of lactate not zero during the resting state?

Answer

(a) Rapid depletion of ATP during strenuous muscular exertion causes the rate of glycolysis to increase dramatically, producing higher cytosolic concentrations of pyruvate and NADH; lactate dehydrogenase converts these to lactate and NAD^+ (lactic acid fermentation).

(b) When energy demands are reduced, the oxidative capacity of the mitochondria is again adequate, and lactate is transformed to pyruvate by lactate dehydrogenase, and the pyruvate is converted to glucose. The rate of the dehydrogenase reaction is slower in this direction because of the limited availability of NAD^+ and because the equilibrium of the reaction is strongly in favor of lactate (conversion of lactate to pyruvate is energy-requiring).

(c) The equilibrium of the lactate dehydrogenase reaction

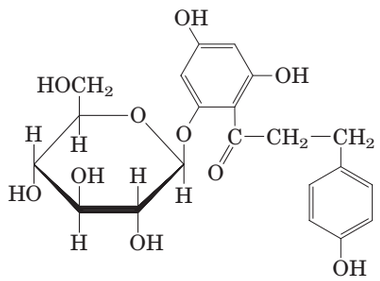
$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \longrightarrow \text{lactate} + \text{NAD}^+$$

is *strongly* in favor of lactate. Thus, even at very low concentrations of NADH and pyruvate, there is a significant concentration of lactate.

27. Relationship between Fructose 1,6-Bisphosphatase and Blood Lactate Levels A congenital defect in the liver enzyme fructose 1,6-bisphosphatase results in abnormally high levels of lactate in the blood plasma. Explain.

Answer In the liver, lactate is converted to pyruvate and then to glucose by gluconeogenesis (see Figs 14–15, 14–16). This pathway includes the glycolytic bypass step catalyzed by fructose 1,6-bisphosphatase (FBPase-1). A defect in this enzyme would prevent the entry of lactate into the gluconeogenic pathway in hepatocytes, causing lactate to accumulate in the blood.

28. Effect of Phloridzin on Carbohydrate Metabolism Phloridzin, a toxic glycoside from the bark of the pear tree, blocks the normal reabsorption of glucose from the kidney tubule, thus causing blood glucose to be almost completely excreted in the urine. In an experiment, rats fed phloridzin and sodium succinate excreted about 0.5 mol of glucose (made by gluconeogenesis) for every 1 mol of sodium succinate ingested. How is the succinate transformed to glucose? Explain the stoichiometry.



Phloridzin

Answer Excretion of glucose promoted by phloridzin causes a drop in blood glucose, which stimulates gluconeogenesis. The ingested succinate enters the mitochondrion via the dicarboxylate transport system and is transformed to oxaloacetate by enzymes of the citric acid cycle. The oxaloacetate passes into the cytosol and is transformed to phosphoenolpyruvate by PEP carboxykinase. Two moles of PEP are then required to produce a mole of glucose by the route outlined in Figure 14–16, consistent with the observed stoichiometry. Note that the rate of glucose production must be much higher than the rate of utilization by tissues because almost 100% of the glucose is excreted.

29. Excess O_2 Uptake during Gluconeogenesis Lactate absorbed by the liver is converted to glucose, with the input of 6 mol of ATP for every mole of glucose produced. The extent of this process in a rat liver preparation can be monitored by administering [^{14}C]lactate and measuring the amount of [^{14}C]glucose produced. Because the stoichiometry of O_2 consumption and ATP production is known (about 5 ATP per O_2), we can predict the extra O_2 consumption above the normal rate when a given amount of lactate is administered. However, when the extra O_2 used in the synthesis of glucose from lactate is actually measured, it is always higher than predicted by known stoichiometric relationships. Suggest a possible explanation for this observation.

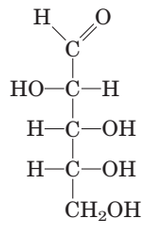
Answer If the catabolic and biosynthetic pathways operate simultaneously, a certain amount of ATP is consumed in "futile cycles" (or "substrate cycles") in which no useful work is done. Examples of such cycles are that between glucose and glucose 6-phosphate and that between fructose 6-phosphate and fructose 1,6-bisphosphate. The net hydrolysis of ATP to ADP and P_i increases the consumption of oxygen, the terminal electron acceptor in oxidative phosphorylation.

30. Role of the Pentose Phosphate Pathway If the oxidation of glucose 6-phosphate via the pentose phosphate pathway were being used primarily to generate NADPH for biosynthesis, the other product, ribose 5-phosphate, would accumulate. What problems might this cause?

Answer At the very least, accumulation of ribose 5-phosphate would tend to force this reaction in the reverse direction by mass action (see Eqn 13–4, p. 493). It might also affect other metabolic reactions that involve ribose 5-phosphate as a substrate or product—such as the pathways of nucleotide synthesis.

Data Analysis Problem

31. Engineering a Fermentation System Fermentation of plant matter to produce ethanol for fuel is one potential method for reducing the use of fossil fuels and thus the CO_2 emissions that lead to global warming. Many microorganisms can break down cellulose then ferment the glucose to ethanol. However, many potential cellulose sources, including agricultural residues and switchgrass, also contain substantial amounts of arabinose, which is not as easily fermented.



D-Arabinose

Escherichia coli is capable of fermenting arabinose to ethanol, but it is not naturally tolerant of high ethanol levels, thus limiting its utility for commercial ethanol production. Another bacterium, *Zymomonas mobilis*, is naturally tolerant of high levels of ethanol but cannot ferment arabinose. Deanda, Zhang, Eddy, and Picataggio (1996) described their efforts to combine the most useful features of these two organisms by introducing the *E. coli* genes for the arabinose-metabolizing enzymes into *Z. mobilis*.

(a) Why is this a simpler strategy than the reverse: engineering *E. coli* to be more ethanol-tolerant?

Deanda and colleagues inserted five *E. coli* genes into the *Z. mobilis* genome: *araA*, coding for L-arabinose isomerase, which interconverts L-arabinose and L-ribulose; *araB*, L-ribulokinase, which uses ATP to phosphorylate L-ribulose at C-5; *araD*, L-ribulose 5-phosphate epimerase, which interconverts L-ribulose 5-phosphate and L-xylulose 5-phosphate; *talB*, transaldolase; and *tktA*, transketolase.

(b) For each of the three *ara* enzymes, briefly describe the chemical transformation it catalyzes and, where possible, name an enzyme discussed in this chapter that carries out an analogous reaction.

The five *E. coli* genes inserted in *Z. mobilis* allowed the entry of arabinose into the nonoxidative phase of the pentose phosphate pathway (Fig. 14–22), where it was converted to glucose 6-phosphate and fermented to ethanol.

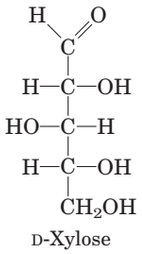
(c) The three *ara* enzymes eventually converted arabinose into which sugar?

(d) The product from part **(c)** feeds into the pathway shown in Figure 14–22. Combining the five *E. coli* enzymes listed above with the enzymes of this pathway, describe the overall pathway for the fermentation of 6 molecules of arabinose to ethanol.

(e) What is the stoichiometry of the fermentation of 6 molecules of arabinose to ethanol and CO_2? How many ATP molecules would you expect this reaction to generate?

(f) *Z. mobilis* uses a slightly different pathway for ethanol fermentation from the one described in this chapter. As a result, the expected ATP yield is only 1 ATP per molecule of arabinose. Although this is less beneficial for the bacterium, it is better for ethanol production. Why?

Another sugar commonly found in plant matter is xylose.



D-Xylose

(g) What additional enzymes would you need to introduce into the modified *Z. mobilis* strain described above to enable it to use xylose as well as arabinose to produce ethanol? You don't need to name the enzymes (they may not even exist in the real world!); just give the reactions they would need to catalyze.

Answer

(a) Ethanol tolerance is likely to involve many more genes, and thus the engineering would be a much more involved project.

(b) L-Arabinose isomerase (the *araA* enzyme) converts an aldose to a ketose by moving the carbonyl of a nonphosphorylated sugar from C-1 to C-2. No analogous enzyme is discussed in this chapter; all the enzymes described here act on phosphorylated sugars. An enzyme that carries out a similar transformation with phosphorylated sugars is phosphohexose isomerase. L-Ribulokinase (*araB*) phosphorylates a sugar at C-5 by transferring the γ phosphate from ATP. Many such reactions are described in this chapter, including the hexokinase reaction. L-Ribulose 5-phosphate epimerase (*araD*) switches the —H and —OH groups on a chiral carbon of a sugar. No analogous reaction is described in the chapter, but it is described in Chapter 20 (see Fig. 20–1, p. 774).

(c) The three *ara* enzymes would convert arabinose to xylulose 5-phosphate by the following pathway:

$$\text{Arabinose} \xrightarrow{\text{L-arabinose isomerase}} \text{L-ribulose} \xrightarrow{\text{L-ribulokinase}} \text{L-ribulose 5-phosphate} \xrightarrow{\text{epimerase}} \text{xylulose 5-phosphate.}$$

(d) The arabinose is converted to xylulose 5-phosphate as in **(c),** which enters the pathway in Figure 14–22; the glucose 6-phosphate product is then fermented to ethanol and CO_2.

(e) 6 molecules of arabinose + 6 molecules of ATP are converted to 6 molecules of xylulose 5-phosphate, which feed into the pathway in Figure 14–22 to yield 5 molecules of glucose 6-phosphate, each of which is fermented to yield 3 ATP (they enter as glucose 6-phosphate, not glucose)—15 ATP in all. Overall, you would expect a yield of 15 ATP – 6 ATP = 9 ATP from the 6 arabinose molecules. The other products are 10 molecules of ethanol and 10 molecules of CO_2.

(f) Given the lower ATP yield, for an amount of growth (i.e., of available ATP) equivalent to growth without the added genes the engineered *Z. mobilis* must ferment more arabinose, and thus it produces more ethanol.

(g) One way to allow the use of xylose would be to add the genes for two enzymes: an analog of the *araD* enzyme that converts xylose to ribose by switching the —H and —OH on C-3, and an analog of the *araB* enzyme that phosphorylates ribose at C-5. The resulting ribose 5-phosphate would feed into the existing pathway.

Reference

Deanda, K., Zhang, M., Eddy, C., & Picataggio, S. (1996) Development of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering. *Appl. Environ. Microbiol.* **62,** 4465–4470.

chapter

15

Principles of Metabolic Regulation

1. Measurement of Intracellular Metabolite Concentrations Measuring the concentrations of metabolic intermediates in a living cell presents great experimental difficulties—usually a cell must be destroyed before metabolite concentrations can be measured. Yet enzymes catalyze metabolic interconversions very rapidly, so a common problem associated with these types of measurements is that the findings reflect not the physiological concentrations of metabolites but the equilibrium concentrations. A reliable experimental technique requires all enzyme-catalyzed reactions to be instantaneously stopped in the intact tissue so that the metabolic intermediates do not undergo change. This objective is accomplished by rapidly compressing the tissue between large aluminum plates cooled with liquid nitrogen (−190 °C), a process called **freeze-clamping.** After freezing, which stops enzyme action instantly, the tissue is powdered and the enzymes are inactivated by precipitation with perchloric acid. The precipitate is removed by centrifugation, and the clear supernatant extract is analyzed for metabolites. To calculate intracellular concentrations, the intracellular volume is determined from the total water content of the tissue and a measurement of the extracellular volume.

The intracellular concentrations of the substrates and products of the phosphofructokinase-1 reaction in isolated rat heart tissue are given in the table below.

Metabolite	Concentration μM*
Fructose 6-phosphate	87.0
Fructose 1,6-bisphosphate	22.0
ATP	11,400
ADP	1,320

Source: From Williamson, J.R. (1965) Glycolytic control mechanisms I: inhibition of glycolysis by acetate and pyruvate in the isolated, perfused rat heart. *J. Biol. Chem.* **240,** 2308–2321.

*Calculated as μmol/mL of intracellular water.

(a) Calculate Q, [fructose 1,6-bisphosphate][ADP]/[fructose 6-phosphate][ATP], for the PFK-1 reaction under physiological conditions.

(b) Given a $\Delta G'^{\circ}$ for the PFK-1 reaction of −14.2 kJ/mol, calculate the equilibrium constant for this reaction.

(c) Compare the values of Q and K'_{eq}. Is the physiological reaction near or far from equilibrium? Explain. What does this experiment suggest about the role of PFK-1 as a regulatory enzyme?

Answer

(a) The mass-action ratio, $Q = \dfrac{(22.0\ \mu\text{M})(1{,}320\ \mu\text{M})}{(87.0\ \mu\text{M})(11{,}400\ \mu\text{M})} = 0.0293$

(b) $\Delta G'^\circ = -RT \ln K'_{eq}$ (RT at 25 °C = 2.48 kJ/mol)

$$\ln K'_{eq} = -\Delta G'^\circ/RT$$
$$= -(-14.2 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$$
$$= 5.73$$
$$K'_{eq} = e^{-5.73} = 308$$

(c) Because K'_{eq} is much greater than Q, it is clear that the PFK-1 reaction does not approach equilibrium in vivo, and thus the product of the reaction, fructose 1,6-bisphosphate, does not approximate an equilibrium concentration. Metabolic pathways are "open systems," operating under near–steady state conditions, with substrates flowing in and products flowing out at all times. Thus, all the fructose 1,6-bisphosphate formed is rapidly used or turned over. The PFK-1–catalyzed reaction, which is the rate-limiting step in glycolysis, is an excellent candidate for the critical regulatory point of the pathway.

2. Are All Metabolic Reactions at Equilibrium?

(a) Phosphoenolpyruvate (PEP) is one of the two phosphoryl group donors in the synthesis of ATP during glycolysis. In human erythrocytes, the steady-state concentration of ATP is 2.24 mM, that of ADP is 0.25 mM, and that of pyruvate is 0.051 mM. Calculate the concentration of PEP at 25 °C, assuming that the pyruvate kinase reaction (see Fig. 13–13) is at equilibrium in the cell.

(b) The physiological concentration of PEP in human erythrocytes is 0.023 mM. Compare this with the value obtained in **(a).** Explain the significance of this difference.

Answer

(a) First, we must calculate the overall $\Delta G'^\circ$ for the pyruvate kinase reaction by breaking this process into two reactions and summing the $\Delta G'^\circ$ values (from Table 13–6):

(1) PEP + $H_2O \longrightarrow$ pyruvate + P_i	$\Delta G'^\circ_1 = -61.9$ kJ/mol
(2) ADP + $P_i \longrightarrow$ ATP + H_2O	$\Delta G'^\circ_2 = 30.5$ kJ/mol
Sum: PEP + ADP $\longrightarrow$ pyruvate + ATP	$\Delta G'^\circ_{sum} = -31.4$ kJ/mol

Assuming the reaction is at equilibrium, we can calculate K'_{eq}:

$$\Delta G'^\circ = -RT \ln K'_{eq}$$
$$\ln K'_{eq} = -\Delta G'^\circ/RT$$
$$= -(-31.4 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$$
$$= 12.7$$
$$K'_{eq} = 3.28 \times 10^5$$

Because $K'_{eq} = \dfrac{[\text{pyruvate}][\text{ATP}]}{[\text{ADP}][\text{PEP}]}$, where all reactants and products are at their equilibrium concentrations,

$$[\text{PEP}] = \frac{[\text{pyruvate}][\text{ATP}]}{[\text{ADP}]\,K'_{eq}}$$
$$= \frac{(5.1 \times 10^{-5}\text{ M})(2.24 \times 10^{-3}\text{ M})}{(2.5 \times 10^{-4}\text{ M})(3.28 \times 10^5)}$$
$$= 1.4 \times 10^{-9}\text{ M}$$

(b) The physiological [PEP] of 0.023 mM is

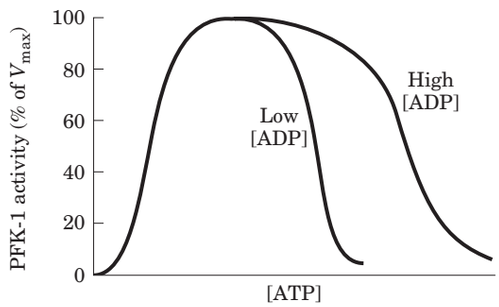
$$\frac{0.023 \times 10^{-3}\text{ M}}{1.4 \times 10^{-9}\text{ M}} = 16{,}000 \text{ times the equilibrium concentration}$$

This reaction, like many others in the cell, is *not* at equilibrium.

3. Effect of O_2 Supply on Glycolytic Rates The regulated steps of glycolysis in intact cells can be identified by studying the catabolism of glucose in whole tissues or organs. For example, the glucose consumption by heart muscle can be measured by artificially circulating blood through an isolated intact heart and measuring the concentration of glucose before and after the blood passes through the heart. If the circulating blood is deoxygenated, heart muscle consumes glucose at a steady rate. When oxygen is added to the blood, the rate of glucose consumption drops dramatically, then is maintained at the new, lower rate. Explain.

Answer In the absence of O_2, the ATP needs of the cell are met by anaerobic glucose metabolism (fermentation) to form lactate. This produces a maximum of 2 ATP per glucose. Because the aerobic metabolism of glucose produces far more ATP per glucose (by oxidative phosphorylation), far less glucose is needed to produce the same amount of ATP. The Pasteur effect was the first demonstration of the primacy of energy production—that is, of ATP levels—in controlling the rate of glycolysis.

4. Regulation of PFK-1 The effect of ATP on the allosteric enzyme PFK-1 is shown below. For a given concentration of fructose 6-phosphate, the PFK-1 activity increases with increasing concentrations of ATP, but a point is reached beyond which increasing the concentration of ATP inhibits the enzyme.



(a) Explain how ATP can be both a substrate and an inhibitor of PFK-1. How is the enzyme regulated by ATP?

(b) In what ways is glycolysis regulated by ATP levels?

(c) The inhibition of PFK-1 by ATP is diminished when the ADP concentration is high, as shown in the illustration. How can this observation be explained?

Answer

(a) In addition to their binding sites for substrate(s), allosteric enzymes have binding sites for regulatory metabolites. Binding of effectors to these regulatory sites modifies the enzyme's activity by altering its V_{max} or K_m value. ATP is a substrate and an allosteric inhibitor of PFK-1. Binding of ATP to the catalytic site increases activity, whereas binding to the allosteric site inhibits activity.

(b) Because ATP is a negative regulator of PFK-1, elevation of [ATP] when energy is abundant inhibits the enzyme and thus the flux of metabolites through the glycolytic pathway.

(c) The graph indicates that increased [ADP] suppresses the inhibition of PFK-1 by ATP. Because the total adenine nucleotide pool is more or less constant in all cells, utilization of ATP leads to an increase in [ADP]. The data show that the activity of the enzyme may be regulated in vivo by the ratio [ATP]/[ADP].

5. Cellular Glucose Concentration The concentration of glucose in human blood plasma is maintained at about 5 mM. The concentration of free glucose inside a myocyte is much lower. Why is the concentration so low in the cell? What happens to glucose after entry into the cell? Glucose is administered intravenously as a food source in certain clinical situations. Given that the transformation of glucose to glucose 6-phosphate consumes ATP, why not administer intravenous glucose 6-phosphate instead?

Answer Glucose enters cells and is immediately exposed to hexokinase, which converts glucose to glucose 6-phosphate using the energy of ATP. This reaction is highly exergonic ($\Delta G'^\circ = -16.7$ kJ/mol), and formation of glucose 6-phosphate is strongly favored. Glucose 6-phosphate is negatively charged and cannot diffuse across the membrane. The glucose transporter is specific for glucose; glucose 6-phosphate cannot leave the cell on this transporter and must be stored (by conversion to glycogen) or metabolized via glycolysis. Intravenous administration of glucose 6-phosphate is not useful because the phosphorylated glucose cannot enter cells on the glucose transporter or by diffusion.

6. Enzyme Activity and Physiological Function The V_{max} of the enzyme glycogen phosphorylase from skeletal muscle is much greater than the V_{max} of the same enzyme from liver tissue.

(a) What is the physiological function of glycogen phosphorylase in skeletal muscle? In liver tissue?

(b) Why does the V_{max} of the muscle enzyme need to be larger than that of the liver enzyme?

Answer

(a) The role of glycogen and glycogen metabolism differs in muscle and liver. *In muscle,* glycogen is broken down to supply energy (ATP), via glycolysis and lactic acid fermentation. Glycogen phosphorylase catalyzes the conversion of stored glycogen to glucose 1-phosphate, which is converted to glucose 6-phosphate and thus enters glycolysis. During strenuous activity, muscle becomes anaerobic and large quantities of glucose 6-phosphate undergo lactic acid fermentation to form the necessary ATP.

In the liver, glycogen is used to maintain the level of glucose in the blood (primarily between meals). In this case, the glucose 6-phosphate is converted to glucose and exported into the bloodstream.

(b) Strenuous muscular activity requires large amounts of ATP, which must be formed rapidly and efficiently, so glycogen phosphorylase must have a high ratio of V_{max}/K_m in muscle. This is not a critical requirement in liver tissue.

7. Glycogen Phosphorylase Equilibrium Glycogen phosphorylase catalyzes the removal of glucose from glycogen. The $\Delta G'^\circ$ for this reaction is 3.1 kJ/mol. **(a)** Calculate the ratio of $[P_i]$ to [glucose 1-phosphate] when the reaction is at equilibrium. (Hint: The removal of glucose units from glycogen does not change the glycogen concentration.) **(b)** The measured ratio $[P_i]$/[glucose 1-phosphate] in myocytes under physiological conditions is more than 100:1. What does this indicate about the direction of metabolite flow through the glycogen phosphorylase reaction in muscle? **(c)** Why are the equilibrium and physiological ratios different? What is the possible significance of this difference?

Answer

(a) First, we need to calculate the equilibrium constant (assuming a temperature of 25 °C):

$$\begin{aligned} \Delta G'^\circ &= -RT \ln K'_{eq} \\ \ln K'_{eq} &= -\Delta G'^\circ/RT \\ &= -(3.1 \text{ kJ/mol})/(2.48 \text{ kJ/mol}) \\ &= -1.2 \\ K'_{eq} &= e^{-1.2} = 0.30 \end{aligned}$$

For the glycogen phosphorylase reaction:

$$\text{Glycogen}_n + P_i \longrightarrow \text{glycogen}_{n-1} + \text{glucose 1-phosphate}$$

$$K'_{eq} = \frac{[\text{glycogen}_{n-1}][\text{glucose 1-phosphate}]}{[\text{glycogen}_n][P_i]}$$

where all reactants are at their equilibrium concentrations. Because the concentration of glycogen remains constant, these terms cancel and the expression becomes

$$K'_{eq} = \frac{[\text{glucose 1-phosphate}]}{[P_i]}$$

This may be arranged to

$$\frac{[P_i]}{[\text{glucose 1-phosphate}]} = \frac{1}{K'_{eq}} = \frac{1}{0.3} = 3.3$$

(b) and **(c)** The high $[P_i]$/[glucose 1-phosphate] ratio in myocytes (>100:1) means that [glucose 1-phosphate] is far below the equilibrium value. The rate at which phosphoglucomutase removes glucose 1-phosphate (by conversion to glucose 6-phosphate, which enters glycolysis) is greater than the rate at which glycogen phosphorylase can produce it. This indicates that the direction of metabolite flow is from glycogen to glucose-1-phosphate, and that the glycogen phosphorylase reaction is the rate-limiting step in glycogen breakdown.

8. Regulation of Glycogen Phosphorylase In muscle tissue, the rate of conversion of glycogen to glucose 6-phosphate is determined by the ratio of phosphorylase *a* (active) to phosphorylase *b* (less active). Determine what happens to the rate of glycogen breakdown if a muscle preparation containing glycogen phosphorylase is treated with **(a)** phosphorylase kinase and ATP; **(b)** PP1; **(c)** epinephrine.

Answer

(a) Treatment with the kinase and ATP converts glycogen phosphorylase to the more active, phosphorylated form, phosphorylase *a;* glycogen breakdown accelerates.

(b) Treatment with the phosphatase PP1 converts the active phosphorylase *a* to the less active phosphorylase *b;* glycogen breakdown slows.

(c) Addition of epinephrine to muscle tissue causes the synthesis of cyclic AMP, which activates phosphorylase kinase. The kinase converts phosphorylase *b* (less active) to phosphorylase *a* (more active); glycogen breakdown accelerates.

9. Glycogen Breakdown in Rabbit Muscle The intracellular use of glucose and glycogen is tightly regulated at four points. To compare the regulation of glycolysis when oxygen is plentiful and when it is depleted, consider the utilization of glucose and glycogen by rabbit leg muscle in two physiological settings: a resting rabbit, with low ATP demands, and a rabbit that sights its mortal enemy, the coyote, and dashes into its burrow. For each setting, determine the relative levels (high, intermediate, or low) of AMP, ATP, citrate, and acetyl-CoA and describe how these levels affect the flow of metabolites through glycolysis by regulating specific enzymes. In periods of stress, rabbit leg muscle produces much of its ATP by anaerobic glycolysis (lactate fermentation) and very little by oxidation of acetyl-CoA derived from fat breakdown.

Answer A primary role of glycolysis is the production of ATP, and the pathway is regulated to ensure efficient ATP formation. The utilization of glycogen and glucose to supply energy is regulated at the following steps: glycogen phosphorylase, phosphofructokinase-1, pyruvate kinase, and entry of acetyl-CoA into the citric acid cycle. In muscle, the primary regulatory metabolites are ATP, AMP, citrate, and acetyl-CoA. ATP is an inhibitor of glycogen phosphorylase and PFK-1; AMP stimulates both. Citrate inhibits PFK-1, and acetyl-CoA inhibits pyruvate kinase. Lack of O_2 leads to elevated levels of NADH, inhibiting pyruvate dehydrogenase and promoting fermentation of pyruvate to lactate.

Under resting conditions, [ATP] is high and [AMP] low because the total adenine nucleotide pool is constant. [Citrate] and [acetyl-CoA] are intermediate because O_2 is not limiting and the citric acid cycle is functioning. Under conditions of active exertion (running), O_2 becomes limiting and ATP synthesis decreases. Consequently, [ATP] is relatively low and [AMP] relatively high, compared with aerobic conditions. [Citrate] and [acetyl-CoA] are low. These changes release the inhibition of glycolysis and stimulate lactic acid production.

10. Glycogen Breakdown in Migrating Birds Unlike the rabbit with its short dash, migratory birds require energy for extended periods of time. For example, ducks generally fly several thousand miles during their annual migration. The flight muscles of migratory birds have a high oxidative capacity and obtain the necessary ATP through the oxidation of acetyl-CoA (obtained from fats) via the citric acid cycle. Compare the regulation of muscle glycolysis during short-term intense activity, as in the fleeing rabbit, and during extended activity, as in the migrating duck. Why must the regulation in these two settings be different?

Answer Migratory birds have a very efficient respiratory system to ensure that O_2 is available to flight muscles under stress (see Box 14–2). Birds also rely on the aerobic oxidation of fat, which produces the greatest amount of energy per gram of fuel. The migratory bird must regulate glycolysis so that glycogen is used only for short bursts of energy, not for the long-term stress of prolonged flight. The sprinting rabbit relies on breakdown of stored (liver) glycogen and anaerobic glycolysis for short-term production of ATP for muscle activity.

The regulation of these two means of ATP production is very different. Under aerobic conditions (see answer to Problem 9), glycolysis is inhibited by the relatively high [ATP], as acetyl-CoA units derived from fat feed into the citric acid cycle and ATP is produced by oxidative phosphorylation. Under anaerobic conditions, glycolysis is stimulated and metabolism of fats does not occur at an appreciable rate because [citrate] and [acetyl-CoA] are low and O_2 (the final acceptor of electrons in oxidative phosphorylation) is absent.

11. Enzyme Defects in Carbohydrate Metabolism Summaries of four clinical case studies follow. For each case, determine which enzyme is defective and designate the appropriate treatment, from the lists provided at the end of the problem. Justify your choices. Answer the questions contained in each case study. (You may need to refer to information in Chapter 14.)

Case A The patient develops vomiting and diarrhea shortly after milk ingestion. A lactose tolerance test is administered. (The patient ingests a standard amount of lactose, and the glucose and galactose concentrations of blood plasma are measured at intervals. In individuals with normal carbohydrate metabolism, the levels increase to a maximum in about 1 hour, then decline.) The patient's blood glucose and galactose concentrations do not increase during the test. Why do blood glucose and galactose increase and then decrease during the test in healthy individuals? Why do they fail to rise in the patient?

Case B The patient develops vomiting and diarrhea after ingestion of milk. His blood is found to have a low concentration of glucose but a much higher than normal concentration of reducing sugars. The urine tests positive for galactose. Why is the concentration of reducing sugar in the blood high? Why does galactose appear in the urine?

Case C The patient complains of painful muscle cramps when performing strenuous physical exercise but has no other symptoms. A muscle biopsy indicates a muscle glycogen concentration much higher than normal. Why does glycogen accumulate?

Case D The patient is lethargic, her liver is enlarged, and a biopsy of the liver shows large amounts of excess glycogen. She also has a lower than normal blood glucose level. What is the reason for the low blood glucose in this patient?

Defective Enzyme

(a) Muscle PFK-1

(b) Phosphomannose isomerase

(c) Galactose 1-phosphate uridylyltransferase

(d) Liver glycogen phosphorylase

(e) Triose kinase
(f) Lactase in intestinal mucosa
(g) Maltase in intestinal mucosa
(h) Muscle debranching enzyme

Treatment

1. Jogging 5 km each day
2. Fat-free diet
3. Low-lactose diet
4. Avoiding strenuous exercise
5. Large doses of niacin (the precursor of NAD^+)
6. Frequent feedings (smaller portions) of a normal diet

Answer

Case A: **(f)** Lactase in the intestinal mucosa hydrolyzes milk lactose to glucose and galactose, so the levels of these sugars increase transiently after milk ingestion. This increase would not occur in a person lacking lactase; the patient would show symptoms of lactose toxicity. The patient should exclude lactose (milk) from the diet (treatment **3**).

Case B: **(c)** Galactose 1-phosphate uridylyltransferase is an enzyme involved in conversion of galactose to glucose, which then can enter glycolysis. Absence of this enzyme leads to accumulation of galactose in the blood and excretion in the urine. A patient with this deficiency should be on a low-lactose diet (treatment **3**).

Case C: **(h)** Without the muscle form of glycogen phosphorylase, glycogen cannot be mobilized to supply ATP during extended exercise, and the result is weakness and cramping during the exercise. Because glycogen continues to be synthesized but is not mobilized, the muscle glycogen level is higher than normal. The patient should avoid strenuous exercise (treatment **4**).

Case D: **(d)** Liver glycogen functions as a source of blood glucose. Accumulation of glycogen and low blood glucose suggest that the liver glycogen phosphorylase is defective or of low activity. The patient should eat light meals regularly and frequently (treatment **6**).

12. Effects of Insufficient Insulin in a Person with Diabetes A man with insulin-dependent diabetes is brought to the emergency room in a near-comatose state. While vacationing in an isolated place, he lost his insulin medication and has not taken any insulin for two days.

(a) For each tissue listed below, is each pathway faster, slower, or unchanged in this patient, compared with the normal level when he is getting appropriate amounts of insulin?

(b) For each pathway, describe at least one control mechanism responsible for the change you predict.

Tissue and Pathways

1. Adipose: fatty acid synthesis
2. Muscle: glycolysis; fatty acid synthesis; glycogen synthesis
3. Liver: glycolysis; gluconeogenesis; glycogen synthesis; fatty acid synthesis; pentose phosphate pathway

Answer

(a) (1) Adipose: fatty acid synthesis slower. (2) Muscle: glycolysis, fatty acid synthesis, and glycogen synthesis slower. (3) Liver: glycolysis faster; gluconeogenesis, glycogen synthesis, and fatty acid synthesis slower; pentose phosphate pathway unchanged.

(b) (1) Adipose and (3) liver: fatty acid synthesis slower because lack of insulin results in inactive acetyl-CoA carboxylase, the first enzyme of fatty acid synthesis. Glycogen synthesis inhibited by cAMP-dependent phosphorylation (thus activation) of glycogen synthase. (2) Muscle: glycolysis slower because GLUT4 is inactive, so glucose uptake is inhibited. (3) Liver: glycolysis slower because the bifunctional PFK-2/FBPase-2 is

converted to the form with active FBPase-2, decreasing [fructose 2,6-bisphosphate], which allosterically stimulates phosphofructokinase and inhibits FBPase-1; this also accounts for the stimulation of gluconeogenesis.

13. Blood Metabolites in Insulin Insufficiency For the patient described in Problem 12, predict the levels of the following metabolites in his blood *before* treatment in the emergency room, relative to levels maintained during adequate insulin treatment: **(a)** glucose; **(b)** ketone bodies; **(c)** free fatty acids.

Answer

(a) Elevated; without insulin, muscle and other tissues cannot take up glucose, because the GLUT4 transporters do not move to the plasma membrane and ingested glucose remains in the blood.

(b) Elevated; accumulation of acetyl-CoA from fatty acid oxidation shifts the thiolase equilibrium toward acetoacetyl-CoA formation, the first step in ketone body formation.

(c) Elevated; to provide fuel for muscle and other tissues, fatty acids are mobilized from adipose and move through the blood to muscle and other tissues.

14. Metabolic Effects of Mutant Enzymes Predict and explain the effect on glycogen metabolism of each of the following defects caused by mutation: **(a)** loss of the cAMP-binding site on the regulatory subunit of protein kinase A (PKA); **(b)** loss of the protein phosphatase inhibitor (inhibitor 1 in Fig. 15–40); **(c)** overexpression of phosphorylase *b* kinase in liver; **(d)** defective glucagon receptors in liver.

Answer

(a) PKA cannot be activated in response to glucagon or epinephrine, and glycogen phosphorylase is not activated.

(b) PP1 remains active, allowing it to dephosphorylate glycogen synthase (activating it) and glycogen phosphorylase (inhibiting it). Glycogen synthesis is stimulated, and glycogen breakdown inhibited.

(c) Phosphorylase remains phosphorylated (active), increasing the breakdown of glycogen and thus depleting the store of liver glycogen.

(d) Gluconeogenesis cannot be stimulated when blood glucose is low, leading to dangerously low blood glucose during periods of fasting.

15. Hormonal Control of Metabolic Fuel Between your evening meal and breakfast, your blood glucose drops and your liver becomes a net producer rather than consumer of glucose. Describe the hormonal basis for this switch, and explain how the hormonal change triggers glucose production by the liver.

Answer The drop in blood glucose triggers release of glucagon by the pancreas. In the liver, glucagon activates glycogen phosphorylase by stimulating its cAMP-dependent phosphorylation and stimulates gluconeogenesis by lowering [fructose 2,6-bisphosphate], thus stimulating FBPase-1, a key enzyme in gluconeogenesis.

16. Altered Metabolism in Genetically Manipulated Mice Researchers can manipulate the genes of a mouse so that a single gene in a single tissue either produces an inactive protein (a "knockout" mouse) or produces a protein that is always (constitutively) active. What effects on metabolism would you predict for mice with the following genetic changes: **(a)** knockout of glycogen debranching enzyme in the liver; **(b)** knockout of hexokinase IV in liver; **(c)** knockout of FBPase-2 in liver; **(d)** constitutively active FBPase-2 in liver; **(e)** constitutively active AMPK in muscle; **(f)** constitutively active ChREBP in liver?

Answer

(a) Reduced capacity to mobilize glycogen; lowered blood glucose between meals

(b) Reduced capacity to lower blood glucose after a carbohydrate meal; elevated blood glucose (see Fig. 15–12)

(c) Reduced fructose 2,6-bisphosphate (F26BP) in liver, stimulating glycolysis and inhibiting gluconeogenesis (see Fig. 15–15)

(d) Reduced F26BP, stimulating gluconeogenesis and inhibiting glycolysis

(e) Increased uptake of fatty acids and glucose; increased oxidation of both (see Fig. 15–6)

(f) Increased conversion of pyruvate to acetyl-CoA; increased fatty acid synthesis

Data Analysis Problem

17. Optimal Glycogen Structure Muscle cells need rapid access to large amounts of glucose during heavy exercise. This glucose is stored in liver and skeletal muscle in polymeric form as particles of glycogen. The typical glycogen particle contains about 55,000 glucose residues (see Fig. 15–33b). Meléndez-Hevia, Waddell, and Shelton (1993) explored some theoretical aspects of the structure of glycogen, as described in this problem.

(a) The cellular concentration of glycogen in liver is about 0.01 μM. What cellular concentration of free glucose would be required to store an equivalent amount of glucose? Why would this concentration of free glucose present a problem for the cell?

Glucose is released from glycogen by glycogen phosphorylase, an enzyme that can remove glucose molecules, one at a time, from one end of a glycogen chain. Glycogen chains are branched (see Figs 15–26 and 15–33b), and the degree of branching—the number of branches per chain—has a powerful influence on the rate at which glycogen phosphorylase can release glucose.

(b) Why would a degree of branching that was too low (i.e., below an optimum level) reduce the rate of glucose release? (Hint: Consider the extreme case of no branches in a chain of 55,000 glucose residues.)

(c) Why would a degree of branching that was too high also reduce the rate of glucose release? (Hint: Think of the physical constraints.)

Meléndez-Hevia and colleagues did a series of calculations and found that two branches per chain (see Fig. 15–33b) was optimal for the constraints described above. This is what is found in glycogen stored in muscle and liver.

To determine the optimum number of glucose residues per chain, Meléndez-Hevia and coauthors considered two key parameters that define the structure of a glycogen particle: t = the number of tiers of glucose chains in a particle (the molecule in Fig. 15–33b has five tiers); g_c = the number of glucose residues in each chain. They set out to find the values of t and g_c that would maximize three quantities: (1) the amount of glucose stored in the particle (G_T) per unit volume; (2) the number of unbranched glucose chains (C_A) per unit volume (i.e., number of chains in the outermost tier, readily accessible to glycogen phosphorylase); and (3) the amount of glucose available to phosphorylase in these unbranched chains (G_{PT}).

(d) Show that $C_A = 2^{t-1}$. This is the number of chains available to glycogen phosphorylase before the action of the debranching enzyme.

(e) Show that C_T, the total number of chains in the particle, is given by $C_T = 2^t - 1$. Thus $G_T = g_c(C_T) = g_c(2^t - 1)$, the total number of glucose residues in the particle.

(f) Glycogen phosphorylase cannot remove glucose from glycogen chains that are shorter than five glucose residues. Show that $G_{PT} = (g_c - 4)(2^{t-1})$. This is the amount of glucose readily available to glycogen phosphorylase.

(g) Based on the size of a glucose residue and the location of branches, the thickness of one tier of glycogen is $0.12g_c$ nm + 0.35 nm. Show that the volume of a particle, V_s, is given by the equation $V_s = \frac{4}{3}\pi t^3(0.12g_c + 0.35)^3$ nm^3.

Meléndez-Hevia and coauthors then determined the optimum values of t and g_c—those that gave the maximum value of a quality function, f, that maximizes G_T, C_A, and G_{PT}, while minimizing V_S: $f = \dfrac{G_T C_A G_{PT}}{V_s}$. They found that the optimum value of g_c is independent of t.

(h) Choose a value of t between 5 and 15 and find the optimum value of g_c. How does this compare with the g_c found in liver glycogen (see Fig. 15–33b)? (Hint: You may find it useful to use a spreadsheet program.)

Answer

(a) Given that each particle contains about 55,000 glucose residues, the equivalent free glucose concentration would be $55{,}000 \times 0.01\ \mu\text{M} = 550$ mM, or 0.55 M. This would present a serious osmotic challenge for the cell! (Body fluids have a substantially lower osmolarity.)

(b) The lower the number of branches, the lower the number of free ends available for glycogen phosphorylase activity, and the slower the rate of glucose release. With no branches, there would be just one site for phosphorylase to act.

(c) The outer tier of the particle would be too crowded with glucose residues for the enzyme to gain access to cleave bonds and release glucose.

(d) The number of chains doubles in each succeeding tier: tier 1 has one chain (2^0), tier 2 has two (2^1), tier 3 has four (2^2), and so on. Thus, for t tiers, the number of chains in the outermost tier, C_A, is 2^{t-1}.

(e) The total number of chains is $2^0 + 2^1 + 2^2 + \ldots 2^{t-1} = 2^t - 1$. Each chain contains g_c glucose molecules, so the total number of glucose molecules, C_T, is $g_c(2^t - 1)$.

(f) Glycogen phosphorylase can release all but four of the glucose residues in a chain of length g_c. Therefore, from each chain in the outer tier it can release $(g_c - 4)$ glucose molecules. Given that there are 2^{t-1} chains in the outer tier, the number of glucose molecules the enzyme can release, G_{PT}, is $(g_c - 4)(2^{t-1})$.

(g) The volume of a sphere is $\frac{4}{3}\pi r^3$. In this case, r is the thickness of one tier times the number of tiers, or $(0.12g_c + 0.35)t$ nm. Thus $V_s = \frac{4}{3}\pi t^3(0.12g_c + 0.35)^3$ nm^3.

(h) You can show algebraically that the value of g_c that maximizes f is independent of t. Choosing $t = 3$:

g_c	C_A	G_T	G_{PT}	V_s	f
5	4	35	4	11	5.8
6	4	42	8	19	9.7
7	4	49	12	24	12
8	4	56	16	28	14
9	4	63	20	32	15
10	4	70	24	34	16
11	4	77	28	36	16
12	4	84	32	38	17
13	4	91	36	40	17
14	4	98	40	41	17
15	4	100	44	42	16
16	4	110	48	43	16

The optimum value of g_c (i.e., at maximum f) is 13. In nature, g_c varies from 12 to 14, which corresponds to f values very close to the optimum. If you choose another value for t, the numbers will differ but the optimal g_c will still be 13.

Reference

Meléndez-Hevia, E., Waddell, T.G., & Shelton, E.D. (1993) Optimization of molecular design in the evolution of metabolism: the glycogen molecule. *Biochem.* J. **295,** 477–483.

chapter

16

The Citric Acid Cycle

1. Balance Sheet for the Citric Acid Cycle The citric acid cycle has eight enzymes: citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinyl-CoA synthetase, succinate dehydrogenase, fumarase, and malate dehydrogenase.

(a) Write a balanced equation for the reaction catalyzed by each enzyme.

(b) Name the cofactor(s) required by each enzyme reaction.

(c) For each enzyme determine which of the following describes the type of reaction(s) catalyzed: condensation (carbon–carbon bond formation); dehydration (loss of water); hydration (addition of water); decarboxylation (loss of CO_2); oxidation-reduction; substrate-level phosphorylation; isomerization.

(d) Write a balanced net equation for the catabolism of acetyl-CoA to CO_2.

Answer

Citrate synthase

(a) Acetyl-CoA + oxaloacetate + $H_2O \longrightarrow$ citrate + CoA

(b) CoA

(c) Condensation

Aconitase

(a) Citrate $\longrightarrow$ isocitrate

(b) No cofactors

(c) Isomerization

Isocitrate dehydrogenase

(a) Isocitrate + $NAD^+ \longrightarrow$ α-ketoglutarate + CO_2 + NADH

(b) NAD^+

(c) Oxidative decarboxylation

α-Ketoglutarate dehydrogenase

(a) α-Ketoglutarate + NAD^+ + CoA $\longrightarrow$ succinyl-CoA + CO_2 + NADH

(b) NAD^+, CoA, thiamine pyrophosphate

(c) Oxidative decarboxylation

Succinyl-CoA synthetase

(a) Succinyl-CoA + P_i + GDP $\longrightarrow$ succinate + CoA + GTP

(b) CoA

(c) Substrate-level phosphorylation and acyl transfer

Succinate dehydrogenase

(a) Succinate + FAD $\longrightarrow$ fumarate + $FADH_2$

(b) FAD

(c) Oxidation

Fumarase

(a) Fumarate + H_2O $\longrightarrow$ malate

(b) No cofactors

(c) Hydration

Malate dehydrogenase

(a) Malate + NAD^+ $\longrightarrow$ oxaloacetate + NADH + H^+

(b) NAD^+

(c) Oxidation

(d) The net equation for the catabolism of acetyl-CoA is

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + P_i + 2H_2O \longrightarrow 2CO_2 + CoA + 3NADH + FADH_2 + GTP + 2H^+$$

2. Net Equation for Glycolysis and the Citric Acid Cycle Write the net biochemical equation for the metabolism of a molecule of glucose by glycolysis and the citric acid cycle, including all cofactors.

Answer

Glycolysis:

$$\text{Glucose} + 2ADP + 2P_i + 2NAD^+ \longrightarrow 2ATP + 2NADH + 2 \text{ pyruvate}$$

Pyruvate dehydrogenase:

$$2 \text{ Pyruvate} + 2NAD^+ + 2CoASH \longrightarrow 2 \text{ acetyl-CoA} + 2CO_2 + 2NADH$$

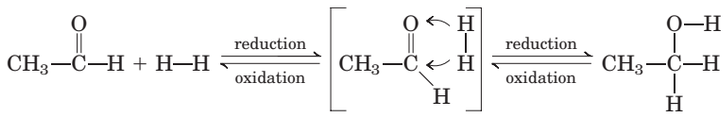
Citric acid cycle:

$$2 \text{ Acetyl-CoA} + 2FAD + 6NAD^+ + 2ADP + 2P_i \longrightarrow 2CoASH + 2FADH_2 + 6NADH + 2ATP + 4CO_2$$

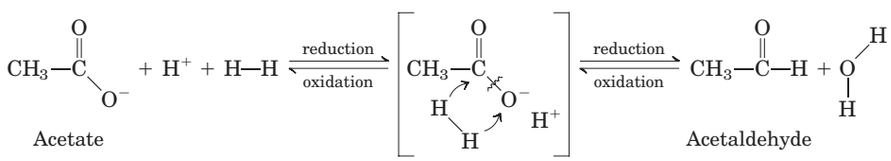
Overall:

$$\text{Glucose} + 4ADP + 4P_i + 10NAD^+ + 2FAD \longrightarrow 4ATP + 10NADH + 2FADH_2 + 6CO_2$$

3. Recognizing Oxidation and Reduction Reactions One biochemical strategy of many living organisms is the stepwise oxidation of organic compounds to CO_2 and H_2O and the conservation of a major part of the energy thus produced in the form of ATP. It is important to be able to recognize oxidation-reduction processes in metabolism. Reduction of an organic molecule results from the hydrogenation of a double bond (Eqn 1, below) or of a single bond with accompanying cleavage (Eqn 2). Conversely, oxidation results from dehydrogenation. In biochemical redox reactions, the coenzymes NAD and FAD dehydrogenate/hydrogenate organic molecules in the presence of the proper enzymes.

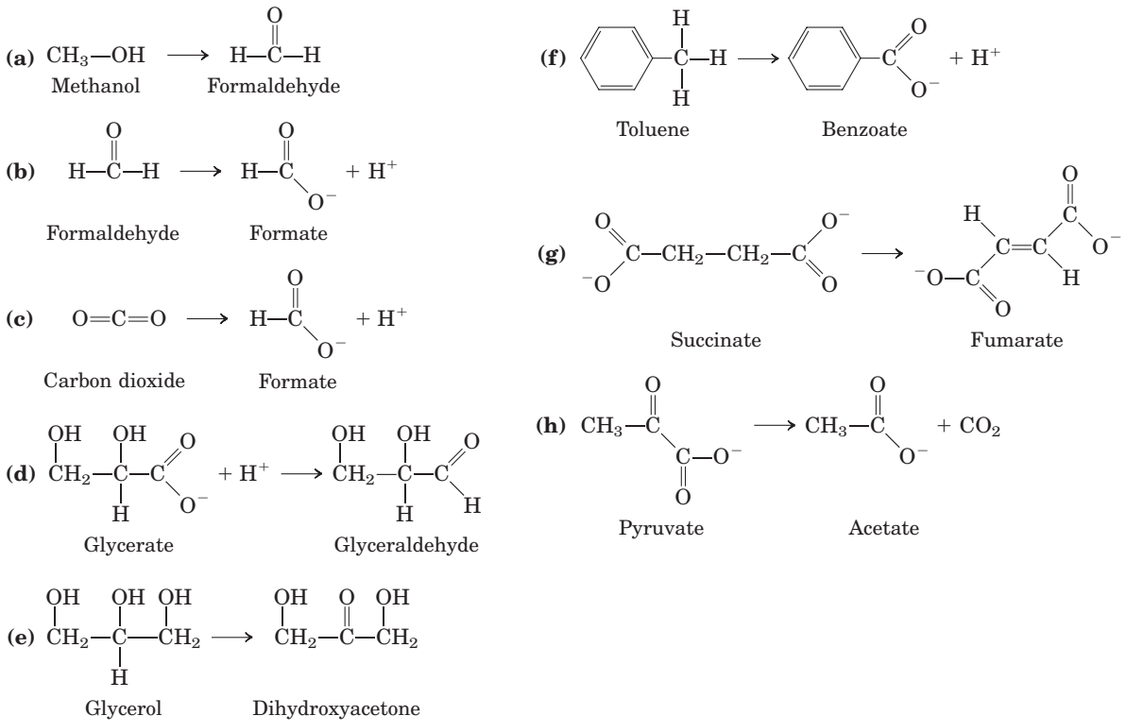


Acetaldehyde Ethanol (1)



Acetate Acetaldehyde (2)

For each of the metabolic transformations in **(a)** through **(h)**, determine whether oxidation or reduction has occurred. Balance each transformation by inserting H—H and, where necessary, H_2O.



Answer Keep in mind that oxidation is the loss of electrons and accompanying H^+, whereas reduction is the gain of electrons (or H—H).

(a) Oxidation: Methanol ⟶ formaldehyde + H—H

(b) Oxidation: Formaldehyde ⟶ formate + H—H

(c) Reduction: CO_2 + H—H ⟶ formate + H^+

(d) Reduction: Glycerate + H—H + H^+ ⟶ glyceraldehyde + H_2O

(e) Oxidation: Glycerol ⟶ dihydroxyacetone + H—H

(f) Oxidation: Toluene + $2H_2O$ ⟶ benzoate + H^+ + 3H—H

(g) Oxidation: Succinate ⟶ fumarate + H—H

(h) Oxidation: Pyruvate + H_2O ⟶ acetate + CO_2 + H—H

4. **Relationship between Energy Release and the Oxidation State of Carbon** A eukaryotic cell can use glucose ($C_6H_{12}O_6$) and hexanoic acid ($C_6H_{14}O_2$) as fuels for cellular respiration. On the basis of their structural formulas, which substance releases more energy per gram on complete combustion to CO_2 and H_2O?

Answer From the structural formulas, we see that the carbon-bound H/C ratio of hexanoic acid (11/6) is higher than that of glucose (7/6). Hexanoic acid is more reduced and yields more energy upon complete combustion to CO_2 and H_2O.

5. **Nicotinamide Coenzymes as Reversible Redox Carriers** The nicotinamide coenzymes (see Fig. 13–24) can undergo reversible oxidation-reduction reactions with specific substrates in the presence of the appropriate dehydrogenase. In these reactions, NADH + H^+ serves as the hydrogen

source, as described in Problem 3. Whenever the coenzyme is oxidized, a substrate must be simultaneously reduced:

$$\underset{\text{Oxidized}}{\text{Substrate}} + \underset{\text{Reduced}}{\text{NADH}} + \text{H}^+ \rightleftharpoons \underset{\text{Reduced}}{\text{product}} + \underset{\text{Oxidized}}{\text{NAD}^+}$$

For each of the reactions in **(a)** through **(f)**, determine whether the substrate has been oxidized or reduced or is unchanged in oxidation state (see Problem 3). If a redox change has occurred, balance the reaction with the necessary amount of NAD^+, NADH, H^+, and H_2O. The objective is to recognize when a redox coenzyme is necessary in a metabolic reaction.

(a) $CH_3CH_2OH \longrightarrow CH_3-C(=O)-H$

Ethanol — Acetaldehyde

(b) $^{2-}O_3PO-CH_2-CH(OH)-C(=O)-OPO_3^{2-} \longrightarrow$

1,3-Bisphosphoglycerate

$^{2-}O_3PO-CH_2-CH(OH)-C(=O)-H + HPO_4^{2-}$

Glyceraldehyde 3-phosphate

(c) $CH_3-C(=O)-COO^- \longrightarrow CH_3-C(=O)-H + CO_2$

Pyruvate — Acetaldehyde

(d) $CH_3-C(=O)-COO^- \longrightarrow CH_3-C(=O)-O^- + CO_2$

Pyruvate — Acetate

(e) $^-OOC-CH_2-C(=O)-COO^- \longrightarrow {}^-OOC-CH_2-CH(OH)-COO^-$

Oxaloacetate — Malate

(f) $CH_3-C(=O)-CH_2-C(=O)-O^- + H^+ \longrightarrow CH_3-C(=O)-CH_3 + CO_2$

Acetoacetate — Acetone

Answer

(a) Oxidized: Ethanol + $NAD^+ \longrightarrow$ acetaldehyde + NADH + H^+

(b) Reduced: 1,3-Bisphosphoglycerate + NADH + $H^+ \longrightarrow$ glyceraldehyde 3-phosphate + NAD^+ + HPO_4^{2-}

(c) Unchanged: Pyruvate + $H^+ \longrightarrow$ acetaldehyde + CO_2

(d) Oxidized: Pyruvate + $NAD^+ \longrightarrow$ acetate + CO_2 + NADH + H^+

(e) Reduced: Oxaloacetate + NADH + $H^+ \longrightarrow$ malate + NAD^+

(f) Unchanged: Acetoacetate + $H^+ \longrightarrow$ acetone + CO_2

6. Pyruvate Dehydrogenase Cofactors and Mechanism Describe the role of each cofactor involved in the reaction catalyzed by the pyruvate dehydrogenase complex.

Answer *TPP:* thiazolium ring adds to α carbon of pyruvate, then stabilizes the resulting carbanion by acting as an electron sink. *Lipoic acid:* oxidizes pyruvate to level of acetate (acetyl-CoA), and activates acetate as a thioester. *CoA-SH:* activates acetate as thioester. *FAD*: oxidizes lipoic acid. *NAD^+*: oxidizes FAD. (See Fig. 16–6.)

7. Thiamine Deficiency Individuals with a thiamine-deficient diet have relatively high levels of pyruvate in their blood. Explain this in biochemical terms.

Answer Thiamine is essential for the formation of thiamine pyrophosphate (TPP), one of the cofactors in the pyruvate dehydrogenase reaction. Without TPP, the pyruvate generated by glycolysis accumulates in cells and enters the blood.

8. Isocitrate Dehydrogenase Reaction What type of chemical reaction is involved in the conversion of isocitrate to α-ketoglutarate? Name and describe the role of any cofactors. What other reaction(s) of the citric acid cycle are of this same type?

Answer Oxidative decarboxylation involving $NADP^+$ or NAD^+ as the electron acceptor; the α-ketoglutarate dehydrogenase reaction is also an oxidative decarboxylation, but its mechanism is different and involves different cofactors: TPP, lipoate, FAD, NAD^+, and CoA-SH.

9. Stimulation of Oxygen Consumption by Oxaloacetate and Malate In the early 1930s, Albert Szent-Györgyi reported the interesting observation that the addition of small amounts of oxaloacetate or malate to suspensions of minced pigeon breast muscle stimulated the oxygen consumption of the preparation. Surprisingly, the amount of oxygen consumed was about seven times more than the amount necessary for complete oxidation (to CO_2 and H_2O) of the added oxaloacetate or malate. Why did the addition of oxaloacetate or malate stimulate oxygen consumption? Why was the amount of oxygen consumed so much greater than the amount necessary to completely oxidize the added oxaloacetate or malate?

Answer Oxygen consumption is a measure of the activity of the first two stages of cellular respiration: glycolysis and the citric acid cycle. Initial nutrients being oxidized are carbohydrates and lipids. Because several intermediates of the citric acid cycle can be siphoned off into biosynthetic pathways, the cycle may slow down for lack of oxaloacetate in the citrate synthase reaction, and acetyl-CoA will accumulate. Addition of oxaloacetate or malate (converted to oxaloacetate by malate dehydrogenase) will stimulate the cycle and allow it to use the accumulated acetyl-CoA. This stimulates respiration. Oxaloacetate is regenerated in the cycle, so addition of oxaloacetate (or malate) stimulates the oxidation of a much larger amount of acetyl-CoA.

10. Formation of Oxaloacetate in a Mitochondrion In the last reaction of the citric acid cycle, malate is dehydrogenated to regenerate the oxaloacetate necessary for the entry of acetyl-CoA into the cycle:

$$\text{L-Malate} + NAD^+ \longrightarrow \text{oxaloacetate} + NADH + H^+ \qquad \Delta G'^\circ = 30.0 \text{ kJ/mol}$$

(a) Calculate the equilibrium constant for this reaction at 25 °C.

(b) Because $\Delta G'^\circ$ assumes a standard pH of 7, the equilibrium constant calculated in **(a)** corresponds to

$$K'_{eq} = \frac{[\text{oxaloacetate}][NADH]}{[\text{L-malate}][NAD^+]}$$

The measured concentration of L-malate in rat liver mitochondria is about 0.20 mM when $[NAD^+]/[NADH]$ is 10. Calculate the concentration of oxaloacetate at pH 7 in these mitochondria.

(c) To appreciate the magnitude of the mitochondrial oxaloacetate concentration, calculate the number of oxaloacetate molecules in a single rat liver mitochondrion. Assume the mitochondrion is a sphere of diameter 2.0 μm.

Answer

(a) $\Delta G'^\circ = -RT \ln K'_{eq}$

$\ln K'_{eq} = -\Delta G'^\circ/RT$

$= -(30.0 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$

$= -12.1$

$K'_{eq} = e^{-12.1} = 5.6 \times 10^{-6}$

(b) Given that

$$K'_{eq} = ([\text{OAA}]_{eq}[\text{NADH}]_{eq})/([\text{malate}]_{eq}[\text{NAD}^+]_{eq})$$

if we hold the values of [malate], [NADH], and [NAD^+] at the values that exist in the cell, we can calculate what [oxaloacetate] must be at equilibrium to give the equilibrium constant calculated in **(a):**

$$\begin{aligned}[\text{oxaloacetate}] &= K'_{eq}\,[\text{malate}][\text{NAD}^+]/[\text{NADH}] \\ &= (5.6 \times 10^{-6})(0.20 \text{ mM})(10) \\ &= 1.1 \times 10^{-5} \text{ mM} = 1.1 \times 10^{-8} \text{ M}\end{aligned}$$

This predicts that [oxaloacetate] at equilibrium would be very low, and the measured concentration is indeed low: less than 10^{-7} M.

(c) The volume of a sphere is $\frac{4}{3}\pi r^3$, thus the volume of a mitochondrion ($r = 1.0 \times 10^{-3}$ mm) is

$$\tfrac{4}{3}(3.14)(1.0 \times 10^{-3} \text{ mm})^3 = 4.2 \times 10^{-9} \text{ mm}^3 = 4.2 \times 10^{-15} \text{ L}$$

Given the concentration of oxaloacetate and Avogadro's number, we can calculate the number of molecules in a mitochondrion:

$$(1.1 \times 10^{-8} \text{ mol/L})(6.02 \times 10^{23} \text{ molecules/mol})(4.2 \times 10^{-15} \text{ L}) = 28 \text{ molecules}$$

11. Cofactors for the Citric Acid Cycle Suppose you have prepared a mitochondrial extract that contains all of the soluble enzymes of the matrix but has lost (by dialysis) all the low molecular weight cofactors. What must you add to the extract so that the preparation will oxidize acetyl-CoA to CO_2?

Answer ADP (or GDP), P_i, CoA-SH, TPP, NAD^+; *not* lipoic acid, which is covalently attached to the isolated enzymes that use it (see Fig. 16–7).

12. Riboflavin Deficiency How would a riboflavin deficiency affect the functioning of the citric acid cycle? Explain your answer.

Answer The flavin nucleotides, FMN and FAD, would not be synthesized. Because FAD is required by the citric acid cycle enzyme succinate dehydrogenase, flavin deficiency would strongly inhibit the cycle.

13. Oxaloacetate Pool What factors might decrease the pool of oxaloacetate available for the activity of the citric acid cycle? How can the pool of oxaloacetate be replenished?

Answer Oxaloacetate might be withdrawn for aspartate synthesis or for gluconeogenesis. Oxaloacetate is replenished by the anaplerotic reactions catalyzed by PEP carboxykinase, PEP carboxylase, malic enzyme, or pyruvate carboxylase (see Fig. 16–15, p. 632).

14. Energy Yield from the Citric Acid Cycle The reaction catalyzed by succinyl-CoA synthetase produces the high-energy compound GTP. How is the free energy contained in GTP incorporated into the cellular ATP pool?

Answer The terminal phosphoryl group in GTP can be transferred to ADP in a reaction catalyzed by nucleoside diphosphate kinase, with an equilibrium constant of 1.0:

$$GTP + ADP \longrightarrow GDP + ATP$$

15. Respiration Studies in Isolated Mitochondria Cellular respiration can be studied in isolated mitochondria by measuring oxygen consumption under different conditions. If 0.01 M sodium malonate is added to actively respiring mitochondria that are using pyruvate as fuel source, respiration soon stops and a metabolic intermediate accumulates.

(a) What is the structure of this intermediate?

(b) Explain why it accumulates.

(c) Explain why oxygen consumption stops.

(d) Aside from removal of the malonate, how can this inhibition of respiration be overcome? Explain.

Answer Malonate is a structural analog of succinate and a competitive inhibitor of succinate dehydrogenase.

(a) Succinate: $^{-}OOC—CH_2—CH_2—COO^{-}$

(b) When succinate dehydrogenase is inhibited, succinate accumulates.

(c) Inhibition of any reaction in a pathway causes the substrate of that reaction to accumulate. Because this substrate is also the product of the preceding reaction, its accumulation changes the effective ΔG of that reaction, and so on for all the preceding steps in the pathway. The net rate of the pathway (or cycle) slows and eventually becomes almost negligible. In the case of the citric acid cycle, ceasing to produce the primary product, NADH, has the effect of stopping electron transfer and consumption of oxygen, the final acceptor of electrons derived from NADH.

(d) Because malonate is a competitive inhibitor, the addition of large amounts of succinate will overcome the inhibition.

16. Labeling Studies in Isolated Mitochondria The metabolic pathways of organic compounds have often been delineated by using a radioactively labeled substrate and following the fate of the label.

(a) How can you determine whether glucose added to a suspension of isolated mitochondria is metabolized to CO_2 and H_2O?

(b) Suppose you add a brief pulse of [3-^{14}C] pyruvate (labeled in the methyl position) to the mitochondria. After one turn of the citric acid cycle, what is the location of the ^{14}C in the oxaloacetate? Explain by tracing the ^{14}C label through the pathway. How many turns of the cycle are required to release all the [3-^{14}C] pyruvate as CO_2?

Answer

(a) If you added uniformly labeled glucose (^{14}C in all carbon atoms), release of labeled CO_2 would indicate that the glucose is metabolized to CO_2 and H_2O.

(b) One turn of the cycle produces oxaloacetate with label equally distributed between C-2 and C-3. The route of the label is from C-3 in pyruvate, to C-2 in acetyl-CoA, to a methylene (—CH_2—) carbon, C-2 or C-4 (see Fig. 16–7), in intermediates to succinate, which is symmetric; from succinate, the label is in C-2 or C-3. The second turn of the cycle releases half the label, and every subsequent turn releases half of what remains, so an infinite number of turns are required to release *all* the labeled carbon.

17. Pathway of CO_2 in Gluconeogenesis In the first bypass step of gluconeogenesis, the conversion of pyruvate to phosphoenolpyruvate (PEP), pyruvate is carboxylated by pyruvate carboxylase to oxaloacetate, which is subsequently decarboxylated to PEP by PEP carboxykinase (Chapter 14). Because the addition of CO_2 is directly followed by the loss of CO_2, you might expect that in tracer experiments, the ^{14}C of $^{14}CO_2$ would not be incorporated into PEP, glucose, or any intermediates in gluconeogenesis.

However, investigators find that when a rat liver preparation synthesizes glucose in the presence of $^{14}CO_2$, ^{14}C slowly appears in PEP and eventually at C-3 and C-4 of glucose. How does the ^{14}C label get into the PEP and glucose? (Hint: During gluconeogenesis in the presence of $^{14}CO_2$, several of the four-carbon citric acid cycle intermediates also become labeled.)

Answer Because pyruvate carboxylase is a mitochondrial enzyme, the [^{14}C]oxaloacetate (OAA) formed by this reaction mixes with the OAA pool of the citric acid cycle. A mixture of [1-^{14}C] and [4-^{14}C] OAA eventually forms by randomization of the C-1 and C-4 positions in the reversible conversions OAA → malate → succinate. [1-^{14}C] OAA leads to formation of [3,4-^{14}C]glucose.

18. [1-^{14}C]Glucose Catabolism An actively respiring bacterial culture is briefly incubated with [1-^{14}C] glucose, and the glycolytic and citric acid cycle intermediates are isolated. Where is the ^{14}C in each of the intermediates listed below? Consider only the initial incorporation of ^{14}C, in the first pass of labeled glucose through the pathways.

(a) Fructose 1,6-bisphosphate
(b) Glyceraldehyde 3-phosphate
(c) Phosphoenolpyruvate
(d) Acetyl-CoA
(e) Citrate
(f) α-Ketoglutarate
(g) Oxaloacetate

Answer Figures 14–2, 14–6, and 16–7 and Box 16–3 outline the fate of all the carbon atoms of glucose. In one pass through the pathways, the label appears at:

(a) C-1
(b) C-3
(c) C-3
(d) C-2 (methyl group)
(e) C-2 (see Box 16–3)
(f) C-4
(g) Equally distributed in C-2 and C-3

19. Role of the Vitamin Thiamine People with beriberi, a disease caused by thiamine deficiency, have elevated levels of blood pyruvate and α-ketoglutarate, especially after consuming a meal rich in glucose. How are these effects related to a deficiency of thiamine?

Answer Thiamine is required for the synthesis of thiamin pyrophosphate (TPP), a prosthetic group in the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes. A thiamin deficiency reduces the activity of these enzyme complexes and causes the observed accumulation of precursors.

20. Synthesis of Oxaloacetate by the Citric Acid Cycle Oxaloacetate is formed in the last step of the citric acid cycle by the NAD^+-dependent oxidation of L-malate. Can a net synthesis of oxaloacetate from acetyl-CoA occur using only the enzymes and cofactors of the citric acid cycle, without depleting the intermediates of the cycle? Explain. How is oxaloacetate that is lost from the cycle (to biosynthetic reactions) replenished?

Answer In the citric acid cycle, the entering acetyl-CoA combines with oxaloacetate to form citrate. One turn of the cycle regenerates oxaloacetate and produces two CO_2 molecules. There is *no* net synthesis of oxaloacetate in the cycle. If any cycle intermediates are channeled into biosynthetic reactions, replenishment of oxaloacetate is essential. Four enzymes can

produce oxaloacetate (or malate) from pyruvate or phosphoenolpyruvate. Pyruvate carboxylase (liver, kidney) and PEP carboxykinase (heart, skeletal muscle) are the most important in animals, and PEP carboxylase is most important in plants, yeast and bacteria. Malic enzyme produces malate from pyruvate in many organisms (see Table 16–2).

21. Oxaloacetate Depletion Mammalian liver can carry out gluconeogenesis using oxaloacetate as the starting material (Chapter 14). Would the operation of the citric acid cycle be affected by extensive use of oxaloacetate for gluconeogenesis? Explain your answer.

Answer Oxaloacetate depletion would tend to inhibit the citric acid cycle. Oxaloacetate is present at relatively low concentrations in mitochondria, and removing it for gluconeogenesis would tend to shift the equilibrium for the citrate synthase reaction toward oxaloacetate. However, anaplerotic reactions (see Fig. 16–15) counter this effect by replacing oxaloacetate.

22. Mode of Action of the Rodenticide Fluoroacetate Fluoroacetate, prepared commercially for rodent control, is also produced by a South African plant. After entering a cell, fluoroacetate is converted to fluoroacetyl-CoA in a reaction catalyzed by the enzyme acetate thiokinase:

$$F{-}CH_2COO^- + CoA\text{-}SH + ATP \longrightarrow F{-}CH_2\underset{\underset{O}{\|}}{C}{-}S\text{-}CoA + AMP + PP_i$$

The toxic effect of fluoroacetate was studied in an experiment using intact isolated rat heart. After the heart was perfused with 0.22 mM fluoroacetate, the measured rate of glucose uptake and glycolysis decreased, and glucose 6-phosphate and fructose 6-phosphate accumulated. Examination of the citric acid cycle intermediates revealed that their concentrations were below normal, except for citrate, with a concentration 10 times higher than normal.

(a) Where did the block in the citric acid cycle occur? What caused citrate to accumulate and the other cycle intermediates to be depleted?

(b) Fluoroacetyl-CoA is enzymatically transformed in the citric acid cycle. What is the structure of the end product of fluoroacetate metabolism? Why does it block the citric acid cycle? How might the inhibition be overcome?

(c) In the heart perfusion experiments, why did glucose uptake and glycolysis decrease? Why did hexose monophosphates accumulate?

(d) Why is fluoroacetate poisoning fatal?

Answer

(a) The block occurs at the aconitase reaction, which normally converts citrate to isocitrate.

(b) Fluoroacetate, an analog of acetate, can be activated to fluoroacetyl-CoA, which condenses with oxaloacetate to form fluorocitrate—the end product of fluoroacetate metabolism. Fluorocitrate is a structural analog of citrate and a strong competitive inhibitor of aconitase. The inhibition can be overcome by addition of large amounts of citrate.

(c) Citrate and fluorocitrate are allosteric inhibitors of phosphofructokinase-1, and as their concentration increases, glycolysis and glucose uptake slow down. Inhibition of PFK-1 causes the accumulation of glucose 6-phosphate and fructose 6-phosphate.

(d) The net effect of fluoroacetate poisoning is to shut down ATP synthesis, aerobic (oxidative) and anaerobic (fermentative).

23. Synthesis of L-Malate in Wine Making The tartness of some wines is due to high concentrations of L-malate. Write a sequence of reactions showing how yeast cells synthesize L-malate from glucose under anaerobic conditions in the presence of dissolved CO_2 (HCO_3^-). Note that the overall reaction for this fermentation cannot involve the consumption of nicotinamide coenzymes or citric acid cycle intermediates.

Answer The glycolytic reactions

$$\text{Glucose} + 2P_i + 2ADP + 2NAD^+ \longrightarrow 2 \text{ pyruvate} + 2ATP + 2NADH + 2H^+ + 2H_2O$$

are followed by the pyruvate carboxylase reaction

$$2 \text{ Pyruvate} + 2CO_2 + 2ATP + 2H_2O \longrightarrow 2 \text{ oxaloacetate} + 2ADP + 2P_i + 4H^+$$

In the citric acid cycle, the malate dehydrogenase reaction

$$2 \text{ Oxaloacetate} + 2NADH + 2H^+ \longrightarrow 2 \text{ L-malate} + 2NAD^+$$

recycles nicotinamide coenzymes under anaerobic conditions. The overall reaction is

$$\text{Glucose} + 2CO_2 \longrightarrow 2 \text{ L-malate} + 4H^+$$

which produces four H^+ per glucose, increasing the acidity and thus the tartness of the wine.

24. Net Synthesis of α-Ketoglutarate α-Ketoglutarate plays a central role in the biosynthesis of several amino acids. Write a sequence of enzymatic reactions that could result in the net synthesis of α-ketoglutarate from pyruvate. Your proposed sequence must not involve the net consumption of other citric acid cycle intermediates. Write an equation for the overall reaction and identify the source of each reactant.

Answer Anaplerotic reactions replenish intermediates in the citric acid cycle. Net synthesis of α-ketoglutarate from pyruvate occurs by the sequential actions of (1) pyruvate carboxylase (which makes extra molecules of oxaloacetate), (2) pyruvate dehydrogenase, and the citric acid cycle enzymes (3) citrate synthase, (4) aconitase, and (5) isocitrate dehydrogenase:

(1) $\text{Pyruvate} + ATP + CO_2 + H_2O \longrightarrow \text{oxaloacetate} + ADP + P_i + H^+$
(2) $\text{Pyruvate} + CoA + NAD^+ \longrightarrow \text{acetyl-CoA} + CO_2 + NADH + H^+$
(3) $\text{Oxaloacetate} + \text{acetyl-CoA} \longrightarrow \text{citrate} + CoA$
(4) $\text{Citrate} \longrightarrow \text{isocitrate}$
(5) $\text{Isocitrate} + NAD^+ \longrightarrow \alpha\text{-ketoglutarate} + CO_2 + NADH + H^+$

Net reaction: $2 \text{ Pyruvate} + ATP + 2NAD^+ + H_2O \longrightarrow \alpha\text{-ketoglutarate} + CO_2 + ADP + P_i + 2NADH + 3H^+$

25. Amphibolic Pathways Explain, giving examples, what is meant by the statement that the citric acid cycle is amphibolic.

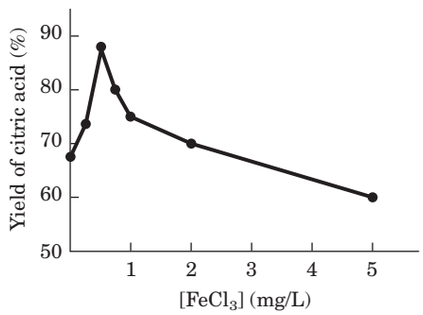
Answer Amphibolic pathways can serve either in energy-yielding catabolic or in energy-requiring biosynthetic processes, depending on the cellular circumstances. For example, the citric acid cycle generates NADH and $FADH_2$ when functioning catabolically. But it can also provide precursors for the synthesis of such products as glutamate and aspartate (from α-ketoglutarate and oxaloacetate, respectively), which in turn serve as precursors for other products, such as glutamine, proline, and asparagine (see Fig. 16–15).

26. Regulation of the Pyruvate Dehydrogenase Complex In animal tissues, the rate of conversion of pyruvate to acetyl-CoA is regulated by the ratio of active, phosphorylated to inactive, unphosphorylated PDH complex. Determine what happens to the rate of this reaction when a preparation of rabbit muscle mitochondria containing the PDH complex is treated with **(a)** pyruvate dehydrogenase kinase, ATP, and NADH; **(b)** pyruvate dehydrogenase phosphatase and Ca^{2+}; **(c)** malonate.

Answer Pyruvate dehydrogenase is regulated by covalent modification and by allosteric inhibitors. The mitochondrial preparation responds as follows: **(a)** Active pyruvate dehydrogenase (dephosphorylated) is converted to inactive pyruvate dehydrogenase (phosphorylated) and the rate of conversion of pyruvate to acetyl-CoA decreases. **(b)** The phosphoryl group on pyruvate dehydrogenase phosphate is removed enzymatically to yield active pyruvate dehydrogenase, which increases the rate of conversion of pyruvate to acetyl-CoA. **(c)** Malonate inhibits succinate dehydrogenase, and citrate accumulates. The accumulated citrate inhibits citrate synthase, and acetyl-CoA accumulates. High levels of acetyl-CoA inhibit pyruvate dehydrogenase, and the rate of conversion of pyruvate to acetyl-CoA is reduced.

27. Commercial Synthesis of Citric Acid Citric acid is used as a flavoring agent in soft drinks, fruit juices, and many other foods. Worldwide, the market for citric acid is valued at hundreds of millions of dollars per year. Commercial production uses the mold *Aspergillus niger,* which metabolizes sucrose under carefully controlled conditions.

(a) The yield of citric acid is strongly dependent on the concentration of $FeCl_3$ in the culture medium, as indicated in the graph. Why does the yield decrease when the concentration of Fe^{3+} is above or below the optimal value of 0.5 mg/L?



(b) Write the sequence of reactions by which *A. niger* synthesizes citric acid from sucrose. Write an equation for the overall reaction.

(c) Does the commercial process require the culture medium to be aerated—that is, is this a fermentation or an aerobic process? Explain.

Answer

(a) Citrate is produced through the action of citrate synthase on oxaloacetate and acetyl-CoA. Although the citric acid cycle does not normally result in an accumulation of intermediates, citrate synthase can be used for net synthesis of citrate when (1) there is a continuous influx of new oxaloacetate and acetyl-CoA, and (2) the transformation of citrate to isocitrate is blocked or at least restricted. *A. niger* grown in a medium rich in sucrose but low in Fe^{3+} meets both requirements. Citrate is transformed to isocitrate by aconitase, an Fe^{3+}-containing enzyme. In an Fe^{3+}-restricted medium, synthesis of aconitase is restricted and thus the breakdown of citrate is partially blocked; citrate accumulates and can be isolated in commercial quantities. Note that *some* aconitase activity is necessary—the mold will not thrive at $[Fe^{3+}]$ below 0.5 mg/L. At higher $[Fe^{3+}]$, however, aconitase is synthesized in increasing amounts; this will lead to a decrease in the yield of citrate as it cycles through the citric acid cycle.

(b) Sucrose + $H_2O \longrightarrow$ glucose + fructose

Glucose + $2P_i$ + 2ADP + $2NAD^+ \longrightarrow$ 2 pyruvate + 2ATP + 2NADH + $2H^+$ + $2H_2O$

Fructose + $2P_i$ + 2ADP + $2NAD^+ \longrightarrow$ 2 pyruvate + 2ATP + 2NADH + $2H^+$ + $2H_2O$

2 Pyruvate + $2NAD^+$ + 2CoA $\longrightarrow$ 2 acetyl-CoA + 2NADH + $2H^+$ + $2CO_2$

2 Pyruvate + $2CO_2$ + 2ATP + $2H_2O \longrightarrow$ 2 oxaloacetate + 2ADP + $2P_i$ + $4H^+$

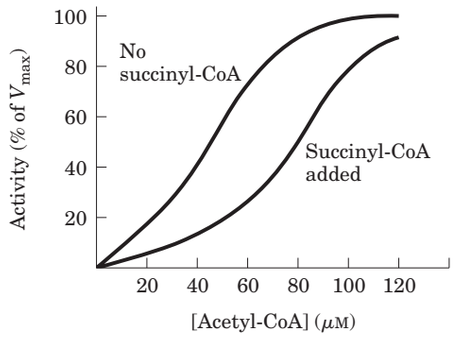
2 Acetyl-CoA + 2 oxaloacetate + $2H_2O \longrightarrow$ 2 citrate + 2CoA

The overall reaction is

$$\text{Sucrose} + H_2O + 2P_i + 2ADP + 6NAD^+ \longrightarrow 2 \text{ citrate} + 2ATP + 6NADH + 10H^+$$

(c) Note that the overall reaction consumes NAD^+. Because the cellular pool of this oxidized coenzyme is limited, it must be recycled by oxidation of NADH via the electron-transfer chain, with consumption of oxygen. Consequently, the overall conversion of sucrose to citrate is an aerobic process and requires molecular oxygen.

28. Regulation of Citrate Synthase In the presence of saturating amounts of oxaloacetate, the activity of citrate synthase from pig heart tissue shows a sigmoid dependence on the concentration of acetyl-CoA, as shown in the graph. When succinyl-CoA is added, the curve shifts to the right and the sigmoid dependence is more pronounced.



On the basis of these observations, suggest how succinyl-CoA regulates the activity of citrate synthase. (Hint: see Fig. 6–34) Why is succinyl-CoA an appropriate signal for regulation of the citric acid cycle? How does the regulation of citrate synthase control the rate of cellular respiration in pig heart tissue?

Answer Succinyl-CoA is an intermediate of the citric acid cycle—the first four-carbon intermediate, formed in the α-ketoglutarate dehydrogenase reaction. Its accumulation signals reduced flux through the cycle, and thus the need for reduced entry of acetyl-CoA into the cycle.

As seen in the graph, succinyl-CoA shifts the half-saturation point, $[S]_{0.5}$ (or $K_{0.5}$), for acetyl-CoA to the right but does not alter V_{max}. This indicates that succinyl-CoA acts as a negative modulator, either directly as a competitive inhibitor with acetyl-CoA or by binding to a site separate from the active site.

Citrate synthase catalyzes the step at which acetyl-CoA enters the cycle, and thus regulation of this enzyme controls the activity of the cycle, the rate of production of reduced coenzymes, and thus the rate of cellular respiration.

29. Regulation of Pyruvate Carboxylase The carboxylation of pyruvate by pyruvate carboxylase occurs at a very low rate unless acetyl-CoA, a positive allosteric modulator, is present. If you have just eaten a meal rich in fatty acids (triacylglycerols) but low in carbohydrates (glucose), how does this regulatory property shut down the oxidation of glucose to CO_2 and H_2O but increase the oxidation of acetyl-CoA derived from fatty acids?

Answer Fatty acid catabolism increases the level of acetyl-CoA, which stimulates pyruvate carboxylase. The resulting increase in oxaloacetate concentration stimulates acetyl-CoA consumption through the citric acid cycle, causing the citrate and ATP concentrations to rise. These metabolites inhibit glycolysis at PFK-1 and inhibit pyruvate dehydrogenase, effectively slowing the utilization of sugars and pyruvate.

30. Relationship between Respiration and the Citric Acid Cycle Although oxygen does not participate directly in the citric acid cycle, the cycle operates only when O_2 is present. Why?

Answer Oxygen is the terminal electron acceptor in oxidative phosphorylation, and thus is needed to recycle NAD^+ from NADH. NADH is produced in greatest quantities by the oxidative reactions of the citric acid cycle. In the absence of O_2, the supply of NAD^+ is depleted, and the accumulated NADH allosterically inhibits pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (see Fig. 16–18).

31. Effect of [NADH]/[NAD^+] on the Citric Acid Cycle How would you expect the operation of the citric acid cycle to respond to a rapid increase in the [NADH]/[NAD^+] ratio in the mitochondrial matrix? Why?

Answer Increased [NADH]/[NAD^+] inhibits the citric acid cycle by mass action at each of the three steps that involve reduction of NAD^+; high [NADH] shifts the equilibrium toward NAD^+. Another way to look at this effect is to consider how an increased ratio of product (NADH) to reactant (NAD^+) affects the free-energy change for any of the three NAD^+-dependent steps of the citric acid cycle. Look, for example, at Equation 13–4 (p. 493).

32. Thermodynamics of Citrate Synthase Reaction in Cells Citrate is formed by the condensation of acetyl-CoA with oxaloacetate, catalyzed by citrate synthase:

$$\text{Oxaloacetate} + \text{acetyl-CoA} + H_2O \longrightarrow \text{citrate} + \text{CoA} + H^+$$

In rat heart mitochondria at pH 7.0 and 25° C, the concentrations of reactants and products are: oxaloacetate, 1 μM; acetyl-CoA, 1 μM; citrate, 220 μM; and CoA, 65 μM. The standard free-energy change for the citrate synthase reaction is −32.2 kJ/mol. What is the direction of metabolite flow through the citrate synthase reaction in rat heart cells? Explain.

Answer The free-energy change of the citrate synthase reaction in the cell is

$$\Delta G = \Delta G'^\circ + RT \ln \frac{[\text{citrate}][\text{CoA}]}{[\text{OAA}][\text{acetyl-CoA}]}$$

$$= -32.2 \text{ kJ/mol} + (2.48 \text{ kJ/mol}) \ln \frac{(220 \times 10^{-6})(65 \times 10^{-6})}{(1 \times 10^{-6})(1 \times 10^{-6})}$$

$$= -8 \text{ kJ/mol}$$

Thus, the citrate synthase reaction is exergonic and proceeds in the direction of citrate formation.

33. Reactions of the Pyruvate Dehydrogenase Complex Two of the steps in the oxidative decarboxylation of pyruvate (steps ④ and ⑤ in Fig. 16–6) do not involve any of the three carbons of pyruvate yet are essential to the operation of the PDH complex. Explain.

Answer The pyruvate dehydrogenase complex can be thought of as performing five enzymatic reactions. The first three (see Fig. 16–6) catalyze the oxidation of pyruvate to acetyl-CoA and reduction of the enzyme. The last two reactions are essential to reoxidize the reduced enzyme, reducing NAD^+ to NADH + H^+. The moiety on the enzyme that is oxidized/reduced is the lipoamide cofactor.

34. Citric Acid Cycle Mutants There are many cases of human disease in which one or another enzyme activity is lacking due to genetic mutation. However, cases in which individuals lack one of the enzymes of the citric acid cycle are extremely rare. Why?

Answer The citric acid cycle is so central to metabolism that a serious defect in any cycle enzyme would probably be lethal to the embryo.

35. Partitioning between the Citric Acid and Glyoxylate Cycles In an organism (such as *E. coli*) that has both the citric acid cycle and the glyoxylate cycle, what determines which of these pathways isocitrate will enter?

Answer Isocitrate can be metabolized by the citric acid cycle or by the glyoxylate cycle. The first enzyme in each pathway is allosterically regulated, so that the accumulation of citric acid cycle intermediates stimulate that cycle while inhibiting the glyoxylate cycle. AMP and ADP, which signal an inadequate reserve of ATP, inhibit the glyoxylate cycle, shifting the use of isocitrate to the energy-producing citric acid cycle. This reciprocal regulation of the two enzymes at the branch point determine which pathway isocitrate will enter.

Data Analysis Problem

36. How the Citric Acid Cycle Was Determined The detailed biochemistry of the citric acid cycle was determined by several researchers over a period of decades. In a 1937 article, Krebs and Johnson summarized their work and the work of others in the first published description of this pathway.

The methods used by these researchers were very different from those of modern biochemistry. Radioactive tracers were not commonly available until the 1940s, so Krebs and other researchers had to use nontracer techniques to work out the pathway. Using freshly prepared samples of pigeon breast muscle, they determined oxygen consumption by suspending minced muscle in buffer in a sealed flask and measuring the volume (in μL) of oxygen consumed under different conditions. They measured levels of substrates (intermediates) by treating samples with acid to remove contaminating proteins, then assaying the quantities of various small organic molecules. The two key observations that led Krebs and colleagues to propose a citric acid *cycle* as opposed to a *linear pathway* (like that of glycolysis) were made in the following experiments.

Experiment I. They incubated 460 mg of minced muscle in 3 mL of buffer at 40 °C for 150 minutes. Addition of *citrate* increased O_2 consumption by 893 μL compared with samples without added citrate. They calculated, based on the O_2 consumed during respiration of other carbon-containing compounds, that the expected O_2 consumption for complete respiration of this quantity of citrate was only 302 μL.

Experiment II. They measured O_2 consumption by 460 mg of minced muscle in 3 mL of buffer when incubated with *citrate* and/or with *1-phosphoglycerol* (glycerol 1-phosphate; this was known to be readily oxidized by cellular respiration) at 40 °C for 140 minutes. The results are shown in the table.

Sample	Substrate(s) added	μL O_2 absorbed
1	No extra	342
2	0.3 mL 0.2 M 1-phosphoglycerol	757
3	0.15 mL 0.02 M citrate	431
4	0.3 mL 0.2 M 1-phosphoglycerol and 0.15 mL 0.02 M citrate	1,385

(a) Why is O_2 consumption a good measure of cellular respiration?

(b) Why does sample 1 (unsupplemented muscle tissue) consume some oxygen?

(c) Based on the results for samples 2 and 3, can you conclude that 1-phosphoglycerol and citrate serve as substrates for cellular respiration in this system? Explain your reasoning.

(d) Krebs and colleagues used the results from these experiments to argue that citrate was "catalytic"—that it helped the muscle tissue samples metabolize 1-phosphoglycerol more completely. How would you use their data to make this argument?

(e) Krebs and colleagues further argued that citrate was not simply consumed by these reactions, but had to be *regenerated*. Therefore, the reactions had to be a *cycle* rather than a linear pathway. How would you make this argument?

Other researchers had found that *arsenate* (AsO_4^{3-}) inhibits α-ketoglutarate dehydrogenase and that *malonate* inhibits succinate dehydrogenase.

(f) Krebs and coworkers found that muscle tissue samples treated with arsenate and citrate would consume citrate only in the presence of oxygen; and under these conditions, oxygen was consumed. Based on the pathway in Figure 16–7, what was the citrate converted to in this experiment, and why did the samples consume oxygen?

In their article, Krebs and Johnson further reported the following. (1) In the presence of arsenate, 5.48 mmol of citrate was converted to 5.07 mmol of α-ketoglutarate. (2) In the presence of malonate, citrate was quantitatively converted to large amounts of succinate and small amounts of α-ketoglutarate. (3) Addition of oxaloacetate in the absence of oxygen led to production of a large amount of citrate; the amount was increased if glucose was also added.

Other workers had found the following pathway in similar muscle tissue preparations:

$$\text{Succinate} \longrightarrow \text{fumarate} \longrightarrow \text{malate} \longrightarrow \text{oxaloacetate} \longrightarrow \text{pyruvate}$$

(g) Based only on the data presented in this problem, what is the order of the intermediates in the citric acid cycle? How does this compare with Figure 16–7? Explain your reasoning.

(h) Why was it important to show the *quantitative* conversion of citrate to α-ketoglutarate?

The Krebs and Johnson article also contains other data that filled in most of the missing components of the cycle. The only component left unresolved was the molecule that reacted with oxaloacetate to form citrate.

Answer

(a) The only reaction in muscle tissue that consumes significant amounts of oxygen is cellular respiration, so O_2 consumption is a good proxy for respiration.

(b) Freshly prepared muscle tissue contains some residual glucose; O_2 consumption is due to oxidation of this glucose.

(c) Yes. Because the amount of O_2 consumed increased when citrate or 1-phosphoglycerol was added, both can serve as substrate for cellular respiration in this system.

(d) *Experiment I*: citrate is causing much more O_2 consumption than would be expected from its complete oxidation. Each molecule of citrate seems to be acting as though it were more than one molecule. The only possible explanation is that each molecule of citrate functions more than once in the reaction—which is how a catalyst operates. *Experiment II*: the key is to calculate the excess O_2 consumed by each sample compared with the control (sample 1).

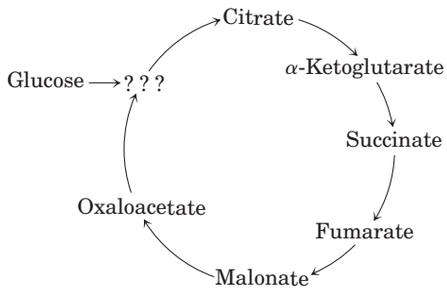
Sample	Substrate(s) added	μL O_2 absorbed	Excess μL O_2 consumed
1	No extra	342	0
2	0.3 mL 0.2 M 1-phosphoglycerol	757	415
3	0.15 mL 0.02 M citrate	431	89
4	0.3 mL 0.2 M 1-phosphoglycerol + 0.15 mL 0.02 M citrate	1,385	1,043

If both citrate and 1-phosphoglycerol were simply substrates for the reaction, you would expect the excess O_2 consumption by sample 4 to be the sum of the individual excess consumptions by samples 2 and 3 (415 μL + 89 μL = 504 μL). However, the excess consumption when both substrates are present is roughly twice this amount (1,043 μL). Thus citrate increases the ability of the tissue to metabolize 1-phosphoglycerol. This behavior is typical of a catalyst. Both experiments (I and II) are required to make this case convincing. Based on experiment I only, citrate is somehow accelerating the reaction, but it is not clear whether it acts by helping substrate metabolism or by some other mechanism. Based on experiment II only, it is not clear which molecule is the catalyst, citrate or 1-phosphoglycerol. Together, the experiments show that citrate is acting as a "catalyst" for the oxidation of 1-phosphoglycerol.

(e) Given that the pathway can consume citrate (see sample 3), if citrate is to act as a catalyst it must be regenerated. If the set of reactions first consumes then regenerates citrate, it must be a circular rather than a linear pathway.

(f) When the pathway is blocked at α-ketoglutarate dehydrogenase, citrate is converted to α-ketoglutarate but the pathway goes no further. Oxygen is consumed by reoxidation of the NADH produced by isocitrate dehydrogenase.

(g)



This differs from Figure 16–7 in that it does not include *cis*-aconitate and isocitrate (between citrate and α-ketoglutarate), or succinyl-CoA, or acetyl-CoA.

(h) Establishing a quantitative conversion was essential to rule out a branched or other, more complex pathway.

Reference

Krebs, H.A. & Johnson, W.A. (1937) The role of citric acid in intermediate metabolism in animal tissues. *Enzymologia* **4,** 148–156. [Reprinted (1980) in *FEBS Lett.* **117** (Suppl.), K2–K10.]

chapter

17

Fatty Acid Catabolism

1. Energy in Triacylglycerols On a per-carbon basis, where does the largest amount of biologically available energy in triacylglycerols reside: in the fatty acid portions or the glycerol portion? Indicate how knowledge of the chemical structure of triacylglycerols provides the answer.

Answer The fatty acids of triacylglycerols are hydrocarbons, with a single carboxyl group. Glycerol, on the other hand, has an —OH group on each carbon and is thus much more highly oxidized than a fatty acid. On oxidation, fatty acids therefore produce far more energy per carbon than does glycerol. Triacylglycerols have an energy of oxidation more than twice that of the same weight of carbohydrates or proteins.

2. Fuel Reserves in Adipose Tissue Triacylglycerols, with their hydrocarbon-like fatty acids, have the highest energy content of the major nutrients.

(a) If 15% of the body mass of a 70.0 kg adult consists of triacylglycerols, what is the total available fuel reserve, in kilojoules and kilocalories, in the form of triacylglycerols? Recall that 1.00 kcal = 4.18 kJ.

(b) If the basal energy requirement is approximately 8,400 kJ/day (2,000 kcal/day), how long could this person survive if the oxidation of fatty acids stored as triacylglycerols were the only source of energy?

(c) What would be the weight loss in pounds per day under such starvation conditions (1 lb = 0.454 kg)?

Answer

(a) Given (in the text) that the energy value of stored triacylglycerol is 38 kJ/g, the available fuel reserve is

$$(0.15)(70.0 \times 10^3 \text{ g})(38 \text{ kJ/g}) = 4.0 \times 10^5 \text{ kJ}$$
$$= 9.6 \times 10^4 \text{ kcal}$$

(b) At a rate of 8.4×10^3 kJ/day, the fuel supply would last

$$(4.0 \times 10^5 \text{ kJ})/(8.4 \times 10^3 \text{ kJ/day}) = 48 \text{ days}$$

(c) If all the triacylglycerol is used over a 48-day period, this represents a rate of weight loss of

$$\frac{(0.15)(70.0 \text{ kg})}{48 \text{ days}} = 0.22 \text{ kg/day}$$

$$\text{or } (0.22 \text{ kg/day})/(0.454 \text{ kg/lb}) = 0.48 \text{ lb/day}$$

3. Common Reaction Steps in the Fatty Acid Oxidation Cycle and Citric Acid Cycle Cells often use the same enzyme reaction pattern for analogous metabolic conversions. For example, the steps in the oxidation of pyruvate to acetyl-CoA and of α-ketoglutarate to succinyl-CoA, although catalyzed by

different enzymes, are very similar. The first stage of fatty acid oxidation follows a reaction sequence closely resembling a sequence in the citric acid cycle. Use equations to show the analogous reaction sequences in the two pathways.

Answer The first three reactions in the β oxidation of fatty acyl–CoA molecules are analogous to three reactions of the citric acid cycle.

The fatty acyl–CoA dehydrogenase reaction is analogous to the succinate dehydrogenase reaction; both are FAD-requiring oxidations:

$$\text{Succinate} + \text{FAD} \longrightarrow \text{fumarate} + \text{FADH}_2$$

$$\text{Fatty acyl–CoA} + \text{FAD} \longrightarrow \textit{trans}\text{-}\Delta^2\text{-enoyl-CoA} + \text{FADH}_2$$

The enoyl-CoA hydratase reaction is analogous to the fumarase reaction; both add water to an olefinic bond:

$$\text{Fumarate} + \text{H}_2\text{O} \longrightarrow \text{malate}$$

$$\textit{trans}\text{-}\Delta^2\text{-Enoyl-CoA} + \text{H}_2\text{O} \longrightarrow \text{L-}\beta\text{-hydroxyacyl-CoA}$$

The β-hydroxyacyl-CoA dehydrogenase reaction is analogous to the malate dehydrogenase reaction; both are NAD-requiring and act on β-hydroxyacyl compounds:

$$\text{Malate} + \text{NAD}^+ \longrightarrow \text{oxaloacetate} + \text{NADH}$$

$$\text{L-}\beta\text{-Hydroxyacyl-CoA} + \text{NAD}^+ \longrightarrow \beta\text{-ketoacyl-CoA} + \text{NADH}$$

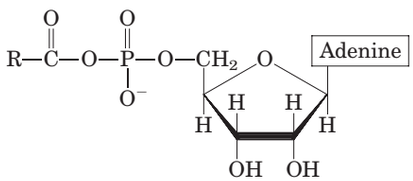
4. *β* Oxidation: How Many Cycles? How many cycles of β oxidation are required for the complete oxidation of activated oleic acid, 18:1(Δ^9)?

Answer 7 cycles; the last releases 2 acetyl-CoA.

5. Chemistry of the Acyl-CoA Synthetase Reaction Fatty acids are converted to their coenzyme A esters in a reversible reaction catalyzed by acyl-CoA synthetase:

$$\text{R—COO}^- + \text{ATP} + \text{CoA} \rightleftharpoons \text{R—}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{—CoA} + \text{AMP} + \text{PP}_\text{i}$$

(a) The enzyme-bound intermediate in this reaction has been identified as the mixed anhydride of the fatty acid and adenosine monophosphate (AMP), acyl-AMP:



Write two equations corresponding to the two steps of the reaction catalyzed by acyl-CoA synthetase.

(b) The acyl-CoA synthetase reaction is readily reversible, with an equilibrium constant near 1. How can this reaction be made to favor formation of fatty acyl–CoA?

Answer Activation of carboxyl groups by ATP could in theory be accomplished by three types of reactions: the formation of acyl-phosphate + ADP; of acyl-ADP + P_i; or of acyl-AMP + PP_i. All these reactions are readily reversible. To create an activation reaction with a highly negative $\Delta G'^\circ$ (effectively irreversible), the third type of reaction can be coupled to a pyrophosphatase reaction, as in the synthesis of fatty acyl–CoA molecules.

(a) $R—COO^- + ATP \longrightarrow$ acyl-AMP + PP_i

Acyl-AMP + CoA $\longrightarrow$ acyl-CoA + AMP

(b) Hydrolysis of PP_i by an inorganic pyrophosphatase pulls the reaction in the direction of fatty acyl–CoA formation.

6. Intermediates in Oleic Acid Oxidation What is the structure of the partially oxidized fatty acyl group that is formed when oleic acid, 18:1(Δ^9), has undergone three cycles of β oxidation? What are the next two steps in the continued oxidation of this intermediate?

Answer After three rounds of β oxidation, the fatty acyl–CoA has been shortened by six carbons with the removal of three acetyl-CoAs. The resulting 12-carbon intermediate is *cis*-Δ^3-dodecanoyl-CoA, with the double bond between the third and fourth carbons from the carboxyl end of the chain. Before another round of β oxidation can occur, that double bond must be moved from Δ^3 to Δ^2, which is catalyzed by Δ^3,Δ^2-enoyl isomerase (see Fig. 17–9). Water is then added to the double bond to form the β-hydroxydodecanoyl-CoA derivative, which can undergo further β oxidation.

7. β Oxidation of an Odd-Chain Fatty Acid What are the direct products of β oxidation of a fully saturated, straight-chain fatty acid of 11 carbons?

Answer 4 acetyl-CoA and 1 propionyl-CoA

8. Oxidation of Tritiated Palmitate Palmitate uniformly labeled with tritium (^3H) to a specific activity of 2.48×10^8 counts per minute (cpm) per micromole of palmitate is added to a mitochondrial preparation that oxidizes it to acetyl-CoA. The acetyl-CoA is isolated and hydrolyzed to acetate. The specific activity of the isolated acetate is 1.00×10^7 cpm/μmol. Is this result consistent with the β-oxidation pathway? Explain. What is the final fate of the removed tritium?

Answer The β-oxidation pathway includes two dehydrogenase enzymes that remove hydrogen (H–H) from the fatty acyl–CoA chain, first at a $—CH_2—CH_2—$ and then at a $—CH_2—CH(OH)—$. The net result of the two reactions is removal of one of the two hydrogens at the point of formation of the enoyl-CoA intermediate. The two other hydrogens in the methyl group of acetyl-CoA come from water.

Palmitate contains 16 carbons, with $(14 \times 2) + 3 = 31$ hydrogens, so each two-carbon unit contains about 4/31 or about 1/8 of the total ^3H present. Thus, the counts per minute expected per acetyl-CoA, with two of the four acetyl hydrogens labeled (the other two arising from unlabeled water), is $(2/4)(2.48 \times 10^8$ cpm/μmol$)(1/8) = 1.6 \times 10^7$ cpm/μmol, somewhat higher than observed. Exchange between β-ketoacyl-CoA and solvent water could cause loss of ^3H.

The final fate of the tritium removed from palmitate is its appearance in water, as reduced carriers ($FADH_2$, NADH) are reoxidized by the mitochondria.

9. Compartmentation in β Oxidation Free palmitate is activated to its coenzyme A derivative (palmitoyl-CoA) in the cytosol before it can be oxidized in the mitochondrion. If palmitate and [^{14}C]coenzyme A are added to a liver homogenate, palmitoyl-CoA isolated from the cytosolic fraction is radioactive, but that isolated from the mitochondrial fraction is not. Explain.

Answer The transport of fatty acid molecules into mitochondria requires a shuttle system involving a fatty acyl–carnitine intermediate. Fatty acids are first converted to fatty acyl–CoA molecules in the cytosol (by the action of acyl–CoA synthetases) then, at the outer mitochondrial membrane, the fatty acyl group is transferred to carnitine (by the action of carnitine acyltransferase I). After transport of fatty acyl–carnitine through the inner membrane, the fatty acyl group is transferred to mitochondrial CoA. The cytosolic and mitochondrial pools of CoA are thus kept separate, and no labeled CoA from the cytosolic pool enters the mitochondrion.

10. Comparative Biochemistry: Energy-Generating Pathways in Birds One indication of the relative importance of various ATP-producing pathways is the V_{max} of certain enzymes of these pathways. The values of V_{max} of several enzymes from the pectoral muscles (chest muscles used for flying) of pigeon and pheasant are listed below.

	V_{max} (μmol substrate/min/g tissue)	
Enzyme	**Pigeon**	**Pheasant**
Hexokinase	3.0	2.3
Glycogen phosphorylase	18.0	120.0
Phosphofructokinase-1	24.0	143.0
Citrate synthase	100.0	15.0
Triacylglycerol lipase	0.07	0.01

(a) Discuss the relative importance of glycogen metabolism and fat metabolism in generating ATP in the pectoral muscles of these birds.

(b) Compare oxygen consumption in the two birds.

(c) Judging from the data in the table, which bird is the long-distance flyer? Justify your answer.

(d) Why were these particular enzymes selected for comparison? Would the activities of triose phosphate isomerase and malate dehydrogenase be equally good bases for comparison? Explain.

Answer

(a) In the pigeon, aerobic oxidation of fatty acids—β oxidation and oxidative phosphorylation—predominates; in the pheasant, anaerobic glycolysis of glycogen predominates. Note the high citrate synthase activity in the pigeon, and the high glycogen phosphorylase and PFK-1 activities in the pheasant.

(b) Using aerobic oxidation, pigeon muscle consumes more oxygen during flight.

(c) The energy available per gram is higher for fat than for glycogen. In addition, anaerobic breakdown of glycogen is limited by tolerance to lactate buildup. Thus the pigeon, using predominantly the oxidative catabolism of fats, is the long-distance flyer.

(d) The enzymes listed in the table (unlike triose phosphate isomerase and malate dehydrogenase) are the regulatory enzymes of their respective pathways and thus limit ATP production rates.

11. Mutant Carnitine Acyltransferase What changes in metabolic pattern would result from a mutation in the muscle carnitine acyltransferase I in which the mutant protein has lost its affinity for malonyl-CoA but not its catalytic activity?

Answer Malonyl-CoA would no longer inhibit fatty acid entry into the mitochondrion and β oxidation, so there might be a futile cycle of simultaneous fatty acid synthesis in the cytosol and fatty acid breakdown in mitochondria. (See Fig. 17–12.)

12. Effect of Carnitine Deficiency An individual developed a condition characterized by progressive muscular weakness and aching muscle cramps. The symptoms were aggravated by fasting, exercise, and a high-fat diet. The homogenate of a skeletal muscle specimen from the patient oxidized added oleate more slowly than did control homogenates, consisting of muscle specimens from healthy individuals. When carnitine was added to the patient's muscle homogenate, the rate of oleate oxidation equaled that in the control homogenates. The patient was diagnosed as having a carnitine deficiency.

(a) Why did added carnitine increase the rate of oleate oxidation in the patient's muscle homogenate?

(b) Why were the patient's symptoms aggravated by fasting, exercise, and a high-fat diet?

(c) Suggest two possible reasons for the deficiency of muscle carnitine in this individual.

Answer

(a) The carnitine-mediated transport of fatty acids into mitochondria is the rate-limiting step in β oxidation (see Fig. 17–6). Carnitine deficiency decreases the rate of transport of fatty acids into mitochondria and thus the rate of β oxidation, so addition of carnitine would increase the rate of oxidation.

(b) Fasting, exercise, and a high-fat diet all cause an increased need for β oxidation of fatty acids and thus an increased demand for carnitine shuttle activity. The symptoms of carnitine deficiency would therefore become more severe under these conditions.

(c) The deficiency of carnitine may result from a dietary deficiency of its precursor, lysine, or from a defect in one of the enzymes that synthesize carnitine from this precursor.

13. Fatty Acids as a Source of Water Contrary to legend, camels do not store water in their humps, which actually consist of large fat deposits. How can these fat deposits serve as a source of water? Calculate the amount of water (in liters) that a camel can produce from 1.0 kg of fat. Assume for simplicity that the fat consists entirely of tripalmitoylglycerol.

Answer Oxidation of fatty acids produces water in significant amounts. From Equation 17–6

$$\text{Palmitoyl-CoA} + 23O_2 + 108P_i + 108\text{ADP} \longrightarrow \text{CoA} + 16CO_2 + 108\text{ATP} + 23H_2O$$

we know that the oxidation of 1 mol of palmitoyl-CoA produces 23 mol of water.

Tripalmitoin (glycerol plus three palmitates in ester linkage) has a molecular weight of 885, so 1 kg of tripalmitoin contains (1.0 kg)(1,000 g/kg)/(885 g/mol) = 1.1 mol. Complete oxidation of the three palmitoyl groups will produce

(1.1 mol tripalmitoin)(3 mol palmitate/mol tripalmitoin)(23 mol H_2O/mol palmitate) = 76 mol H_2O

Thus, the volume of water produced (ignoring the contribution of glycerol oxidation) is

$$(76\text{ mol})(18\text{ g/mol})(1\text{ kg}/1{,}000\text{ g})(1\text{ L/kg}) = 1.4\text{ L}$$

Note: in reality, this may be an overestimate. The fatty acyl groups of the triacylglycerol in the camel's fat may be less highly reduced than palmitate.

14. Petroleum as a Microbial Food Source Some microorganisms of the genera *Nocardia* and *Pseudomonas* can grow in an environment where hydrocarbons are the only food source. These bacteria oxidize straight-chain aliphatic hydrocarbons, such as octane, to their corresponding carboxylic acids:

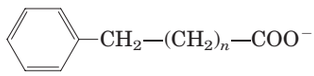
$$CH_3(CH_2)_6CH_3 + NAD^+ + O_2 \rightleftharpoons CH_3(CH_2)_6COOH + NADH + H^+$$

How could these bacteria be used to clean up oil spills? What would be some of the limiting factors to the efficiency of this process?

Answer By oxidizing hydrocarbons to their corresponding fatty acids, these microbes can obtain all their energy from β oxidation and oxidative phosphorylation, converting the hydrocarbons to CO_2 and H_2O. Theoretically, oil spills could be broken down by treatment with these microbes.

Because of the extreme hydrophobicity of hydrocarbons, close contact between substrate and bacterial enzymes might be difficult to achieve; under field conditions (e.g., an oil spill), detergents are often added to improve this contact. In addition, other nutrients, such as nitrogen or phosphorus, may be limiting for the bacterial populations, and these elements are often added to foster the growth of the hydrocarbon-oxidizers.

15. Metabolism of a Straight-Chain Phenylated Fatty Acid A crystalline metabolite was isolated from the urine of a rabbit that had been fed a straight-chain fatty acid containing a terminal phenyl group:



A 302 mg sample of the metabolite in aqueous solution was completely neutralized by 22.2 mL of 0.100 M NaOH.

(a) What is the probable molecular weight and structure of the metabolite?

(b) Did the straight-chain fatty acid contain an even or an odd number of methylene (—CH_2—) groups (i.e., is n even or odd)? Explain.

Answer

(a) 22.2 mL of 0.1 M NaOH is equivalent to $(22.2 \times 10^{-3}\ \text{L})\ (0.100\ \text{mol/L}) = 22.2 = 10^{-4}$ mol of unknown metabolite (assuming that it contains only one carboxyl group) in the 302 mg sample. Thus, the M_r of the metabolite is

$$\frac{302 \times 10^{-3}\ \text{g}}{22.2 \times 10^{-4}\ \text{mol}} = 136$$

This is the M_r of phenylacetic acid.

(b) Because β oxidation removes two-carbon units, and the end product is a two-carbon unit, the original fatty acyl chain must have had an even number of methylene groups (with the phenyl group counted as equivalent to a terminal methyl group). An odd-numbered fatty acid would have produced phenylpropionate.

16. Fatty Acid Oxidation in Uncontrolled Diabetes When the acetyl-CoA produced during β oxidation in the liver exceeds the capacity of the citric acid cycle, the excess acetyl-CoA forms ketone bodies—acetone, acetoacetate, and D-β-hydroxybutyrate. This occurs in severe, uncontrolled diabetes: because the tissues cannot use glucose, they oxidize large amounts of fatty acids instead. Although acetyl-CoA is not toxic, the mitochondrion must divert the acetyl-CoA to ketone bodies. What problem would arise if acetyl-CoA were not converted to ketone bodies? How does the diversion to ketone bodies solve the problem?

Answer Individuals with uncontrolled diabetes oxidize large quantities of fat because they cannot use glucose efficiently. This leads to a decrease in activity of the citric acid cycle (see Problem 17) and an increase in the pool of acetyl-CoA. If acetyl-CoA were not converted to ketone bodies, the CoA pool would become depleted. Because the mitochondrial CoA pool is small, liver mitochondria recycle CoA by condensing two acetyl-CoA molecules to form acetoacetyl-CoA + CoA (see Fig. 17–18). The acetoacetyl-CoA is converted to other ketones, and the CoA is recycled for use in the β-oxidation pathway and energy production.

17. Consequences of a High-Fat Diet with No Carbohydrates Suppose you had to subsist on a diet of whale blubber and seal blubber, with little or no carbohydrate.

(a) What would be the effect of carbohydrate deprivation on the utilization of fats for energy?

(b) If your diet were totally devoid of carbohydrate, would it be better to consume odd- or even-numbered fatty acids? Explain.

Answer

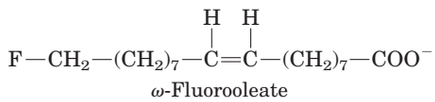
(a) Pyruvate, formed from glucose via glycolysis, is the main source of the oxaloacetate needed to replenish citric acid cycle intermediates (see Table 16–2). In the absence of carbohydrate in the diet, the oxaloacetate level drops and the citric acid cycle slows. This increases the rate of β oxidation of fatty acids and leads to ketosis.

(b) The last cycle of β oxidation produces two acetyl-CoA molecules from an even-numbered fatty acid, or propionyl-CoA + acetyl-CoA from an odd-numbered fatty acid. Propionyl-CoA can be converted to succinyl-CoA (see Fig. 17–11), which when converted to oxaloacetate stimulates the citric acid cycle and relieves the conditions leading to ketosis. Thus, it would be better to consume odd-numbered fatty acids.

18. Even- and Odd-Chain Fatty Acids in the Diet In a laboratory experiment, two groups of rats are fed two different fatty acids as their sole source of carbon for a month. The first group gets heptanoic acid (7:0), and the second gets octanoic acid (8:0). After the experiment, a striking difference is seen between the two groups. Those in the first group are healthy and have gained weight, whereas those in the second group are weak and have lost weight as a result of losing muscle mass. What is the biochemical basis for this difference?

Answer The β oxidation of heptanoic acid (which has an odd number of carbons) produces the three-carbon intermediate propionyl-CoA, which can be converted by propionyl-CoA carboxylase to methylmalonyl-CoA, then to succinyl-CoA. This four-carbon product of fatty acid oxidation can then be converted to oxaloacetate in the citric acid cycle, and the oxaloacetate can be used for gluconeogenesis—thus providing the animal with carbohydrate as well as energy from fatty acid oxidation. Animals fed octanoic acid (with an even number of carbons) degrade it completely to acetyl-CoA by three rounds of β oxidation. This provides energy via the citric acid cycle but does not provide starting material for gluconeogenesis. These animals are therefore deficient in glucose, the primary fuel for the brain and an intermediate in many biosynthetic pathways.

19. Metabolic Consequences of Ingesting ω-Fluorooleate The shrub *Dichapetalum toxicarium,* native to Sierra Leone, produces ω-fluorooleate, which is highly toxic to warm-blooded animals.



ω-Fluorooleate

This substance has been used as an arrow poison, and powdered fruit from the plant is sometimes used as a rat poison (hence the plant's common name, ratsbane). Why is this substance so toxic? (Hint: review Chapter 16, Problem 22.)

Answer Oxidation of ω-fluorooleate in the β-oxidation pathway forms fluoroacetyl-CoA in the last pass through the sequence. Entry of fluoroacetyl-CoA into the citric acid cycle produces fluorocitrate, a powerful inhibitor of the enzyme aconitase. As a result of this inhibition, the citric acid cycle shuts down and the flow of reducing equivalents to oxidative phosphorylation is fatally impaired.

20. Mutant Acetyl-CoA Carboxylase What would be the consequences for fat metabolism of a mutation in acetyl-CoA carboxylase that replaced the Ser residue normally phosphorylated by AMPK to an Ala residue? What might happen if the same Ser were replaced by Asp? (Hint: See Fig. 17–12.)

Answer The Ser-to-Ala change would produce an enzyme that could not be inhibited by phosphorylation by AMPK. The first step in fatty acid synthesis would be constantly turned on, and the malonyl-CoA produced by acetyl-CoA carboxylase would inhibit entry of fatty acids into mitochondria, shutting down β oxidation. The Ser-to-Asp mutation would put a negatively charged Asp residue in the position occupied by Ⓟ-Ser in the inhibited wild-type enzyme. This might mimic the effect of a phosphorylated Ser residue, shutting down acetyl-CoA carboxylase, inhibiting fatty acid synthesis, and stimulating β oxidation.

21. Effect of PDE Inhibitor on Adipocytes How would an adipocyte's response to epinephrine be affected by the addition of an inhibitor of cAMP phosphodiesterase (PDE)? (Hint: See Fig. 12–4.)

Answer Response to glucagon or epinephrine would be prolonged because cAMP, once formed, would persist, stimulating protein kinase A for a longer period and leading to longer-lasting mobilization of fatty acids in adipocytes.

22. Role of FAD as Electron Acceptor Acyl-CoA dehydrogenase uses enzyme-bound FAD as a prosthetic group to dehydrogenate the α and β carbons of fatty acyl–CoA. What is the advantage of using FAD as an electron acceptor rather than NAD^+? Explain in terms of the standard reduction potentials for the Enz-FAD/$FADH_2$ ($E'° = -0.219$ V) and NAD^+/NADH ($E'° = -0.320$ V) half-reactions.

Answer Enz-FAD, having a more positive standard reduction potential, is a better electron acceptor than NAD^+, and the reaction is driven in the direction of fatty acyl–CoA oxidation (a negative free-energy change). This more favorable free-energy change is obtained at the expense of 1 ATP; only 1.5 ATP molecules are formed per $FADH_2$ oxidized in the respiratory chain, compared with 2.5 ATP per NADH.

23. β Oxidation of Arachidic Acid How many turns of the fatty acid oxidation cycle are required for complete oxidation of arachidic acid (see Table 10–1) to acetyl-CoA?

Answer Arachidic acid is a 20-carbon saturated fatty acid. Nine cycles of the β-oxidation pathway are required for its oxidation, producing 10 molecules of acetyl-CoA, the last two in the ninth turn.

24. Fate of Labeled Propionate If [3-^{14}C]propionate (^{14}C in the methyl group) is added to a liver homogenate, ^{14}C-labeled oxaloacetate is rapidly produced. Draw a flow chart for the pathway by which propionate is transformed to oxaloacetate, and indicate the location of the ^{14}C in oxaloacetate.

Answer Propionate is first converted to the CoA derivative. Figure 17–11 shows the three-step pathway that converts propionyl-CoA to succinyl-CoA, which can be summarized as follows. Use these descriptions to prepare your own flow diagram.

1. Propionyl-CoA carboxylase uses CO_2 and ATP to form D-methylmalonyl-CoA by carboxylation at C-2 of the propionyl group.
2. Methylmalonyl-CoA epimerase shifts the CoA thioester from C-1 (of the original propionyl group) to the newly added carboxylate, making the product L-methylmalonyl-CoA.
3. Methylmalonyl-CoA mutase moves the carboxy-CoA group from C-2 to C-3 within the original propionyl unit, forming succinyl-CoA.
4. Once succinyl-CoA is formed, the citric acid cycle can convert it to oxaloacetate.

Given the stereochemistry of these reactions, the [^{14}C]-label is equilibrated at C-2 and C-3 of the oxaloacetate.

25. Phytanic Acid Metabolism When phytanic acid uniformly labeled with ^{14}C is fed to a mouse, radioactivity can be detected in malate, a citric acid cycle intermediate, within minutes. Draw a metabolic pathway that could account for this. Which of the carbon atoms in malate would contain ^{14}C label?

Answer Phytanic acid is degraded to pristanic acid by the pathway shown in Figure 17–17. Pristanic acid undergoes β oxidation, with each round yielding propionyl-CoA (not acetyl-CoA, as for a straight-chain fatty acid). Degradation of uniformly labeled phytanic acid produces

propionyl-CoA labeled in all three carbons of propionate. Propionyl-CoA is converted to succinyl-CoA by the series of reactions shown in Figure 17–11. The C-2 and C-3 of the succinyl moiety are labeled, and either C-1 or C-4 as well. When this succinate is converted to malate in the citric acid cycle, the malate is labeled at C-2 and C-3, and labeled half as much at C-1 and C-4.

26. Sources of H_2O Produced in β Oxidation The complete oxidation of palmitoyl-CoA to carbon dioxide and water is represented by the overall equation

$$\text{Palmitoyl-CoA} + 23O_2 + 108P_i + 108\text{ADP} \longrightarrow \text{CoA} + 16CO_2 + 108\text{ATP} + 23H_2O$$

Water is also produced in the reaction

$$\text{ADP} + P_i \longrightarrow \text{ATP} + H_2O$$

but is not included as a product in the overall equation. Why?

Answer ATP hydrolysis in the cell's energy-requiring reactions uses water, in the reaction

$$\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i$$

In a cell at steady state, for every mole of ATP hydrolyzed, a mole of ATP is formed by condensation of ADP + P_i. There is no *net* change in [ATP] and thus no *net* production of H_2O.

27. Biological Importance of Cobalt In cattle, deer, sheep, and other ruminant animals, large amounts of propionate are produced in the rumen through the bacterial fermentation of ingested plant matter. Propionate is the principal source of glucose for these animals, via the route propionate → oxaloacetate → glucose. In some areas of the world, notably Australia, ruminant animals sometimes show symptoms of anemia with concomitant loss of appetite and retarded growth, resulting from an inability to transform propionate to oxaloacetate. This condition is due to a cobalt deficiency caused by very low cobalt levels in the soil and thus in plant matter. Explain.

Answer One of the enzymes necessary for the conversion of propionate to oxaloacetate is methylmalonyl-CoA mutase (see Fig. 17–11). This enzyme requires as an essential cofactor the cobalt-containing coenzyme B_{12}, which is synthesized from vitamin B_{12}. A cobalt deficiency in animals would result in coenzyme B_{12} deficiency.

28. Fat Loss during Hibernation Bears expend about 25×10^6 J/day during periods of hibernation, which may last as long as seven months. The energy required to sustain life is obtained from fatty acid oxidation. How much weight loss (in kilograms) has occurred after seven months? How might ketosis be minimized during hibernation? (Assume the oxidation of fat yields 38 kJ/g.)

Answer If the catabolism of fat yields 38 kJ/g, or 3.8×10^4 kJ/kg, and the bear expends 25×10^6 J/day, or 2.5×10^4 kJ/day, then the bear will lose

$$(2.5 \times 10^4 \text{ kJ/day})/(3.8 \times 10^4 \text{ kJ/kg}) = 0.66 \text{ kg/day}$$

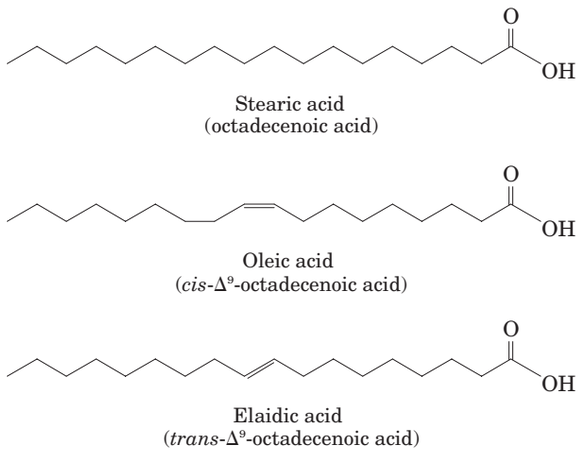
and in 7 months, or 210 days, will lose

$$0.66 \text{ kg/day} \times 210 \text{ days} = 140 \text{ kg}$$

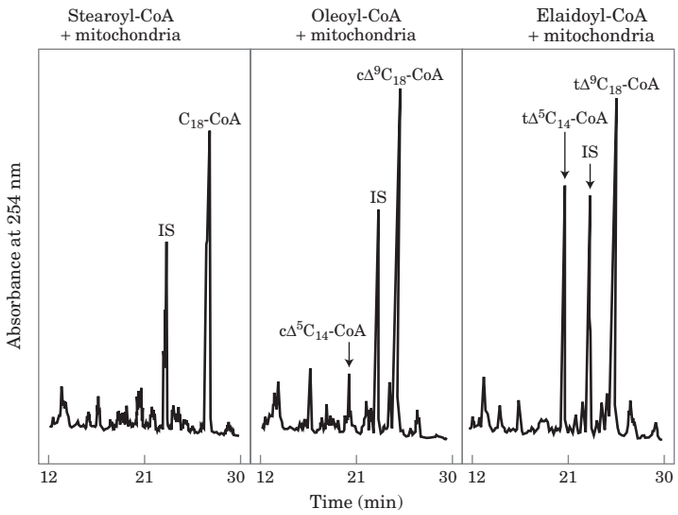
To minimize ketosis, a slow but steady degradation of nonessential proteins would provide three-, four-, and five-carbon products essential to the formation of glucose by gluconeogenesis. This would avoid the inhibition of the citric acid cycle that occurs when oxaloacetate is withdrawn from the cycle to be used for gluconeogenesis. The citric acid cycle could continue to degrade acetyl-CoA, rather than shunting it into ketone body formation.

Data Analysis Problem

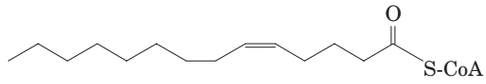
29. *β* Oxidation of Trans Fats Unsaturated fats with trans double bonds are commonly referred to as "trans fats." There has been much discussion about the effects of dietary trans fats on health. In their investigations of the effects of trans fatty acid metabolism on health, Yu and colleagues (2004) showed that a model trans fatty acid was processed differently from its cis isomer. They used three related 18-carbon fatty acids to explore the difference in β oxidation between cis and trans isomers of the same-size fatty acid.



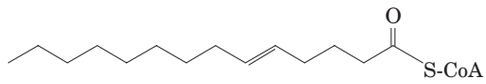
The researchers incubated the coenzyme A derivative of each acid with rat liver mitochondria for 5 minutes, then separated the remaining CoA derivatives in each mixture by HPLC (high-performance liquid chromatography). The results are shown below, with separate panels for the three experiments.



In the figure, IS indicates an internal standard (pentadecanoyl-CoA) added to the mixture, after the reaction, as a molecular marker. The researchers abbreviated the CoA derivatives as follows: stearoyl-CoA, C_{18}-CoA; *cis*-Δ^5-tetradecenoyl-CoA, cΔ^5C_{14}-CoA; oleoyl-CoA, cΔ^9C_{18}-CoA; *trans*-Δ^5-tetradecenoyl-CoA, tΔ^5C_{14}-CoA; and elaidoyl-CoA, tΔ^9C_{18}- CoA.



cis-Δ^5-Tetradecenoyl-CoA



trans-Δ^5-Tetradecenoyl-CoA

(a) Why did Yu and colleagues need to use CoA derivatives rather than the free fatty acids in these experiments?

(b) Why were no lower molecular weight CoA derivatives found in the reaction with stearoyl-CoA?

(c) How many rounds of β oxidation would be required to convert the oleoyl-CoA and the elaidoyl-CoA to *cis*-Δ^5-tetradecenoyl-CoA and *trans*-Δ^5-tetradecenoyl-CoA, respectively?

There are two forms of the enzyme acyl-CoA dehydrogenase (see Fig. 17–8a): long-chain acyl-CoA dehydrogenase (LCAD) and very-long-chain acyl-CoA dehydrogenase (VLCAD). Yu and coworkers measured the kinetic parameters of both enzymes. They used the CoA derivatives of three fatty acids: tetradecanoyl-CoA (C_{14}-CoA), *cis*-Δ^5-tetradecenoyl-CoA ($c\Delta^5C_{14}$-CoA), and *trans*-Δ^5-tetradecenoyl-CoA ($t\Delta^5C_{14}$-CoA). The results are shown below. (See Chapter 6 for definitions of the kinetic parameters.)

	LCAD			VLCAD		
	C_{14}-CoA	$c\Delta^5C_{14}$-CoA	$t\Delta^5C_{14}$-CoA	C_{14}-CoA	$c\Delta^5C_{14}$-CoA	$t\Delta^5C_{14}$-CoA
V_{max}	3.3	3.0	2.9	1.4	0.32	0.88
K_m	0.41	0.40	1.6	0.57	0.44	0.97
k_{cat}	9.9	8.9	8.5	2.0	0.42	1.12
k_{cat}/K_m	24	22	5	4	1	1

(d) For LCAD, the K_m differs dramatically for the cis and trans substrates. Provide a plausible explanation for this observation in terms of the structures of the substrate molecules. (Hint: You may want to refer to Fig. 10–2.)

(e) The kinetic parameters of the two enzymes are relevant to the differential processing of these fatty acids *only* if the LCAD or VLCAD reaction (or both) is the rate-limiting step in the pathway. What evidence is there to support this assumption?

(f) How do these different kinetic parameters explain the different levels of the CoA derivatives found after incubation of rat liver mitochondria with stearoyl-CoA, oleoyl-CoA, and elaidoyl-CoA (shown in the three-panel figure)?

Yu and coworkers measured the substrate specificity of rat liver mitochondrial thioesterase, which hydrolyzes acyl-CoA to CoA and free fatty acid (see Chapter 21). This enzyme was approximately twice as active with C_{14}-CoA thioesters as with C_{18}-CoA thioesters.

(g) Other research has suggested that free fatty acids can pass through membranes. In their experiments, Yu and colleagues found *trans*-Δ^5-tetradecenoic acid outside mitochondria (i.e., in the medium) that had been incubated with elaidoyl-CoA. Describe the pathway that led to this extramitochondrial *trans*-Δ^5-tetradecenoic acid. Be sure to indicate where in the cell the various transformations take place, as well as the enzymes that catalyze the transformations.

(h) It is often said in the popular press that "trans fats are not broken down by your cells and instead accumulate in your body." In what sense is this statement correct and in what sense is it an oversimplification?

Answer

(a) Fatty acids are converted to their CoA derivatives by enzymes in the cytoplasm; the acyl-CoAs are then imported into mitochondria for oxidation. Given that the researchers were using isolated mitochondria, they had to use CoA derivatives.

(b) Stearoyl-CoA was rapidly converted to 9 acetyl-CoA by the β-oxidation pathway. All intermediates reacted rapidly and none were detectable at significant levels.

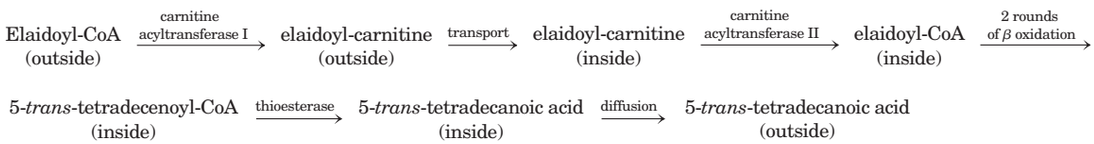
(c) Two rounds. Each round removes two carbon atoms, thus two rounds convert an 18-carbon to a 14-carbon fatty acid and 2 acetyl-CoA.

(d) The K_m is higher for the trans isomer than for the cis, so a higher concentration of trans isomer is required for the same rate of breakdown. Roughly speaking, the trans isomer binds less well than the cis, probably because differences in shape, even though not at the target site for the enzyme, affect substrate binding to the enzyme.

(e) The substrate for LCAD/VLCAD builds up differently, depending on the particular substrate; this is expected for the rate-limiting step in a pathway.

(f) The kinetic parameters show that the trans isomer is a poorer substrate than the cis for LCAD, but there is little difference for VLCAD. Because it is a poorer substrate, the trans isomer accumulates to higher levels than the cis.

(g) One possible pathway is shown below (indicating "inside" and "outside" mitochondria).



(h) It is correct insofar as trans fats are broken down less efficiently than cis fats, and thus trans fats may "leak" out of mitochondria. It is incorrect to say that trans fats are not broken down by cells; they are broken down, but at a slower rate than cis fats.

Reference

Yu, W., Liang, X., Ensenauer, R., Vockley, J., Sweetman, L., & Schultz, H. (2004) Leaky β-oxidation of a *trans*-fatty acid. *J. Biol. Chem.* **279,** 52,160–52,167.

chapter

18

Amino Acid Oxidation and the Production of Urea

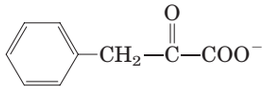
1. Products of Amino Acid Transamination Name and draw the structure of the α-keto acid resulting when each of the following amino acids undergoes transamination with α-ketoglutarate: **(a)** aspartate, **(b)** glutamate, **(c)** alanine, **(d)** phenylalanine.

Answer

(a) $^{-}OOC-CH_2-C(=O)-COO^{-}$ Oxaloacetate

(b) $^{-}OOC-CH_2-CH_2-C(=O)-COO^{-}$ α-Ketoglutarate

(c) $CH_3-C(=O)-COO^{-}$ Pyruvate

(d)  Phenylpyruvate

2. Measurement of Alanine Aminotransferase Activity The activity (reaction rate) of alanine aminotransferase is usually measured by including an excess of pure lactate dehydrogenase and NADH in the reaction system. The rate of alanine disappearance is equal to the rate of NADH disappearance measured spectrophotometrically. Explain how this assay works.

Answer The measurement of the activity of alanine aminotransferase by measurement of the reaction of its product with lactate dehydrogenase is an example of a "coupled" assay. The product of the transamination (pyruvate) is rapidly consumed in the subsequent "indicator reaction," catalyzed by an excess of lactate dehydrogenase. The dehydrogenase uses the cofactor NADH, the disappearance of which is conveniently measured by observing the rate of decrease in NADH absorption at 340 nm. Thus, the rate of disappearance of NADH is a measure of the rate of the aminotransferase reaction, *if NADH and lactate dehydrogenase are added in excess.*

3. Alanine and Glutamine in the Blood Normal human blood plasma contains all the amino acids required for the synthesis of body proteins, but not in equal concentrations. Alanine and glutamine are present in much higher concentrations than any other amino acids. Suggest why.

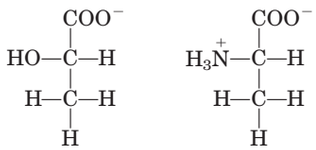
Answer Muscle tissue can convert amino acids to their keto acids plus ammonia, then oxidize the keto acids to produce ATP for muscle contraction. However, urea cannot be formed in

muscle. Alanine and glutamine transport amino groups in the bloodstream to the liver (see Fig. 18–2) from muscle and other nonhepatic tissues. In muscle, amino groups from all amino acids are transferred to pyruvate or glutamate to form alanine or glutamine, and these latter amino acids are transported to the liver.

4. **Distribution of Amino Nitrogen** If your diet is rich in alanine but deficient in aspartate, will you show signs of aspartate deficiency? Explain.

   Answer No; aspartate is readily formed by the transfer of the amino group of alanine to oxaloacetate. Cellular levels of aminotransferases are sufficient to provide all of the amino acids in this fashion, if the α-keto acids are available.

5. **Lactate versus Alanine as Metabolic Fuel: The Cost of Nitrogen Removal** The three carbons in lactate and alanine have identical oxidation states, and animals can use either carbon source as a metabolic fuel. Compare the net ATP yield (moles of ATP per mole of substrate) for the complete oxidation (to CO_2 and H_2O) of lactate versus alanine when the cost of nitrogen excretion as urea is included.



Lactate Alanine

   Answer Lactate and alanine are converted to pyruvate by their respective dehydrogenases, lactate dehydrogenase and alanine dehydrogenase, producing pyruvate and NADH + H^+ and, in the case of alanine, NH_4^+. Complete oxidation of 1 mol of pyruvate to CO_2 and H_2O produces 12.5 mol of ATP via the citric acid cycle and oxidative phosphorylation (see Table 16–1). In addition, the NADH from each dehydrogenase reaction produces 2.5 mol of ATP per mole of NADH reoxidized. Thus oxidation produces 15 mol of ATP per mole of lactate. Urea formation uses the equivalent of 4 mol of ATP per mole of urea formed (Fig. 18–10), or 2 mol of ATP per mol of NH_4^+. Subtracting this value from the energy yield of alanine results in 13 mol of ATP per mole of alanine oxidized.

6. **Ammonia Toxicity Resulting from an Arginine-Deficient Diet** In a study conducted some years ago, cats were fasted overnight then given a single meal complete in all amino acids except arginine. Within 2 hours, blood ammonia levels increased from a normal level of 18 μg/L to 140 μg/L, and the cats showed the clinical symptoms of ammonia toxicity. A control group fed a complete amino acid diet or an amino acid diet in which arginine was replaced by ornithine showed no unusual clinical symptoms.
   (a) What was the role of fasting in the experiment?
   (b) What caused the ammonia levels to rise in the experimental group? Why did the absence of arginine lead to ammonia toxicity? Is arginine an essential amino acid in cats? Why or why not?
   (c) Why can ornithine be substituted for arginine?

   Answer
   (a) Fasting resulted in lowering of blood glucose levels. Subsequent feeding of an arginine-free diet led to a rapid catabolism of all the ingested amino acids, especially the glucogenic ones. This catabolism was exacerbated by the lack of an essential amino acid, which prevented protein synthesis.
   (b) Oxidative deamination of amino acids caused the elevation of ammonia levels. In addition, the lack of arginine (an intermediate in the urea cycle) slowed the conversion of ammonia to urea. Arginine (or ornithine) synthesis in the cat is not sufficient to meet the needs imposed by the stress of this experiment, suggesting that arginine is an essential amino acid.

(c) Ornithine (or citrulline) can be substituted for arginine because it also is an intermediate in the urea cycle.

7. Oxidation of Glutamate Write a series of balanced equations, and an overall equation for the net reaction, describing the oxidation of 2 mol of glutamate to 2 mol of α-ketoglutarate and 1 mol of urea.

Answer

H_2O + glutamate + $NAD^+ \longrightarrow$ α-ketoglutarate + NH_4^+ + NADH + H^+
NH_4^+ + 2ATP + H_2O + $CO_2 \longrightarrow$ carbamoyl phosphate + 2ADP + P_i + $3H^+$
Carbamoyl phosphate + ornithine $\longrightarrow$ citrulline + P_i + H^+
Citrulline + aspartate + ATP $\longrightarrow$ argininosuccinate + AMP + PP_i + H^+
Argininosuccinate $\longrightarrow$ arginine + fumarate
Fumarate + $H_2O \longrightarrow$ malate
Malate + $NAD^+ \longrightarrow$ oxaloacetate + NADH + H^+
Oxaloacetate + glutamate $\longrightarrow$ aspartate + α-ketoglutarate
Arginine + $H_2O \longrightarrow$ urea + ornithine

The sum of these reactions is

$$2 \text{ Glutamate} + CO_2 + 4H_2O + 2NAD^+ + 3ATP \longrightarrow 2\ \alpha\text{-ketoglutarate} + 2NADH + 7H^+ + \text{urea} + 2ADP + AMP + PP_i + 2P_i \quad \textbf{(1)}$$

Three additional reactions need to be considered:

$$AMP + ATP \longrightarrow 2ADP \quad \textbf{(2)}$$
$$O_2 + 8H^+ + 2NADH + 6ADP + 6P_i \longrightarrow 2NAD^+ + 6ATP + 8H_2O \quad \textbf{(3)}$$
$$H_2O + PP_i \longrightarrow 2P_i + H^+ \quad \textbf{(4)}$$

Summing the last four equations:

$$2 \text{ Glutamate} + CO_2 + O_2 + 2ADP + 2P_i \longrightarrow 2\ \alpha\text{-ketoglutarate} + \text{urea} + 3H_2O + 2ATP$$

8. Transamination and the Urea Cycle Aspartate aminotransferase has the highest activity of all the mammalian liver aminotransferases. Why?

Answer The second amino group introduced into urea is transferred from aspartate. This amino acid is generated in large quantities by transamination between oxaloacetate and glutamate (and many other amino acids), catalyzed by aspartate aminotransferase. Approximately one half of all the amino groups excreted as urea must pass through the aspartate aminotransferase reaction, and liver contains higher levels of this aminotransferase than of any other.

9. The Case against the Liquid Protein Diet A weight-reducing diet heavily promoted some years ago required the daily intake of "liquid protein" (soup of hydrolyzed gelatin), water, and an assortment of vitamins. All other food and drink were to be avoided. People on this diet typically lost 10 to 14 lb in the first week.

(a) Opponents argued that the weight loss was almost entirely due to water loss and would be regained very soon after a normal diet was resumed. What is the biochemical basis for this argument?

(b) A number of people on this diet died. What are some of the dangers inherent in the diet and how can they lead to death?

Answer

(a) A person on a diet consisting only of protein must use amino acids as the principal source of metabolic fuel. Because the catabolism of amino acids requires the removal of

nitrogen as urea, the process consumes large quantities of water to dilute and excrete the urea in the urine. Furthermore, electrolytes in the "liquid protein" must be diluted with water and excreted. If this abnormally large daily water loss through the kidney is not balanced by a sufficient water intake, a net loss of body water results.

(b) When considering the nutritional benefits of protein, keep in mind the total amount of amino acids needed for protein synthesis and the distribution of amino acids in the dietary protein. Gelatin contains a nutritionally unbalanced distribution of amino acids. As large amounts of gelatin are ingested and the excess amino acids are catabolized, the capacity of the urea cycle may be exceeded, leading to ammonia toxicity. This is further complicated by the dehydration that may result from excretion of large quantities of urea. A combination of these two factors could produce coma and death.

10. Ketogenic Amino Acids Which amino acids are exclusively ketogenic?

Answer Lysine and leucine are exclusively ketogenic. These amino acids are degraded entirely to acetyl-CoA and acetoacetyl-CoA, and no parts of their carbon skeletons can be used for glucose synthesis. Leucine is especially common in proteins. Its degradation makes a substantial contribution to ketosis under starvation conditions.

11. A Genetic Defect in Amino Acid Metabolism: A Case History A two-year-old child was taken to the hospital. His mother said that he vomited frequently, especially after feedings. The child's weight and physical development were below normal. His hair, although dark, contained patches of white. A urine sample treated with ferric chloride ($FeCl_3$) gave a green color characteristic of the presence of phenylpyruvate. Quantitative analysis of urine samples gave the results shown in the table.

	Concentration (mM)	
Substance	**Patient's urine**	**Normal urine**
Phenylalanine	7.0	0.01
Phenylpyruvate	4.8	0
Phenyllactate	10.3	0

(a) Suggest which enzyme might be deficient in this child. Propose a treatment.

(b) Why does phenylalanine appear in the urine in large amounts?

(c) What is the source of phenylpyruvate and phenyllactate? Why does this pathway (normally not functional) come into play when the concentration of phenylalanine rises?

(d) Why does the boy's hair contain patches of white?

Answer

(a) Because phenylalanine (and its related phenylketones) accumulate in this patient, it is likely that the first enzyme in phenylalanine catabolism, phenylalanine hydroxylase (also called phenylalanine-4-monooxygenase), is defective or missing (see Fig. 18–23). The most appropriate treatment for patients with this disease, known as phenylketonuria (PKU), is to establish a low-phenylalanine diet that provides just enough of the amino acid to meet the needs for protein synthesis.

(b) Phenylalanine appears in the urine because high levels of this amino acid accumulate in the bloodstream and the body attempts to dispose of it.

(c) Phenylalanine is converted to phenylpyruvate by transamination, a reaction that has an equilibrium constant of about 1.0. Phenyllactate is formed from phenylpyruvate by reduction (see Fig. 18–25). This pathway is of importance only when phenylalanine hydroxylase is defective.

(d) The normal catabolic pathway of phenylalanine is through tyrosine, a precursor of melanin, the dark pigment normally present in hair. Decreased tyrosine levels in patients with phenylketonuria result in varying degrees of pigment loss.

12. Role of Cobalamin in Amino Acid Catabolism Pernicious anemia is caused by impaired absorption of vitamin B_{12}. What is the effect of this impairment on the catabolism of amino acids? Are all amino acids equally affected? (Hint: see Box 17–2.)

Answer The catabolism of the carbon skeletons of valine, isoleucine, and methionine is impaired because of the absence of a functional methylmalonyl-CoA mutase. This enzyme requires coenzyme B_{12} as a cofactor, and a deficiency of this vitamin leads to elevated methylmalonic acid levels (methylmalonic acidemia). The symptoms and effects of this deficiency are severe (see Table 18–2 and Box 18–2).

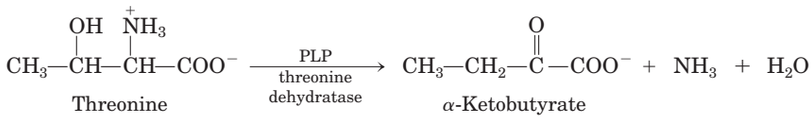
13. Vegetarian Diets Vegetarian diets can provide high levels of antioxidants and a lipid profile that can help prevent coronary disease. However, there can be some associated problems. Blood samples were taken from a large group of volunteer subjects who were vegans (strict vegetarians: no animal products), lactovegetarians (vegetarians who eat dairy products), or omnivores (individuals with a normal, varied diet including meat). In each case, the volunteers had followed the diet for several years. The blood levels of both homocysteine and methylmalonate were elevated in the vegan group, somewhat lower in the lactovegetarian group, and much lower in the omnivore group. Explain.

Answer The vegan diet lacks vitamin B_{12}, leading to the increase in homocysteine and methylmalonate (reflecting the deficiencies in methionine synthase and methylmalonic acid mutase, respectively) in individuals on the diet for several years. Dairy products provide some vitamin B_{12} in the lactovegetarian diet.

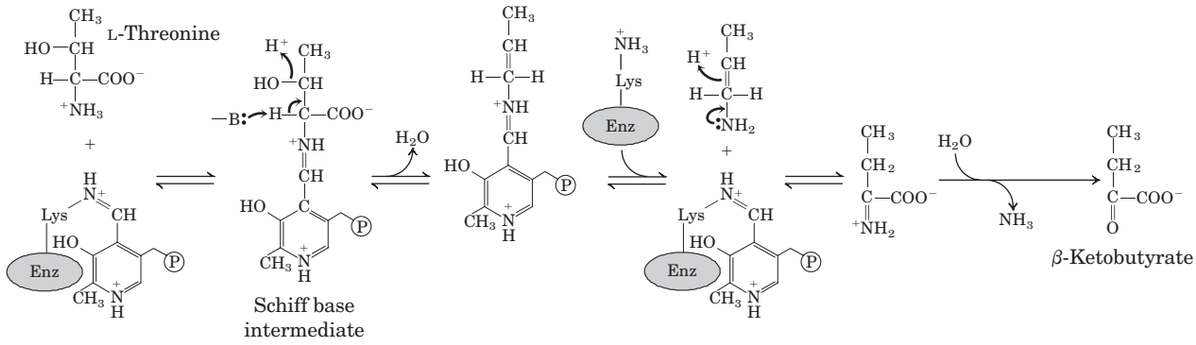
14. Pernicious Anemia Vitamin B_{12} deficiency can arise from a few rare genetic diseases that lead to low B_{12} levels despite a normal diet that includes B_{12}-rich meat and dairy sources. These conditions cannot be treated with dietary B_{12} supplements. Explain.

Answer The genetic forms of pernicious anemia generally arise as a result of defects in the pathway that mediates absorption of dietary vitamin B_{12} (see Box 17–2, p. 658). Because dietary supplements are not absorbed in the intestine, these conditions are treated by injecting supplementary B_{12} directly into the bloodstream.

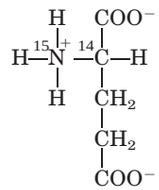
15. Pyridoxal Phosphate Reaction Mechanisms Threonine can be broken down by the enzyme threonine dehydratase, which catalyzes the conversion of threonine to α-ketobutyrate and ammonia. The enzyme uses PLP as a cofactor. Suggest a mechanism for this reaction, based on the mechanisms in Figure 18–6. Note that this reaction includes an elimination at the β carbon of threonine.



Answer The mechanism is identical to that for serine dehydratase (see Fig. 18–20a, p. 693) except that the extra methyl group of threonine is retained, yielding α-ketobutyrate instead of pyruvate.



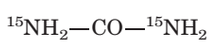
16. Pathway of Carbon and Nitrogen in Glutamate Metabolism When [2-^{14}C, ^{15}N] glutamate undergoes oxidative degradation in the liver of a rat, in which atoms of the following metabolites will each isotope be found: **(a)** urea, **(b)** succinate, **(c)** arginine, **(d)** citrulline, **(e)** ornithine, **(f)** aspartate?



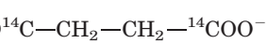
Labeled glutamate

Answer

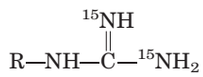
(a) The amino groups of urea contain ^{15}N, a result of glutamate dehydrogenase producing $^{15}NH_4^+$ or of a transaminase producing ^{15}N-labeled aspartate.



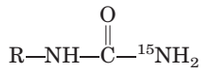
(b) After loss of the amino group, the [2-^{14}C] α-ketoglutarate is metabolized in the citric acid cycle. Succinate thus formed is labeled in the carboxyl groups.



(c) The arginine formed in the urea cycle contains ^{15}N in both guanidino nitrogens.

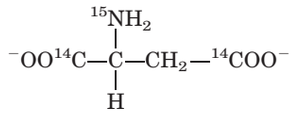


(d) Citrulline formed in the urea cycle contains ^{15}N in the carboxamide group.



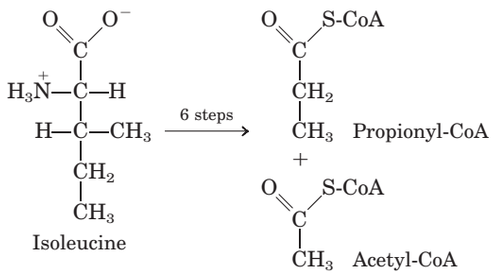
(e) No labeled N is found in ornithine.

(f) Aspartate contains ^{15}N in its amino group as a result of transamination from glutamate. It also contains ^{14}C in its carboxyl groups as a result of succinate conversion to oxaloacetate (as in **(b)**).

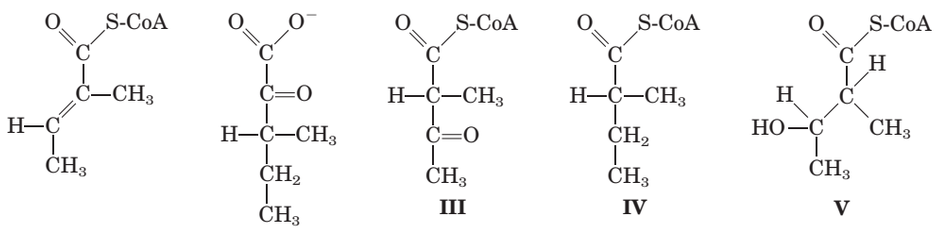


Note: in **(c), (d),** and **(e),** these urea cycle intermediates will contain low levels of ^{14}C as a result of a very weak synthesis of ornithine from glutamate.

17. Chemical Strategy of Isoleucine Catabolism Isoleucine is degraded in six steps to propionyl-CoA and acetyl-CoA.



(a) The chemical process of isoleucine degradation includes strategies analogous to those used in the citric acid cycle and the β oxidation of fatty acids. The intermediates of isoleucine degradation (I to V) shown below are not in the proper order. Use your knowledge and understanding of the citric acid cycle and β-oxidation pathway to arrange the intermediates in the proper metabolic sequence for isoleucine degradation.



(b) For each step you propose, describe the chemical process, provide an analogous example from the citric acid cycle or β-oxidation pathway (where possible), and indicate any necessary cofactors.

Answer

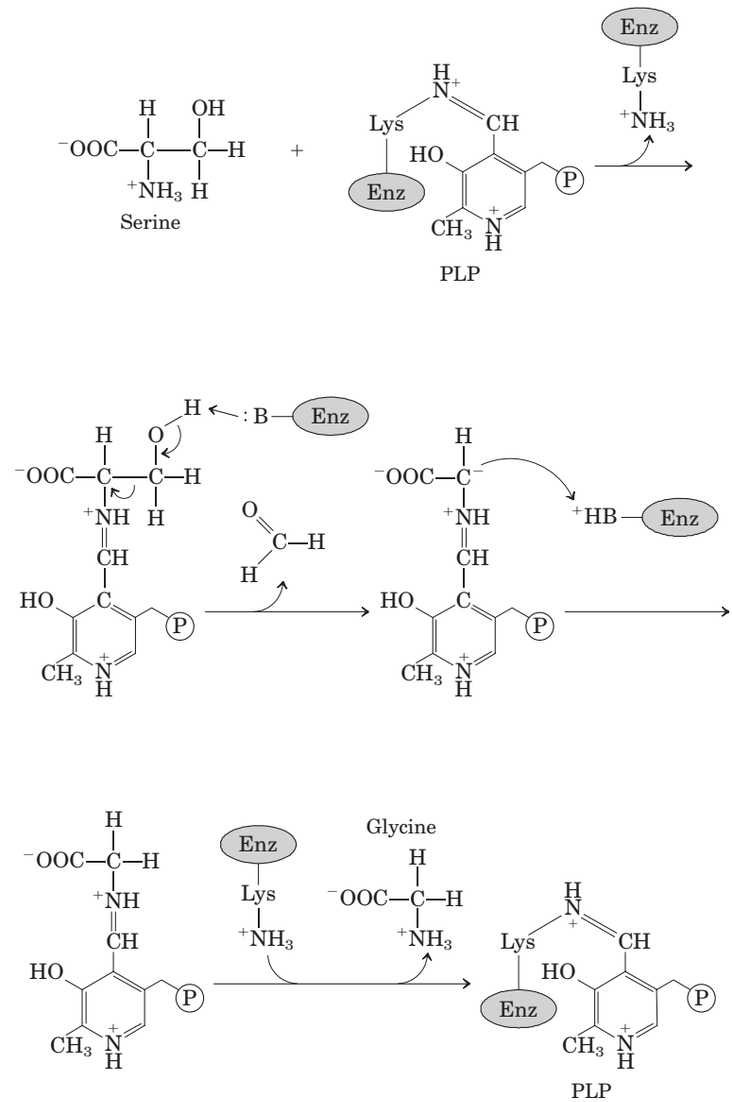
1 2 3 4 5 6

(a) Isoleucine → II → IV → I → V → III → acetyl-CoA + propionyl-CoA

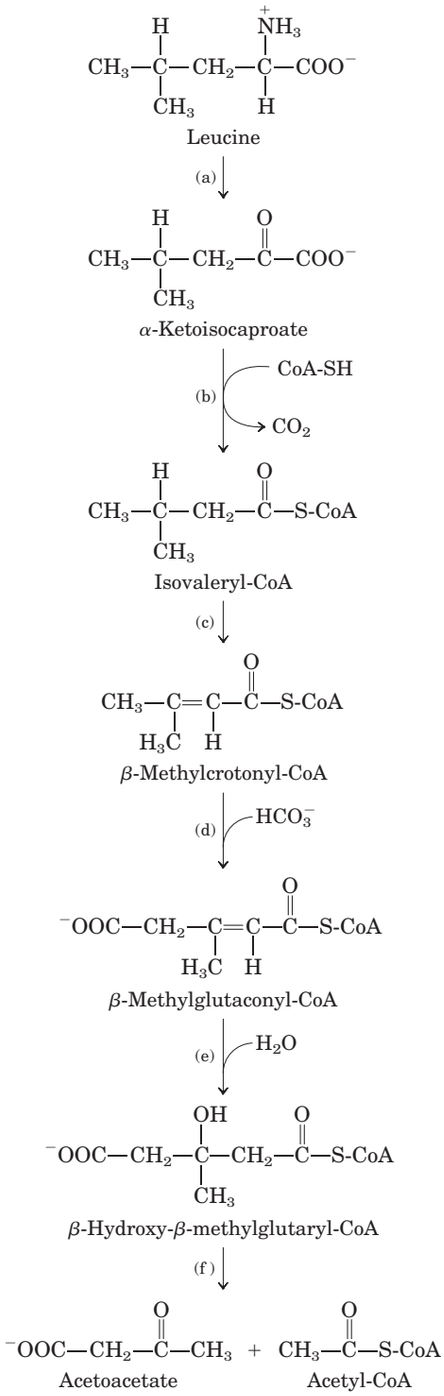
(b) Step 1 is a transamination that has no analogous reaction; it requires PLP. Step 2 is an oxidative decarboxylation similar to the pyruvate dehydrogenase reaction; it requires NAD^+, TPP, lipoate, FAD. Step 3 is an oxidation similar to the succinate dehydrogenase reaction; it requires FAD. Step 4 is a hydration analogous to the fumarase reaction; no cofactor is required. Step 5 is an oxidation analogous to the malate dehyrogenase reaction of the citric acid cycle; it requires NAD^+. Step 6 is a thiolysis analogous to the final cleavage step of β oxidation catalyzed by thiolase; it requires CoA.

18. Role of Pyridoxal Phosphate in Glycine Metabolism The enzyme serine hydroxymethyltransferase requires pyridoxal phosphate as cofactor. Propose a mechanism for the reaction catalyzed by this enzyme, in the direction of serine degradation (glycine production). (Hint: see Figs 18–19 and 18–20b.)

Answer See the mechanism below. The formaldehyde produced in the second step reacts rapidly with tetrahydrofolate at the enzyme active site to produce N^5,N^{10}-methylene tetrahydrofolate (see Fig. 18–17).



19. Parallel Pathways for Amino Acid and Fatty Acid Degradation The carbon skeleton of leucine is degraded by a series of reactions closely analogous to those of the citric acid cycle and β oxidation. For each reaction, **(a)** through **(f),** indicate its type, provide an analogous example from the citric acid cycle or β-oxidation pathway (where possible), and note any necessary cofactors.



Answer

(a) Transamination; no analogies in either pathway; requires PLP.

(b) Oxidative decarboxylation; analogous to oxidative decarboxylation of pyruvate to acetyl-CoA prior to entry into the citric acid cycle, and of α-ketoglutarate to succinyl-CoA in the citric acid cycle; requires NAD^+, FAD, lipoate, thiamine pyrophosphate.

(c) Dehydrogenation (oxidation); analogous to dehydrogenation of succinate to fumarate in the citric acid cycle and of fatty acyl–CoA to enoyl-CoA in β oxidation; requires FAD.

(d) Carboxylation; no analogous reaction in the citric acid cycle or β oxidation; requires ATP and biotin.

(e) Hydration; analogous to hydration of fumarate to malate in the citric acid cycle and of enoyl-CoA to 3-hydroxyacyl-CoA in β oxidation; no cofactors.

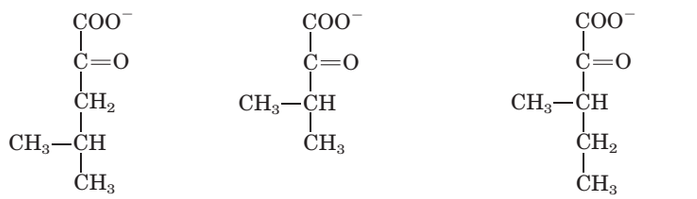
(f) Reverse aldol reaction; analogous to reverse of citrate synthase reaction in the citric acid cycle and identical to cleavage of β-hydroxy-β-methylglutaryl-CoA in formation of ketone bodies; no cofactors.

Data Analysis Problem

20. Maple Syrup Urine Disease Figure 18–28 shows the pathway for the degradation of branched-chain amino acids and the site of the biochemical defect that causes maple syrup urine disease. The initial findings that eventually led to the discovery of the defect in this disease were presented in three papers published in the late 1950s and early 1960s. This problem traces the history of the findings from initial clinical observations to proposal of a biochemical mechanism.

Menkes, Hurst, and Craig (1954) presented the cases of four siblings, all of whom died following a similar course of symptoms. In all four cases, the mother's pregnancy and the birth had been normal. The first 3 to 5 days of each child's life were also normal. But soon thereafter each child began having convulsions, and the children died between the ages of 11 days and 3 months. Autopsy showed considerable swelling of the brain in all cases. The children's urine had a strong, unusual "maple syrup" odor, starting from about the third day of life.

Menkes (1959) reported data collected from six more children. All showed symptoms similar to those described above, and died within 15 days to 20 months of birth. In one case, Menkes was able to obtain urine samples during the last months of the infant's life. When he treated the urine with 2,4-dinitrophenylhydrazone, which forms colored precipitates with keto compounds, he found three α-keto acids in unusually large amounts:



α-Ketoisocaproate α-Ketoisovalerate α-Keto-β-methyl-n-valerate

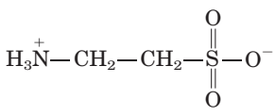
(a) These α-keto acids are produced by the deamination of amino acids. For each of the α-keto acids above, draw and name the amino acid from which it was derived.

Dancis, Levitz, and Westall (1960) collected further data that led them to propose the biochemical defect shown in Figure 18–28. In one case, they examined a patient whose urine first showed the maple syrup odor when he was 4 months old. At the age of 10 months (March 1956), the child was admitted to the hospital because he had a fever, and he showed grossly retarded motor development.

At the age of 20 months (January 1957), he was readmitted and was found to have the degenerative neurological symptoms seen in previous cases of maple syrup urine disease; he died soon after. Results of his blood and urine analyses are shown in the table below, along with normal values for each component.

	Urine (mg/24 h)			Plasma (mg/ml)	
	Normal	Patient		Normal	Patient
Amino acid(s)		Mar. 1956	Jan. 1957		Jan. 1957
Alanine	5–15	0.2	0.4	3.0–4.8	0.6
Asparagine and glutamine	5–15	0.4	0	3.0–5.0	2.0
Aspartic acid	1–2	0.2	1.5	0.1–0.2	0.04
Arginine	1.5–3	0.3	0.7	0.8–1.4	0.8
Cystine	2–4	0.5	0.3	1.0–1.5	0
Glutamic acid	1.5–3	0.7	1.6	1.0–1.5	0.9
Glycine	20–40	4.6	20.7	1.0–2.0	1.5
Histidine	8–15	0.3	4.7	1.0–1.7	0.7
Isoleucine	2–5	2.0	13.5	0.8–1.5	2.2
Leucine	3–8	2.7	39.4	1.7–2.4	14.5
Lysine	2–12	1.6	4.3	1.5–2.7	1.1
Methionine	2–5	1.4	1.4	0.3–0.6	2.7
Ornithine	1–2	0	1.3	0.6–0.8	0.5
Phenylalanine	2–4	0.4	2.6	1.0–1.7	0.8
Proline	2–4	0.5	0.3	1.5–3.0	0.9
Serine	5–15	1.2	0	1.3–2.2	0.9
Taurine	1–10	0.2	18.7	0.9–1.8	0.4
Threonine	5–10	0.6	0	1.2–1.6	0.3
Tryptophan	3–8	0.9	2.3	Not measured	0
Tyrosine	4–8	0.3	3.7	1.5–2.3	0.7
Valine	2–4	1.6	15.4	2.0–3.0	13.1

(b) The table includes taurine, an amino acid not normally found in proteins. Taurine is often produced as a by-product of cell damage. Its structure is:



Based on its structure and the information in this chapter, what is the most likely amino acid precursor of taurine? Explain your reasoning.

(c) Compared with the normal values given in the table, which amino acids showed significantly elevated levels in the patient's blood in January 1957? Which ones in the patient's urine?

Based on their results and their knowledge of the pathway shown in Figure 18–28, Dancis and coauthors concluded: "although it appears most likely to the authors that the primary block is in the metabolic degradative pathway of the branched-chain amino acids, this cannot be considered established beyond question."

(d) How do the data presented here support this conclusion?

(e) Which data presented here do *not* fit this model of maple syrup urine disease? How do you explain these seemingly contradictory data?

(f) What data would you need to collect to be more secure in your conclusion?

Answer

(a) Leucine; valine; isoleucine

(b) Cysteine (derived from cystine). If cysteine were decarboxylated as shown in Figure 18–6, it would yield $H_3N^+—CH_2—CH_2—SH$, which could be oxidized to taurine.

(c) The January 1957 blood shows significantly elevated levels of isoleucine, leucine, methionine, and valine; the January 1957 urine, significantly elevated isoleucine, leucine, taurine, and valine.

(d) All patients had high levels of isoleucine, leucine, and valine in both blood and urine, suggesting a defect in the breakdown of these amino acids. Given that the urine also contained high levels of the keto forms of these three amino acids, the block in the pathway must occur after deamination but before dehydrogenation (as shown in Fig. 18–28).

(e) The model does not explain the high levels of methionine in blood and taurine in urine. The high taurine levels may be due to the death of brain cells during the end stage of the disease. However, the reason for high levels of methionine in blood are unclear; the pathway of methionine degradation is not linked with the degradation of branched-chain amino acids. Increased methionine could be a secondary effect of buildup of the other amino acids. It is important to keep in mind that the January 1957 samples were from an individual who was dying, so comparing blood and urine results with those of a healthy individual may not be appropriate.

(f) The following information is needed (and was eventually obtained by other workers): (1) The dehydrogenase activity is significantly reduced or missing in individuals with maple syrup urine disease. (2) The disease is inherited as a single-gene defect. (3) The defect occurs in a gene encoding all or part of the dehydrogenase. (4) The genetic defect leads to production of inactive enzyme.

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Menkes, J.H., Hurst, P.L., & Craig J.M. (1954) A new syndrome: progressive familial infantile cerebral dysfunction associated with an unusual urinary substance. *Pediatrics* **14,** 462-466.

Oxidative Phosphorylation and Photophosphorylation

chapter

19

1. Oxidation-Reduction Reactions The NADH dehydrogenase complex of the mitochondrial respiratory chain promotes the following series of oxidation-reduction reactions, in which Fe^{3+} and Fe^{2+} represent the iron in iron-sulfur centers, Q is ubiquinone, QH_2 is ubiquinol, and E is the enzyme:

(1) $NADH + H^+ + \text{E-FMN} \longrightarrow NAD^+ + \text{E-FMNH}_2$

(2) $\text{E-FMNH}_2 + 2Fe^{3+} \longrightarrow \text{E-FMN} + 2Fe^{2+} + 2H^+$

(3) $2Fe^{2+} + 2H^+ + Q \longrightarrow 2Fe^{3+} + QH_2$

Sum: $NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$

For each of the three reactions catalyzed by the NADH dehydrogenase complex, identify **(a)** the electron donor, **(b)** the electron acceptor, **(c)** the conjugate redox pair, **(d)** the reducing agent, and **(e)** the oxidizing agent.

Answer Oxidation-reduction reactions require an electron donor and an electron acceptor. Recall that electron donors are reducing agents; electron acceptors are oxidizing agents.

(1) NADH is the electron donor **(a)** and the reducing agent **(d);** E-FMN is the electron acceptor **(b)** and the oxidizing agent **(e);** NAD^+/NADH and E-FMN/E-FMNH_2 are conjugate redox pairs **(c).**

(2) E-FMNH_2 is the electron donor **(a)** and reducing agent **(d);** Fe^{3+} is the electron acceptor **(b)** and oxidizing agent **(e);** E-FMN/E-FMNH_2 and Fe^{3+}/Fe^{2+} are redox pairs **(c).**

(3) Fe^{2+} is the electron donor **(a)** and reducing agent **(d);** Q is the electron acceptor **(b)** and oxidizing agent **(e);** and Fe^{3+}/Fe^{2+} and Q/QH_2 are redox pairs **(c).**

2. All Parts of Ubiquinone Have a Function In electron transfer, only the quinone portion of ubiquinone undergoes oxidation-reduction; the isoprenoid side chain remains unchanged. What is the function of this chain?

Answer The long isoprenoid side chain makes ubiquinone very soluble in lipids and allows it to diffuse in the semifluid membrane. This is important because ubiquinone transfers electrons from Complexes I and II to Complex III, all of which are embedded in the inner mitochondrial membrane.

3. Use of FAD Rather Than NAD^+ in Succinate Oxidation All the dehydrogenases of glycolysis and the citric acid cycle use NAD^+ ($E'°$ for NAD^+/NADH is -0.32 V) as electron acceptor except succinate dehydrogenase, which uses covalently bound FAD ($E'°$ for FAD/$FADH_2$ in this enzyme is 0.050 V). Suggest why FAD is a more appropriate electron acceptor than NAD^+ in the dehydrogenation of succinate, based on the $E'°$ values of fumarate/succinate ($E'° = 0.031$), NAD^+/NADH, and the succinate dehydrogenase FAD/$FADH_2$.

Answer From the difference in standard reduction potential ($\Delta E'°$) for each pair of half-reactions, we can calculate the $\Delta G'°$ values for the oxidation of succinate using NAD^+ and oxidation using E-FAD.

For NAD^+:

$$\begin{aligned}\Delta G'° &= -n\mathcal{F}\Delta E'° \\ &= -2(96.5\ \text{kJ/V} \cdot \text{mol})(-0.32\ \text{V} - 0.031\ \text{V}) \\ &= \ \ 68\ \text{kJ/mol}\end{aligned}$$

For E-FAD:

$$\begin{aligned}\Delta G'° &= -2(96.5\ \text{kJ/V} \cdot \text{mol})(0.050\ \text{V} - 0.031\ \text{V}) \\ &= -3.7\ \text{kJ/mol}\end{aligned}$$

The oxidation of succinate by E-FAD is favored by the negative standard free-energy change, which is consistent with a K'_{eq} of >1. Oxidation by NAD^+ would require a large, positive, standard free-energy change and have a K'_{eq} favoring the synthesis of succinate.

4. Degree of Reduction of Electron Carriers in the Respiratory Chain The degree of reduction of each carrier in the respiratory chain is determined by conditions in the mitochondrion. For example, when NADH and O_2 are abundant, the steady-state degree of reduction of the carriers decreases as electrons pass from the substrate to O_2. When electron transfer is blocked, the carriers before the block become more reduced and those beyond the block become more oxidized (see Fig. 19–6). For each of the conditions below, predict the state of oxidation of ubiquinone and cytochromes *b*, c_1, *c*, and $a + a_3$.

(a) Abundant NADH and O_2, but cyanide added

(b) Abundant NADH, but O_2 exhausted

(c) Abundant O_2, but NADH exhausted

(d) Abundant NADH and O_2

Answer As shown in Figure 19–6, the oxidation-reduction state of the carriers in the electron-transfer system varies with the conditions.

(a) Cyanide inhibits cytochrome oxidase ($a + a_3$); all carriers become reduced.

(b) In the absence of O_2, no terminal electron acceptor is present; all carriers become reduced.

(c) In the absence of NADH, no carrier can be reduced; all carriers become oxidized.

(d) These are the usual conditions for an aerobic, actively metabolizing cell; the early carriers (e.g., Q) are somewhat reduced, while the late ones (e.g., cytochrome *c*) are oxidized.

5. Effect of Rotenone and Antimycin A on Electron Transfer Rotenone, a toxic natural product from plants, strongly inhibits NADH dehydrogenase of insect and fish mitochondria. Antimycin A, a toxic antibiotic, strongly inhibits the oxidation of ubiquinol.

(a) Explain why rotenone ingestion is lethal to some insect and fish species.

(b) Explain why antimycin A is a poison.

(c) Given that rotenone and antimycin A are equally effective in blocking their respective sites in the electron-transfer chain, which would be a more potent poison? Explain.

Answer

(a) The inhibition of NADH dehydrogenase by rotenone decreases the rate of electron flow through the respiratory chain, which in turn decreases the rate of ATP production. If this reduced rate is unable to meet its ATP requirements, the organism dies.

(b) Antimycin A strongly inhibits the oxidation of reduced Q in the respiratory chain, severely limiting the rate of electron transfer and ATP production.

(c) Electrons flow into the system at Complex I from the NAD^+-linked reactions and at Complex II from succinate and fatty acyl–CoA through FAD (see Figs. 19–8 and 19–16). Antimycin A inhibits electron flow (through Q) from *all* these sources, whereas rotenone inhibits flow only through Complex I. Thus, antimycin A is a more potent poison.

6. Uncouplers of Oxidative Phosphorylation In normal mitochondria the rate of electron transfer is tightly coupled to the demand for ATP. When the rate of use of ATP is relatively low, the rate of electron transfer is low; when demand for ATP increases, electron-transfer rate increases. Under these conditions of tight coupling, the number of ATP molecules produced per atom of oxygen consumed when NADH is the electron donor—the P/O ratio–is about 2.5.

(a) Predict the effect of a relatively low and a relatively high concentration of uncoupling agent on the rate of electron transfer and the P/O ratio.

(b) Ingestion of uncouplers causes profuse sweating and an increase in body temperature. Explain this phenomenon in molecular terms. What happens to the P/O ratio in the presence of uncouplers?

(c) The uncoupler 2,4-dinitrophenol was once prescribed as a weight-reducing drug. How could this agent, in principle, serve as a weight-reducing aid? Uncoupling agents are no longer prescribed because some deaths occurred following their use. Why might the ingestion of uncouplers lead to death?

Answer Uncouplers of oxidative phosphorylation stimulate the rate of electron flow but not ATP synthesis.

(a) At relatively low levels of an uncoupling agent, P/O ratios drop somewhat, but the cell can compensate for this by increasing the rate of electron flow; ATP levels can be kept relatively normal. At high levels of uncoupler, P/O ratios approach zero and the cell cannot maintain ATP levels.

(b) As amounts of an uncoupler increase, the P/O ratio decreases and the body struggles to make sufficient ATP by oxidizing more fuel. The heat produced by this increased rate of oxidation raises the body temperature. The P/O ratio is affected as noted in **(a).**

(c) Increased activity of the respiratory chain in the presence of an uncoupler requires the degradation of additional energy stores (glycogen and fat). By oxidizing more fuel in an attempt to produce the same amount of ATP, the organism loses weight. If the P/O ratio nears zero, the lack of ATP will be lethal.

7. Effects of Valinomycin on Oxidative Phosphorylation When the antibiotic valinomycin is added to actively respiring mitochondria, several things happen: the yield of ATP decreases, the rate of O_2 consumption increases, heat is released, and the pH gradient across the inner mitochondrial membrane increases. Does valinomycin act as an uncoupler or an inhibitor of oxidative phosphorylation? Explain the experimental observations in terms of the antibiotic's ability to transfer K^+ ions across the inner mitochondrial membrane.

Answer The observed effects are consistent with the action of an uncoupler—that is, an agent that causes the free energy released in electron transfer to appear as heat rather than in ATP. In respiring mitochondria, H^+ ions are translocated out of the matrix during electron transfer, creating a proton gradient and an electrical potential across the membrane. A significant portion of the free energy used to synthesize ATP originates from this electric potential. Valinomycin combines with K^+ ions to form a complex that passes through the inner mitochondrial membrane. So, as a proton is translocated out by electron transfer, a K^+ ion moves in, and the potential across the membrane is lost. This reduces the yield of ATP per mole of protons flowing through ATP synthase (F_oF_1). In other words, electron transfer and phosphorylation become uncoupled. In response to the decreased efficiency of ATP synthesis, the rate of electron transfer increases markedly. This results in an increase in the H^+ gradient, in oxygen consumption, and in the amount of heat released.

8. **Mode of Action of Dicyclohexylcarbodiimide (DCCD)** When DCCD is added to a suspension of tightly coupled, actively respiring mitochondria, the rate of electron transfer (measured by O_2 consumption) and the rate of ATP production dramatically decrease. If a solution of 2,4-dinitrophenol is now added to the preparation, O_2 consumption returns to normal but ATP production remains inhibited.
   (a) What process in electron transfer or oxidative phosphorylation is affected by DCCD?
   (b) Why does DCCD affect the O_2 consumption of mitochondria? Explain the effect of 2,4-dinitrophenol on the inhibited mitochondrial preparation.
   (c) Which of the following inhibitors does DCCD most resemble in its action: antimycin A, rotenone, or oligomycin?

   Answer
   (a) DCCD inhibits ATP synthesis. In tightly coupled mitochondria, this inhibition leads to inhibition of electron transfer also.
   (b) A decrease in electron transfer causes a decrease in O_2 consumption. 2,4-Dinitrophenol uncouples electron transfer from ATP synthesis, allowing respiration to increase. No ATP is synthesized and the P/O ratio decreases.
   (c) DCCD and oligomycin inhibit ATP synthesis (see Table 19–4).

9. **Compartmentalization of Citric Acid Cycle Components** Isocitrate dehydrogenase is found only in the mitochondrion, but malate dehydrogenase is found in both the cytosol and mitochondrion. What is the role of cytosolic malate dehydrogenase?

   Answer Malate dehydrogenase catalyzes the conversion of malate to oxaloacetate in the citric acid cycle, which takes place in the mitochondrion, and also plays a key role in the transport of reducing equivalents across the inner mitochondrial membrane via the malate-aspartate shuttle (Fig. 19–29). This shuttle requires the presence of malate dehydrogenase in the cytosol and the mitochondrial matrix.

10. **The Malate–α-Ketoglutarate Transport System** The transport system that conveys malate and α-ketoglutarate across the inner mitochondrial membrane (see Fig. 19–29) is inhibited by *n*-butylmalonate. Suppose *n*-butylmalonate is added to an aerobic suspension of kidney cells using glucose exclusively as fuel. Predict the effect of this inhibitor on **(a)** glycolysis, **(b)** oxygen consumption, **(c)** lactate formation, and **(d)** ATP synthesis.

    Answer NADH produced in the cytosol cannot cross the inner mitochondrial membrane, but must be oxidized if glycolysis is to continue. Reducing equivalents from NADH enter the mitochondrion by way of the malate-aspartate shuttle. NADH reduces oxaloacetate to form malate and NAD^+, and the malate is transported into the mitochondrion. Cytosolic oxidation of glucose can continue, and the malate is converted back to oxaloacetate and NADH in the mitochondrion (see Fig. 19–29).

(a) If *n*-butylmalonate, an inhibitor of the malate–α-ketoglutarate transporter, is added to cells, NADH accumulates in the cytosol. This forces glycolysis to operate anaerobically, with reoxidation of NADH in the lactate dehydrogenase reaction.

(b) Because reducing equivalents from the oxidation reactions of glycolysis do not enter the mitochondrion, oxygen consumption slows and eventually ceases.

(c) The end product of anaerobic glycolysis, lactate, accumulates.

(d) ATP is not formed aerobically because the cells have converted to anaerobic glycolysis. Overall, ATP synthesis decreases drastically, to 2 ATP per glucose molecule.

11. Cellular ADP Concentration Controls ATP Formation Although ADP and P_i are required for the synthesis of ATP, the rate of synthesis depends mainly on the concentration of ADP, not P_i. Why?

Answer The steady-state concentration of P_i in the cell is much higher than that of ADP. As the ADP concentration rises as a result of ATP consumption, there is little change in $[P_i]$, so P_i cannot serve as a regulator.

12. Time Scales of Regulatory Events in Mitochondria Compare the likely time scales for the adjustments in respiratory rate caused by **(a)** increased [ADP] and **(b)** reduced pO_2. What accounts for the difference?

Answer In **(a),** respiratory control by ADP, the increase in respiratory rate is limited by the rate of diffusion of ADP, and the response would be expected to occur in fractions of a millisecond. The adjustment to **(b),** hypoxia mediated by HIF-1, requires a change in concentration of several proteins, the result of increased synthesis or degradation. The time scale for protein synthesis or degradation is typically many seconds to hours—much longer than the time required for changes in substrate concentration.

13. The Pasteur Effect When O_2 is added to an anaerobic suspension of cells consuming glucose at a high rate, the rate of glucose consumption declines greatly as the O_2 is used up, and accumulation of lactate ceases. This effect, first observed by Louis Pasteur in the 1860s, is characteristic of most cells capable of aerobic and anaerobic glucose catabolism.

(a) Why does the accumulation of lactate cease after O_2 is added?

(b) Why does the presence of O_2 decrease the rate of glucose consumption?

(c) How does the onset of O_2 consumption slow down the rate of glucose consumption? Explain in terms of specific enzymes.

Answer The addition of oxygen to an anaerobic suspension allows cells to convert from fermentation to oxidative phosphorylation as a mechanism for reoxidizing NADH and making ATP. Because ATP synthesis is much more efficient under aerobic conditions, the amount of glucose needed will decrease (the Pasteur effect). This decreased utilization of glucose in the presence of oxygen can be demonstrated in any tissue that is capable of aerobic and anaerobic glycolysis.

(a) Oxygen allows the tissue to convert from lactic acid fermentation to respiratory electron transfer and oxidative phosphorylation as the mechanism for NADH oxidation.

(b) Cells produce much more ATP per glucose molecule oxidized aerobically, so less glucose is needed.

(c) As [ATP] rises, phosphofructokinase-1 is inhibited, thus slowing the rate of glucose entry into the glycolytic pathway.

14. Respiration-Deficient Yeast Mutants and Ethanol Production Respiration-deficient yeast mutants (p^-; "petites") can be produced from wild-type parents by treatment with mutagenic agents. The mutants lack cytochrome oxidase, a deficit that markedly affects their metabolic behavior. One striking effect is that fermentation is not suppressed by O_2—that is, the mutants lack the Pasteur effect (see Problem 13). Some companies are very interested in using these mutants to ferment wood chips to ethanol for energy use. Explain the advantages of using these mutants rather than wild-type yeast for large-scale ethanol production. Why does the absence of cytochrome oxidase eliminate the Pasteur effect?

Answer The absence of cytochrome oxidase prevents these mutants from oxidizing the products of fermentation (ethanol, acetate, lactate, or glycerol) via the normal respiratory route. These mutants do not have a working citric acid cycle because they cannot reoxidize NADH through the O_2-dependent electron-transfer chain. Thus, catabolism of glucose stops at the ethanol stage, even in the presence of oxygen. The ability to carry out these fermentations in the presence of oxygen is a major practical advantage because completely anaerobic conditions are difficult to maintain. The Pasteur effect—the decrease in glucose consumption that occurs when oxygen is introduced—is not observed in the absence of an active citric acid cycle and electron-transfer chain.

15. Advantages of Supercomplexes for Electron Transfer There is growing evidence that mitochondrial Complexes I, II, III, and IV are part of a larger supercomplex. What might be the advantage of having all four complexes within a supercomplex?

Answer When electron-carrying complexes are bound together in a supercomplex, electron flow between complexes occurs in a solid state; this electron movement is kinetically favored compared with the situation in which electron flow depends on each complex diffusing to and colliding with the next complex in the chain.

16. How Many Protons in a Mitochondrion? Electron transfer translocates protons from the mitochondrial matrix to the external medium, establishing a pH gradient across the inner membrane (outside more acidic than inside). The tendency of protons to diffuse back into the matrix is the driving force for ATP synthesis by ATP synthase. During oxidative phosphorylation by a suspension of mitochondria in a medium of pH 7.4, the pH of the matrix has been measured as 7.7.

(a) Calculate $[H^+]$ in the external medium and in the matrix under these conditions.

(b) What is the outside-to-inside ratio of $[H^+]$? Comment on the energy inherent in this concentration difference. (Hint: see Eqn 11–4, p. 396)

(c) Calculate the number of protons in a respiring liver mitochondrion, assuming its inner matrix compartment is a sphere of diameter 1.5 μm.

(d) From these data, is the pH gradient alone sufficient to generate ATP?

(e) If not, suggest how the necessary energy for synthesis of ATP arises.

Answer

(a) Using the equation $\text{pH} = -\log [H^+]$, we can calculate external $[H^+] = 10^{-7.4} = 4.0 \times 10^{-8}$ M; and internal $[H^+] = 10^{-7.7} = 2.0 \times 10^{-8}$ M.

(b) From **(a),** the ratio is 2:1. We can calculate the free energy inherent in this *concentration* difference across the membrane. Assuming a temperature of 25 °C:

$$\begin{aligned}\Delta G &= RT \ln (C_2/C_1)\\ &= (2.48 \text{ kJ/mol}) \ln 2\\ &= -1.7 \text{ kJ/mol}\end{aligned}$$

(c) Given that the volume of the mitochondrion $= \frac{4}{3}\pi(0.75 \times 10^{-3} \text{ mm})^3$ and $[H^+] = 2.0 \times 10^{-8}$ M, the number of protons is

$$\frac{(1.33)(3.14)(0.75 \times 10^{-3} \text{ mm})^3(2.0 \times 10^{-8} \text{ mol/L})(6.02 \times 10^{23} \text{ protons/mol})}{(10^6 \text{ mm}^3\text{/L})} = 21 \text{ protons}$$

(d) No; the energy available from the H^+ concentration gradient, $2.3\Delta pH\,RT = 2.3(0.3)$ (2.48 kJ/mol) = 1.7 kJ/mol, is insufficient to synthesize 1 mol of ATP.

(e) The total energy inherent in the pH gradient is the sum of the energy due to the concentration gradient and the energy due to the charge separation. The overall transmembrane electrical potential is the main factor in producing a sufficiently large ΔG_t (see Eqns 19–8 and 19–9).

17. Rate of ATP Turnover in Rat Heart Muscle Rat heart muscle operating aerobically fills more than 90% of its ATP needs by oxidative phosphorylation. Each gram of tissue consumes O_2 at the rate of 10.0 μmol/min, with glucose as the fuel source.

(a) Calculate the rate at which the heart muscle consumes glucose and produces ATP.

(b) For a steady-state concentration of ATP of 5.0 μmol/g of heart muscle tissue, calculate the time required (in seconds) to completely turn over the cellular pool of ATP. What does this result indicate about the need for tight regulation of ATP production? (Note: Concentrations are expressed as micromoles per gram of muscle tissue because the tissue is mostly water.)

Answer ATP turns over very rapidly in all types of tissues and cells.

(a) Glucose oxidation requires 6 mol of O_2 per mol of glucose. Therefore, glucose is consumed at the rate of (10.0 μmol/min · g)/6 = 1.7 μmol/min · g of tissue. If each glucose produces 32 ATP (see Table 19–5), the muscle produces ATP at the rate of (1.7 μmol glucose/min · g)(32 ATP/glucose) = 54 μmol/min · g, or 0.91 μmol/s · g.

(b) It takes (5.0 μmol/g)/(0.91 μmol/s · g) = 5.5 s to produce 5.0 μmol of ATP per gram, so the entire pool of ATP must be regenerated (turned over) every 5.5 s. In order to do this, the cell must regulate ATP synthesis precisely.

18. Rate of ATP Breakdown in Flight Muscle ATP production in the flight muscle of the fly *Lucilia sericata* results almost exclusively from oxidative phosphorylation. During flight, 187 mL of O_2/hr · g of body weight is needed to maintain an ATP concentration of 7.0 μmol/g of flight muscle. Assuming that flight muscle makes up 20% of the weight of the fly, calculate the rate at which the flight-muscle ATP pool turns over. How long would the reservoir of ATP last in the absence of oxidative phosphorylation? Assume that reducing equivalents are transferred by the glycerol 3-phosphate shuttle and that O_2 is at 25 °C and 101.3 kPa (1 atm).

Answer Using the gas laws ($PV = nRT$), we can calculate that 187 mL of O_2 contains

$$n = PV/RT = (1\text{ atm})(0.187\text{ L})/(0.08205\text{ L} \cdot \text{atm/mol} \cdot \text{K})(298\text{ K}) = 7650\ \mu\text{mol of } O_2$$

Thus, the rate of oxygen consumption by flight muscle is

$$(7650\ \mu\text{mol/hr})/(1\text{ g})(0.2)(3600\text{ s/hr}) = 10.6\ \mu\text{mol/s} \cdot \text{g}$$

Assuming a yield of 30 ATP per glucose (see Table 19–5; assume the use of the glycerol 3-phosphate shuttle), and given 6 O_2 consumed per glucose, the amount of ATP formed is

$$[(30\text{ ATP/glucose})/(6\ O_2\text{/glucose})](10.6\ \mu\text{mol } O_2\text{/s} \cdot \text{g}) = 53\ \mu\text{mol/s} \cdot \text{g}$$

Thus, a reservoir of 7.0 μmol/g would last (7.0 μmol/g)/(53 μmol/s · g) = 0.13 s.

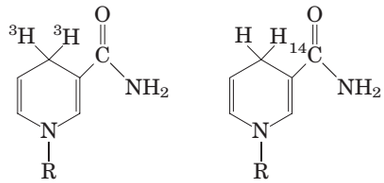
19. Mitochondrial Disease and Cancer Mutations in the genes that encode certain mitochondrial proteins are associated with a high incidence of some types of cancer. How might defective mitochondria lead to cancer?

Answer Reactive oxygen species react with macromolecules, including DNA. If a mitochondrial defect leads to increased production of ROS, the nuclear genes that encode proto-oncogenes (pp. 473, 474) can be damaged, producing oncogenes and leading to unregulated cell division and cancer.

20. Variable Severity of a Mitochondrial Disease Individuals with a disease caused by a specific defect in the mitochondrial genome may have symptoms ranging from mild to severe. Explain why.

Answer The explanation is probably heteroplasmy. In some individuals, a mitochondrial mutation may affect only a small proportion of cells and tissues, because most mitochondria in these cells and tissues have normal genomes and the few mutant mitochondria do not significantly compromise the ability to produce ATP. In other individuals, the chance distribution of mitochondrial genomes during cell division has led to a high degree of heteroplasmy in which many cells have a majority of defective mitochondria, resulting in more severe symptoms.

21. Transmembrane Movement of Reducing Equivalents Under aerobic conditions, extramitochondrial NADH must be oxidized by the mitochondrial electron-transfer chain. Consider a preparation of rat hepatocytes containing mitochondria and all the cytosolic enzymes. If [4-^{3}H]NADH is introduced, radioactivity soon appears in the mitochondrial matrix. However, if [7-^{14}C]NADH is introduced, no radioactivity appears in the matrix. What do these observations reveal about the oxidation of extramitochondrial NADH by the electron-transfer chain?



[4-^{3}H]NADH [7-^{14}C]NADH

Answer The malate-aspartate shuttle transfers electrons and protons from the cytoplasm into the mitochondrion. Neither NAD^+ nor NADH passes through the inner membrane, thus the labeled NAD moiety of [7-^{14}C]NADH remains in the cytosol. The ^{3}H on [4-^{3}H]NADH enters the mitochondrion via the malate-aspartate shuttle (see Fig. 19–29). In the cytosol, [4-^{3}H]NADH transfers its ^{3}H to oxaloacetate to form [^{3}H]malate, which enters the mitochondrion via the malate–α-ketoglutarate transporter, then donates the ^{3}H to NAD^+ to form [4-^{3}H]NADH in the matrix.

22. High Blood Alanine Level Associated with Defects in Oxidative Phosphorylation Most individuals with genetic defects in oxidative phosphorylation are found to have relatively high concentrations of alanine in their blood. Explain this in biochemical terms.

Answer In these individuals, the usual route for pyruvate metabolism—conversion to acetyl-CoA and entry into the citric acid cycle—is slowed by the decreased capacity for carrying electrons from NADH to oxygen. Accumulation of pyruvate in the tissues shifts the equilibrium for pyruvate-alanine transaminase, resulting in elevated concentrations of alanine in tissues and blood.

23. NAD Pools and Dehydrogenase Activities Although both pyruvate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase use NAD^+ as their electron acceptor, the two enzymes do not compete for the same cellular NAD pool. Why?

Answer Pyruvate dehydrogenase is located in the mitochondrion, and glyceraldehyde 3-phosphate dehydrogenase in the cytosol. Because the mitochondrial and cytosolic pools of NAD are separated by the inner mitochondrial membrane, the enzymes do not compete for the same NAD pool. However, reducing equivalents are transferred from one nicotinamide coenzyme pool to the other via shuttle mechanisms (see Problem 21).

24. Diabetes as a Consequence of Mitochondrial Defects Glucokinase is essential in the metabolism of glucose in pancreatic β cells. Humans with two defective copies of the glucokinase gene exhibit a severe, neonatal diabetes, whereas those with only one defective copy of the gene have a much milder form of the disease (mature onset diabetes of the young, MODY2). Explain this difference in terms of the biology of the β cell.

Answer In β cells in which both copies of glucokinase are defective, the rate of glycolytic ATP production does not increase when blood glucose rises, and thus these cells cannot produce high enough concentrations of ATP to affect the ATP-dependent K^+ channel that indirectly regulates insulin secretion. With one functional copy of glucokinase, β cells can respond to very high glucose concentrations by producing suprathreshold concentrations of ATP, triggering insulin release.

25. Effects of Mutations in Mitochondrial Complex II Single nucleotide changes in the gene for succinate dehydrogenase (Complex II) are associated with midgut carcinoid tumors. Suggest a mechanism to explain this observation.

Answer Defects in Complex II result in increased production of ROS, damage to DNA, and mutations that lead to unregulated cell division (cancer). It is not clear why the cancer tends to occur in the midgut.

26. Photochemical Efficiency of Light at Different Wavelengths The rate of photosynthesis, measured by O_2 production, is higher when a green plant is illuminated with light of wavelength 680 nm than with light of 700 nm. However, illumination by a combination of light of 680 nm and 700 nm gives a higher rate of photosynthesis than light of either wavelength alone. Explain.

Answer Plants have two photosystems. Photosystem I absorbs light maximally at 700 nm and catalyzes cyclic photophosphorylation and $NADP^+$ reduction (see Fig. 19–56). Photosystem II absorbs light maximally at 680 nm, splits H_2O to O_2 and H^+, and donates electrons and H^+ to PSI. Therefore, light of 680 nm is better in promoting O_2 production, but maximum photosynthetic rates are observed only when plants are illuminated with light of both wavelengths.

27. Balance Sheet for Photosynthesis In 1804 Theodore de Saussure observed that the total weights of oxygen and dry organic matter produced by plants is greater than the weight of carbon dioxide consumed during photosynthesis. Where does the extra weight come from?

Answer Because the general reaction for plant photosynthesis is

$$CO_2 + H_2O \longrightarrow O_2 + \text{organic matter}$$

the extra weight must come from the water consumed in the overall reaction.

28. Role of H_2S in Some Photosynthetic Bacteria Illuminated purple sulfur bacteria carry out photosynthesis in the presence of H_2O and $^{14}CO_2$, but only if H_2S is added and O_2 is absent. During the course of photosynthesis, measured by formation of [^{14}C]carbohydrate, H_2S is converted to elemental sulfur, but no O_2 is evolved. What is the role of the conversion of H_2S to sulfur? Why is no O_2 evolved?

Answer Purple sulfur bacteria use H_2S as a source of electrons and protons:

$$H_2S \longrightarrow S + 2H^+ + 2e^-$$

The electrons are "activated" by a light energy–capturing photosystem. These cells produce their ATP by photophosphorylation and their NADPH from H_2S oxidation. Because H_2O is not split, O_2 is not evolved (photosystem II is absent).

29. Boosting the Reducing Power of Photosystem I by Light Absorption When photosystem I absorbs red light at 700 nm, the standard reduction potential of P700 changes from 0.40 V to about −1.2 V. What fraction of the absorbed light is trapped in the form of reducing power?

Answer For a change in standard reduction potential, ΔE, of 0.4 V − (−1.2 V), the free-energy change per electron is

$$\begin{aligned}\Delta G'^\circ &= n\mathcal{F}\Delta E'^\circ \\ &= -(96.48\ \text{kJ/V} \cdot \text{mol})(-1.6\ \text{V}) \\ &= 150\ \text{kJ/mol}\end{aligned}$$

Two photons are absorbed per electron elevated to a higher energy level, which for 700 nm light is equivalent to 2(170 kJ/mol) = 340 kJ/mol (see Fig. 19–46). Thus, the fraction of light energy trapped as reducing power is

$$(150\ \text{kJ/mol})/(340\ \text{kJ/mol}) = 0.44, \text{ or } 44\%$$

30. Electron Flow through Photosystems I and II Predict how an inhibitor of electron passage through pheophytin would affect electron flow through **(a)** photosystem II and **(b)** photosystem I. Explain your reasoning.

Answer

(a) Electron flow through PSII would stop, as there would be no way for electrons to move from PSII to the cytochrome b_6f complex and all the electron acceptors in PSII would very quickly be reduced.

(b) Electron flow through PSI would probably slow. The supply of electrons from PSII would be blocked, but with operation of the cyclic pathway, some electron flow through PSI could continue.

31. Limited ATP Synthesis in the Dark In a laboratory experiment, spinach chloroplasts are illuminated in the absence of ADP and P_i, then the light is turned off and ADP and P_i are added. ATP is synthesized for a short time in the dark. Explain this finding.

Answer Illumination of chloroplasts in the absence of ADP and P_i sets up a proton gradient across the thylakoid membrane. When ADP and P_i are added, ATP synthesis is driven by the gradient. In the absence of continuous illumination, the gradient soon becomes exhausted and ATP synthesis stops.

32. Mode of Action of the Herbicide DCMU When chloroplasts are treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, or diuron), a potent herbicide, O_2 evolution and photophosphorylation cease. Oxygen evolution, but not photophosphorylation, can be restored by addition of an external electron acceptor, or Hill reagent. How does DCMU act as a weed killer? Suggest a location for the inhibitory action of this herbicide in the scheme shown in Figure 19–56. Explain.

Answer DCMU must inhibit the electron-transfer system linking photosystem II and photosystem I at a position ahead of the first site of ATP production. DCMU competes with PQ_B for electrons from PQ_A (Table 19–4). Addition of a Hill reagent allows H_2O to be split and O_2 to be evolved, but electrons are pulled out of the system before the point of ATP synthesis and before the production of NADPH. DCMU kills plants by inhibiting ATP production.

33. Effect of Venturicidin on Oxygen Evolution Venturicidin is a powerful inhibitor of the chloroplast ATP synthase, interacting with the CF_o part of the enzyme and blocking proton passage through the CF_oCF_1 complex. How would venturicidin affect oxygen evolution in a suspension of well-illuminated chloroplasts? Would your answer change if the experiment were done in the presence of an uncoupling reagent such as 2,4-dinitrophenol (DNP)? Explain.

Answer Oxygen evolution requires continuing passage of electrons through PSII. Electrons will continue to flow through PSII and the cytochrome b_6f complex until the energetic cost of pumping a proton across the thylakoid membrane exceeds the energy available from absorption of a photon. This point is soon reached when proton flow through CF_oCF_1 is blocked by venturicidin, and oxygen evolution ceases. Addition of an uncoupling agent provides a route for protons to move through the thylakoid membrane, dissipating the energy of the proton gradient. Electrons can now continue to move through PSII and the cytochrome b_6f complex, and oxygen is produced in the water-splitting reaction.

34. Bioenergetics of Photophosphorylation The steady-state concentrations of ATP, ADP, and P_i in isolated spinach chloroplasts under full illumination at pH 7.0 are 120.0, 6.0, and 700.0 μM, respectively.

(a) What is the free-energy requirement for the synthesis of 1 mol of ATP under these conditions?

(b) The energy for ATP synthesis is furnished by light-induced electron transfer in the chloroplasts. What is the minimum voltage drop necessary (during transfer of a pair of electrons) to synthesize ATP under these conditions? (You may need to refer to Eqn 13–7, p. 515.)

Answer

(a) $$\Delta G = \Delta G'^\circ + RT \ln \frac{[\text{ATP}]}{[\text{ADP}][P_i]}$$

$$= 30.5 \text{ kJ/mol} + (2.48 \text{ kJ/mol}) \ln \frac{1.2 \times 10^{-4}}{(6.0 \times 10^{-6})\,(7.0 \times 10^{-4})}$$

$$= 30.5 \text{ kJ/mol} + 25.4 \text{ kJ/mol} = 55.9 \text{ kJ/mol}$$

$$= 56 \text{ kJ/mol (two significant figures)}$$

(b) $$\Delta G = -n\mathcal{F}\Delta E$$

$$\Delta E = -\Delta G/n\mathcal{F}$$

$$= \frac{-56 \text{ kJ/mol}}{-2(96.48 \text{ kJ/V} \cdot \text{mol})}$$

$$= 0.29 \text{ V}$$

35. Light Energy for a Redox Reaction Suppose you have isolated a new photosynthetic microorganism that oxidizes H_2S and passes the electrons to NAD^+. What wavelength of light would provide enough energy for H_2S to reduce NAD^+ under standard conditions? Assume 100% efficiency in the photochemical event, and use E'° of -243 mV for H_2S and -320 mV for NAD^+. See Figure 19–46 for the energy equivalents of wavelengths of light.

Answer First, calculate the standard free-energy change ($\Delta G'^\circ$) of the redox reaction

$$NAD^+ + H_2S \longrightarrow NADH + S^- + H^+$$

Because $\Delta E'^\circ = -320 \text{ mV} - (-243 \text{ mV}) = -77 \text{ mV}$,

$$\Delta G'^\circ = -n\mathcal{F}\Delta E'^\circ$$

$$= (-2)(96.48 \text{ kJ/V} \cdot \text{mol})(-0.077 \text{ V}) = 15 \text{ kJ/mol}$$

This is the minimum energy needed to drive the reduction of NAD^+ by H_2S. Inspection of Figure 19–46 shows that the energy in a "mole" of photons (an einstein) in the visible part of the spectrum ranges from 170 to 300 kJ. Any visible light should have sufficient energy to drive the reduction of NADH by H_2S. In principle, and assuming 100% efficiency, even infrared light should have enough energy to drive this reaction.

36. Equilibrium Constant for Water-Splitting Reactions The coenzyme $NADP^+$ is the terminal electron acceptor in chloroplasts, according to the reaction

$$2H_2O + 2NADP^+ \longrightarrow 2NADPH + 2H^+ + O_2$$

Use the information in Table 19–2 to calculate the equilibrium constant for this reaction at 25 °C. (The relationship between K'_{eq} and $\Delta G'^\circ$ is discussed on p. 492.) How can the chloroplast overcome this unfavorable equilibrium?

Answer Using standard reduction potentials from Table 19–2, $\Delta E'^\circ$ for the reaction is $-0.324\text{ V} - 0.816\text{ V} = -1.140\text{ V}$.

$$\begin{aligned}\Delta G'^\circ &= -n\mathcal{F}\Delta E'^\circ \\ &= -4(96.48\text{ kJ/V}\cdot\text{mol})(-1.140\text{ V}) \\ &= 440\text{ kJ/mol}\end{aligned}$$

(Note that $n = 4$ because 4 electrons are required to produce 1 mol of O_2.)

$$\begin{aligned}\Delta G'^\circ &= -RT\ln K'_{eq} \\ \ln K'_{eq} &= -\Delta G'^\circ/RT \\ &= (-440\text{ kJ/mol})/(2.48\text{ kJ/mol}) \\ &= -177 \\ K'_{eq} &= e^{-177} = 1.35 \times 10^{-77}\end{aligned}$$

The equilibrium is clearly very unfavorable. In chloroplasts, the input of light energy overcomes this barrier.

37. Energetics of Phototransduction During photosynthesis, eight photons must be absorbed (four by each photosystem) for every O_2 molecule produced:

$$2H_2O + 2NADP^+ + 8\text{ photons} \longrightarrow 2NADPH + 2H^+ + O_2$$

Assuming that these photons have a wavelength of 700 nm (red) and that the absorption and use of light energy are 100% efficient, calculate the free-energy change for the process.

Answer From Problem 36, $\Delta G'^\circ$ for the production of 1 mol of O_2 in this reaction is 440 kJ/mol. A light input of 8 photons (700 nm) is equivalent to (8)(170 kJ/einstein) = 1360 kJ/einstein (see Fig. 19–46). (An einstein is a "mole" of photons.) The overall standard free-energy change of the reaction is

$$\Delta G'^\circ = (440 - 1360)\text{ kJ/mol} = -920\text{ kJ/mol}$$

38. Electron Transfer to a Hill Reagent Isolated spinach chloroplasts evolve O_2 when illuminated in the presence of potassium ferricyanide (a Hill reagent), according to the equation

$$2H_2O + 4Fe^{3+} \longrightarrow O_2 + 4H^+ + 4Fe^{2+}$$

where Fe^{3+} represents ferricyanide and Fe^{2+}, ferrocyanide. Is NADPH produced in this process? Explain.

Answer No NADPH is produced. Artificial electron acceptors can remove electrons from the photosynthetic system and stimulate O_2 production. Ferricyanide competes with the cytochrome b_6f complex for electrons and removes them from the system. Consequently, P700 (of photosystem I) does not receive any electrons that can be activated for $NADP^+$ reduction. However, O_2 is evolved because all components of photosystem II are oxidized (see Fig. 19–56).

39. How Often Does a Chlorophyll Molecule Absorb a Photon? The amount of chlorophyll *a* (M_r 892) in a spinach leaf is about 20 μg/cm^2 of leaf. In noonday sunlight (average energy reaching the leaf is 5.4 J/cm^2 · min), the leaf absorbs about 50% of the radiation. How often does a single chlorophyll molecule absorb a photon? Given that the average lifetime of an excited chlorophyll molecule in vivo is 1 ns, what fraction of the chlorophyll molecules are excited at any one time?

Answer The leaf absorbs light in units of photons that vary in energy from 170 to 300 kJ/einstein, depending on wavelength (see Fig. 19–46). The leaf absorbs light energy at the rate of $0.5(5.4 \text{ J/cm}^2 \cdot \text{min}) = 2.7 \text{ J/cm}^2 \cdot \text{min}$. Assuming an average energy of 270 kJ/einstein, this rate of light absorption is

$$(2.7 \times 10^{-3} \text{ kJ/cm}^2 \cdot \text{min})/(270 \text{ kJ/einstein}) = 1 \times 10^{-5} \text{ einstein/cm}^2 \cdot \text{min}$$

The concentration of chlorophyll in the leaf is

$$(20 \times 10^{-6} \text{ g/cm}^2)/(892 \text{ g/mol}) = 2 \times 10^{-8} \text{ mol/cm}^2$$

Thus, 1 mol of chlorophyll absorbs 1 einstein of photons every

$$(2 \times 10^{-8} \text{ mol/cm}^2)/(1 \times 10^{-5} \text{ einstein/cm}^2 \cdot \text{min}) = 2 \times 10^{-3} \text{ min} = 0.1 \text{ s}$$

Because excitation lasts about 1 ns = 1×10^{-9} s, the fraction of chlorophylls excited at any one time is $(1 \times 10^{-9} \text{ s})/(0.1 \text{ s}) = 1 \times 10^{-8}$, or one in every 10^8 molecules.

40. Effect of Monochromatic Light on Electron Flow The extent to which an electron carrier is oxidized or reduced during photosynthetic electron transfer can sometimes be observed directly with a spectrophotometer. When chloroplasts are illuminated with 700 nm light, cytochrome *f,* plastocyanin, and plastoquinone are oxidized. When chloroplasts are illuminated with 680 nm light, however, these electron carriers are reduced. Explain.

Answer Light at 700 nm activates electrons in P700 and $NADP^+$ is reduced (see Fig. 19–56). This drains all the electrons from the electron-transfer system between photosystems II and I, because light at 680 nm is not available to replace electrons by activating PSII. When light at 680 nm activates PSII (but not PSI), all the carriers between the two systems become reduced because no electrons are excited in PSI.

41. Function of Cyclic Photophosphorylation When the [NADPH]/[$NADP^+$] ratio in chloroplasts is high, photophosphorylation is predominantly cyclic (see Fig. 19–56). Is O_2 evolved during cyclic photophosphorylation? Is NADPH produced? Explain. What is the main function of cyclic photophosphorylation?

Answer Neither O_2 nor NADPH is produced. At high [NADPH]/[$NADP^+$] ratios, electron transfer from reduced ferredoxin to $NADP^+$ is inhibited and the electrons are diverted into the cytochrome b_6f complex. These electrons return to P700 and ATP is synthesized by photophosphorylation. Because electrons are not lost from P700, none are needed from PSII. Thus, H_2O is not split and O_2 is not produced. In addition, NADPH is not formed because the electrons return to P700. The function of cyclic photophosphorylation is to produce ATP.

Data Analysis Problem

42. Photophosphorylation: Discovery, Rejection, and Rediscovery In the 1930s and 1940s, researchers were beginning to make progress toward understanding the mechanism of photosynthesis. At the time, the role of "energy-rich phosphate bonds" (today, "ATP") in glycolysis and cellular respiration was just becoming known. There were many theories about the mechanism of photosynthesis, especially about the role of light. This problem focuses on what was then called the "primary photochemical process"—that is, on what it is, exactly, that the energy from captured light produces in the photosynthetic cell. Interestingly, one important part of the modern model of photosynthesis was proposed early on, only to be rejected, ignored for several years, then finally revived and accepted.

In 1944, Emerson, Stauffer, and Umbreit proposed that "the function of light energy in photosynthesis is the formation of 'energy-rich' phosphate bonds" (p. 107). In their model (hereafter, the "Emerson model"), the free energy necessary to drive both CO_2 fixation *and* reduction came from these "energy-rich phosphate bonds" (i.e., ATP), produced as a result of light absorption by a chlorophyll-containing protein.

This model was explicitly rejected by Rabinowitch (1945). After summarizing Emerson and coauthors' findings, Rabinowitch stated: "Until more positive evidence is provided, we are inclined to consider as more convincing a general argument against this hypothesis, which can be derived from energy considerations. Photosynthesis is eminently a problem of energy *accumulation*. What good can be served, then, by converting light quanta (even those of red light, which amount to about 43 kcal per Einstein) into 'phosphate quanta' of only 10 kcal per mole? This appears to be a start in the wrong direction—toward *dissipation* rather than toward accumulation of energy" (Vol. I, p. 228). This argument, along with other evidence, led to the abandonment of the Emerson model until the 1950s, when it was found to be correct—albeit in a modified form.

For each piece of information from Emerson and coauthors' article presented in (**a**) through (**d**) below, answer the following three questions:

1. How does this information support the Emerson model, in which light energy is used directly by chlorophyll *to make ATP*, and the ATP then provides the energy to drive CO_2 fixation and reduction?
2. How would Rabinowitch explain this information, based on his model (and most other models of the day), in which light energy is used directly by chlorophyll *to make reducing compounds*? Rabinowitch wrote: "Theoretically, there is no reason why *all* electronic energy contained in molecules excited by the absorption of light should not be available for oxidation-reduction" (Vol. I, p. 152). In this model, the reducing compounds are then used to fix and reduce CO_2, and the energy for these reactions comes from the large amounts of free energy released by the reduction reactions.
3. How is this information explained by our modern understanding of photosynthesis?

(a) Chlorophyll contains a Mg^{2+} ion, which is known to be an essential cofactor for many enzymes that catalyze phosphorylation and dephosphorylation reactions.

(b) A crude "chlorophyll protein" isolated from photosynthetic cells showed phosphorylating activity.

(c) The phosphorylating activity of the "chlorophyll protein" was inhibited by light.

(d) The levels of several different phosphorylated compounds in photosynthetic cells changed dramatically in response to light exposure. (Emerson and coworkers were not able to identify the specific compounds involved.)

As it turned out, the Emerson and Rabinowitch models were both partly correct and partly incorrect.

(e) Explain how the two models relate to our current model of photosynthesis.

In his rejection of the Emerson model, Rabinowitch went on to say: "The difficulty of the phosphate storage theory appears most clearly when one considers the fact that, in weak light, eight or ten quanta of light are sufficient to reduce one molecule of carbon dioxide. If each quantum should produce one molecule of high-energy phosphate, the accumulated energy would be only 80–100 kcal per Einstein—while photosynthesis requires *at least* 112 kcal per mole, and probably more, because of losses in irreversible partial reactions" (Vol. 1, p. 228).

(f) How does Rabinowitch's value of 8 to 10 photons per molecule of CO_2 reduced compare with the value accepted today? You need to consult Chapter 20 for some of the information required here.

(g) How would you rebut Rabinowitch's argument, based on our current knowledge about photosynthesis?

Answer

(a) (1) The presence of Mg^{2+} supports the hypothesis that chlorophyll is directly involved in catalysis of the phosphorylation reaction: ADP + $P_i \rightarrow$ ATP. (2) Many enzymes (or other proteins) that contain Mg^{2+} are not phosphorylating enzymes, so the presence of Mg^{2+} in chlorophyll does not prove its role in phosphorylation reactions. (3) The presence of Mg^{2+} is essential to chlorophyll's photochemical properties: light absorption and electron transfer.

(b) (1) Enzymes catalyze reversible reactions, so an isolated enzyme that can, under certain laboratory conditions, catalyze removal of a phosphoryl group could probably, under different conditions (such as in cells), catalyze addition of a phosphoryl group. So it is plausible that chlorophyll could be involved in the phosphorylation of ADP. (2) There are two possible explanations: the chlorophyll protein is a phosphatase only and does not catalyze ADP phosphorylation under cellular conditions, or the crude preparation contains a contaminating phosphatase activity that is unconnected to the photosynthetic reactions. (3) It is likely that the preparation was contaminated with a nonphotosynthetic phosphatase activity.

(c) (1) This light inhibition is what one would expect if the chlorophyll protein catalyzed the reaction ADP + P_i + light $\rightarrow$ ATP. Without light, the reverse reaction, a dephosphorylation, would be favored. In the presence of light, energy is provided and the equilibrium would shift to the right, reducing the phosphatase activity. (2) This inhibition must be an artifact of the isolation or assay methods. (3) It is unlikely that the crude preparation methods in use at the time preserved intact chloroplast membranes, so the inhibition must be an artifact.

(d) (1) In the presence of light, ATP is synthesized and other phosphorylated intermediates are consumed. (2) In the presence of light, glucose is produced and is metabolized by cellular respiration to produce ATP, with changes in the levels of phosphorylated intermediates. (3) In the presence of light, ATP is produced and other phosphorylated intermediates are consumed.

(e) Light energy is used to produce ATP (as in the Emerson model) *and* is used to produce reducing power (as in the Rabinowitch model).

(f) The approximate stoichiometry for photophosphorylation (Chapter 19) is that 8 photons yield 2 NADPH and about 3 ATP. Two NADPH and 3 ATP are required to reduce 1 CO_2 (Chapter 20). Thus, at a minimum, 8 photons are required per CO_2 molecule reduced. This is in good agreement with Rabinowitch's value.

(g) Because the energy of light is used to produce *both* ATP and NADPH, each photon absorbed contributes more than just 1 ATP for photosynthesis. The process of energy extraction from light is more efficient than Rabinowitch supposed, and plenty of energy is available for this process—even with red light.

Reference

Emerson, R.L., Stauffer, J.F., & Umbreit, W.W. (1944) Relationships between phosphorylation and photosynthesis in *Chlorella. Am. J. Botany* **31,** 107–120.

Rabinowitch, E.I. (1945) *Photosynthesis and Related Processes,* Interscience Publishers, New York.

chapter

20 Carbohydrate Biosynthesis in Plants and Bacteria

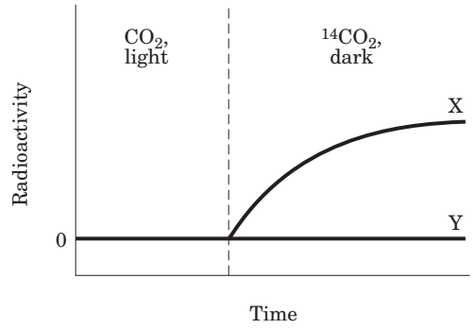
1. **Segregation of Metabolism in Organelles** What are the advantages to the plant cell of having different organelles to carry out different reaction sequences that share intermediates?

   Answer Within organelles, reaction intermediates and enzymes can be maintained at different levels from those in the cytosol and in other organelles. For example, the ATP/ADP ratio is lower in mitochondria than in the cytosol because the role of adenine nucleotides in the mitochondrial matrix is to accept a phosphoryl group, whereas the role in the cytosol is to donate a phosphoryl group. Similarly, different NADH/NAD^+ and NADPH/$NADP^+$ ratios reflect the reductive (biosynthetic) functions of the cytosol and the oxidative (catabolic) functions of the mitochondrial matrix. By segregating reaction sequences that share intermediates, the cell can regulate catabolic and anabolic processes separately.

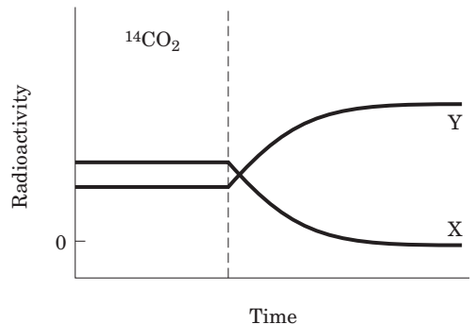
2. **Phases of Photosynthesis** When a suspension of green algae is illuminated in the absence of CO_2 and then incubated with $^{14}CO_2$ in the dark, $^{14}CO_2$ is converted to [^{14}C]glucose for a brief time. What is the significance of this observation with regard to the CO_2-assimilation process, and how is it related to the light reactions of photosynthesis? Why does the conversion of $^{14}CO_2$ to [^{14}C]glucose stop after a brief time?

   Answer This observation suggests that photosynthesis occurs in two phases: (1) a light-dependent phase that generates ATP and NADPH, which are essential for CO_2 fixation, and (2) a light-independent (dark) phase, in which these energy-rich components are used for synthesis of glucose. In the absence of additional illumination, the supplies of NADPH and ATP become exhausted and CO_2 fixation ceases.

3. **Identification of Key Intermediates in CO_2 Assimilation** Calvin and his colleagues used the unicellular green alga *Chlorella* to study the carbon-assimilation reactions of photosynthesis. They incubated $^{14}CO_2$ with illuminated suspensions of algae and followed the time course of appearance of ^{14}C in two compounds, X and Y, under two sets of conditions. Suggest the identities of X and Y, based on your understanding of the Calvin cycle.
   (a) Illuminated *Chlorella* were grown with unlabeled CO_2, then the light was turned off and $^{14}CO_2$ was added (vertical dashed line in graph (a)). Under these conditions, X was the first compound to become labeled with ^{14}C; Y was unlabeled.
   (b) Illuminated *Chlorella* cells were grown with $^{14}CO_2$. Illumination was continued until all the $^{14}CO_2$ had disappeared (vertical dashed line in graph (b)). Under these conditions, X became labeled quickly but lost its radioactivity with time, whereas Y became more radioactive with time.



(a)



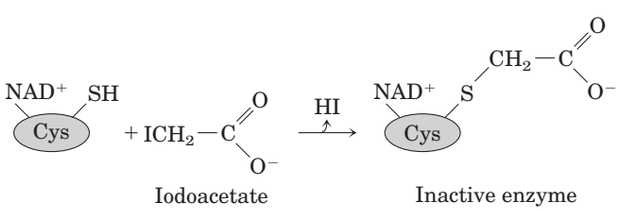
(b)

Answer Compound X is 3-phosphoglycerate and compound Y is ribulose 1,5-bisphosphate (see Fig. 20–4).

(a) Illumination of *Chlorella* in the presence of unlabeled CO_2 gives rise to steady-state levels of ribulose 1,5–bisphosphate, 3–phosphoglycerate, ATP, and NADPH. When the light is turned off, the production of ATP and NADPH ceases, but the Calvin cycle continues briefly until the residual ATP and NADPH are exhausted. Once this occurs, conversion of 3-phosphoglycerate (Stage 2 in Fig. 20–4) to hexoses, which depends on ATP and NADPH, is blocked. Thus, $^{14}CO_2$ added at the time the light is turned off is transformed primarily to 3-phosphoglycerate, but not to other intermediates of the cycle such as ribulose 1,5-bisphosphate.

(b) Illumination of *Chlorella* in the presence of $^{14}CO_2$ gives rise to steady-state levels of ^{14}C-labeled 3-phosphoglycerate and ribulose 1,5-bisphosphate. If the concentration of CO_2 is rapidly decreased, none is available for the ribulose 1,5-bisphosphate carboxylase reaction, and this constitutes a block at the fixation step (Stage 1 in Fig. 20–4). Because this experiment is carried out under conditions of constant illumination, the steps requiring ATP and NADPH are not blocked and all labeled 3-phosphoglycerate (X) can be converted to labeled ribulose 1,5-bisphosphate (Y). This results in a decrease in labeled X and a commensurate increase in labeled Y.

4. Regulation of the Calvin Cycle Iodoacetate reacts irreversibly with the free —SH groups of Cys residues in proteins.



Iodoacetate Inactive enzyme

Predict which Calvin cycle enzyme(s) would be inhibited by iodoacetate, and explain why.

Answer Ribulose 5-phosphate kinase, fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase, and glyceraldehyde 3-phosphate dehydrogenase would be inhibited. All have mechanisms requiring activation by reduction of a critical disulfide bond to a pair of —SH groups. Iodoacetate reacts irreversibly with free —SH groups.

5. **Thioredoxin in Regulation of Calvin Cycle Enzymes** Motohashi and colleagues used thioredoxin as a hook to fish out from plant extracts the proteins that are activated by thioredoxin. To do this, they prepared a mutant thioredoxin in which one of the reactive Cys residues was replaced with a Ser. Explain why this modification was necessary for their experiments. [Motohashi, K., Kondoh, A., Stumpp, M.T., & Hisabori, T. (2001) Comprehensive survey of proteins targeted by chloroplast thioredoxin. *Proc. Natl. Acad. Sci. USA* **98,** 11,224-11,229.]

   Answer The several Calvin cycle enzymes that are regulated by the effect of light on ferredoxin reduction are activated when reduced ferredoxin passes electrons to thioredoxin, reducing a disulfide bond to two —SH groups. For thioredoxin to play its catalytic role in the disulfide exchange reaction that activates the Calvin cycle enzymes, it must have both of its reactive —SH groups. The mutant thioredoxin, in which one of the reactive Cys residues is converted to a Ser (which lacks an —SH group), cannot form an internal disulfide, as it has only one —SH group. When this mutant thioredoxin interacts with one of the enzymes regulated by disulfide reduction, it forms a disulfide bond with that enzyme, but the next step, the reduction of the pair of Cys residues in the enzyme, cannot occur because the thioredoxin cannot form a disulfide. Consequently, thioredoxin remains covalently attached to the enzyme, and when the thioredoxin is isolated, the enzyme comes along, "hooked" on thioredoxin.

6. **Comparison of the Reductive and Oxidative Pentose Phosphate Pathways** The *reductive* pentose phosphate pathway generates a number of intermediates identical to those of the *oxidative* pentose phosphate pathway (Chapter 14). What role does each pathway play in cells where it is active?

   Answer The *reductive* pentose phosphate pathway regenerates ribulose 1,5-bisphosphate from triose phosphates produced during photosynthesis, in a series of reactions involving sugars of three, four, five, six, and seven carbons and the enzymes transaldolase and transketolase. The oxidative pentose phosphate pathway plays a different metabolic role: it provides NADPH for reductive biosynthesis and pentose phosphates for nucleotide synthesis.

7. **Photorespiration and Mitochondrial Respiration** Compare the oxidative photosynthetic carbon cycle (C_2 cycle), also called *photorespiration,* with the *mitochondrial respiration* that drives ATP synthesis. Why are both processes referred to as respiration? Where in the cell do they occur, and under what circumstances? What is the path of electron flow in each?

   Answer Both processes are called "respiration" because both consume O_2 and produce CO_2. Mitochondrial respiration, or cellular respiration, occurs only in mitochondria, providing most of the ATP required by the cell. In the mitochondrion, electrons derived from oxidation of various fuels in the matrix are passed through a chain of carriers in the inner mitochondrial membrane to O_2 (see Fig. 19–16); the proton gradient thus generated provides the energy for ATP synthesis. Photorespiration takes place only in plants. It is the long series of reactions, involving enzymes in the chloroplasts and peroxisomes, in which the glycolate produced when rubisco "fixes" molecular oxygen is converted to phosphoglycerate. In this reaction series (see Fig. 20–21), one-fourth of the carbons in glycolate are released as CO_2, and O_2 is consumed in the reaction catalyzed by glycolic acid oxidase. This amount of O_2 consumption is added to that occurring in the rubisco reaction. Photorespiration is an expensive side reaction of photosynthesis, resulting from the lack of specificity of rubisco.

8. Rubisco and the Composition of the Atmosphere N. E. Tolbert has argued that the dual specificity of rubisco for CO_2 and O_2 is not simply a leftover from evolution in a low-oxygen environment. He suggests that the relative activities of the carboxylase and oxygenase activities of rubisco actually have set, and now maintain, the ratio of CO_2 to O_2 in the earth's atmosphere. Discuss the pros and cons of this hypothesis, in molecular terms and in global terms. How does the existence of C_4 organisms bear on the hypothesis? [Tolbert, N.E. (1994) The role of photosynthesis and photorespiration in regulating atmospheric CO_2 and O_2. In *Regulation of Atmospheric CO_2 and O_2 by Photosynthetic Carbon Metabolism* (Tolbert, N.E. & Preiss, J., eds), pp. 8–33, Oxford University Press, New York.]

Answer This hypothesis assumes that the rubisco reaction is the most important chemical reaction in determining the composition of the earth's atmosphere. This may once have been true, but in the modern world, many other processes, such as the burning of fossil fuels and destruction of tropical forests (by burning and other means), probably have at least as large an impact on atmospheric composition. To the extent that C4 plants contribute to global CO_2 fixation, they also tend to counterbalance the effect of rubisco on the balance of CO_2 and O_2 in the atmosphere.

9. Role of Sedoheptulose 1,7-Bisphosphatase What effect on the cell and the organism might result from a defect in sedoheptulose 1,7-bisphosphatase in **(a)** a human hepatocyte and **(b)** the leaf cell of a green plant?

Answer

(a) In a human hepatocyte, NADPH produced by the pentose phosphate pathway—for which sedoheptulose 1,7-bisphosphatase activity is essential—is needed for the reductive steps in the synthesis of fatty acids, triacylglycerols, and sterols. Without this source of NADPH, all these processes would be inhibited. Cells would be unable to synthesize lipids and other reduced products.

(b) The lack of sedoheptulose 1,7-bisphosphatase would prevent the functioning of the Calvin cycle; the enzyme is essential in the series of reactions that convert triose phosphates to ribulose 1,5-bisphosphate. Without generation of ribulose 1,5-bisphosphate, the Calvin cycle is effectively blocked; there is no acceptor for CO_2 in the first step in that pathway. The leaf cell would therefore be incapable of fixing CO_2.

10. Pathway of CO_2 Assimilation in Maize If a maize (corn) plant is illuminated in the presence of $^{14}CO_2$, after about 1 second, more than 90% of all the radioactivity incorporated in the leaves is found at C-4 of malate, aspartate, and oxaloacetate. Only after 60 seconds does ^{14}C appear at C-1 of 3-phosphoglycerate. Explain.

Answer In maize, CO_2 is fixed by the C_4 pathway elucidated by Hatch and Slack. Phosphoenolpyruvate is rapidly carboxylated to oxaloacetate, some of which undergoes transamination to aspartate but most of which is reduced to malate in the mesophyll cells. Only after subsequent decarboxylation of labeled malate does $^{14}CO_2$ enter the Calvin cycle for conversion to glucose. The rate of entry into the cycle is limited by the rate of the rubisco-catalyzed reaction.

11. Identifying CAM Plants Given some $^{14}CO_2$ and all the tools typically present in a biochemistry research lab, how would you design a simple experiment to determine whether a plant was a typical C_4 plant or a CAM plant?

Answer The distinguishing features of CAM metabolism are the initial fixation of CO_2 at night and the storage of this fixed CO_2 in the vacuole until the next morning, when photosynthesis begins again. One could therefore measure the amount of $^{14}CO_2$ fixed in leaves during an hour of darkness, and the amount fixed during an hour of bright illumination. The CAM plant will take up much more CO_2 at night than a typical C_4 plant. One could also measure the concentration of organic acids in the vacuoles by titrating an extract of leaves; the CAM plant will have (in darkness) a much higher level of titratable acidity due to the malic acid stored in the vacuoles.

12. Chemistry of Malic Enzyme: Variation on a Theme Malic enzyme, found in the bundle-sheath cells of C_4 plants, carries out a reaction that has a counterpart in the citric acid cycle. What is the analogous reaction? Explain your choice.

Answer Malic enzyme catalyzes oxidative decarboxylation of a hydroxycarboxylic acid in the C_4 pathway:

$$\underset{\text{Malate}}{{}^{-}OOC{-}CH(OH){-}CH_2{-}COO^{-}} + NADP^{+} \longrightarrow \underset{\text{Pyruvate}}{{}^{-}OOC{-}CO{-}CH_3} + CO_2 + NADPH + H^{+}$$

which is analogous to the reaction catalyzed in the citric acid cycle by the enzyme isocitrate dehydrogenase:

$$\text{Isocitrate} + NAD^{+} \longrightarrow \alpha\text{-ketoglutarate} + NADH + H^{+}$$

13. The Cost of Storing Glucose as Starch Write the sequence of steps and the net reaction required to calculate the cost, in ATP molecules, of converting a molecule of cytosolic glucose 6-phosphate to starch and back to glucose 6-phosphate. What fraction of the maximum number of ATP molecules available from complete catabolism of glucose 6-phosphate to CO_2 and H_2O does this cost represent?

Answer The reactions for formation of starch from glucose 6-phosphate are:

(1) Glucose 6-phosphate $\rightarrow$ glucose 1-phosphate

(2) ATP + glucose 1-phosphate $\rightarrow$ ADP-glucose + PP_i

(3) ADP-glucose + $\text{starch}_n \rightarrow$ ADP + starch_{n+1}

The reactions for breakdown of starch are:

(4) $\text{Starch}_{n+1} + P_i \rightarrow \text{starch}_n$ + glucose 1-phosphate

(5) Glucose 1-phosphate $\rightarrow$ glucose 6-phosphate

The sum of reactions (1) through (5) is

(6) ATP + $P_i \rightarrow PP_i$ + ADP

Because it takes one ATP to convert ADP to ATP, we need to add

(7) ADP + ATP $\rightarrow$ ATP + ADP

The net reaction is the sum of reactions (6) and (7):

(8) ATP + $P_i \rightarrow$ ADP + PP_i

So, 1 mol of ATP is expended to store 1 mol of glucose 6-phosphate as starch. A mole of glucose 6-phosphate, when fully oxidized via glycolysis and the citric acid cycle, yields 31 to 33 mol of ATP, so the cost of storage is 1/33, or about 3.3% of the energy available.

14. Inorganic Pyrophosphatase The enzyme inorganic pyrophosphatase contributes to making many biosynthetic reactions that generate inorganic pyrophosphate essentially irreversible in cells. By keeping the concentration of PP_i very low, the enzyme "pulls" these reactions in the direction of PP_i formation. The synthesis of ADP-glucose in chloroplasts is one reaction that is pulled in the forward direction by this mechanism. However, the synthesis of UDP-glucose in the plant cytosol, which produces PP_i, is readily reversible in vivo. How do you reconcile these two facts?

Answer [PP_i] is high in the plant cell cytosol because the cytosol lacks inorganic pyrophosphatase, the enzyme that degrades PP_i to 2 P_i. Plants are unique in this regard. Animal cells have pyrophosphatase in their cytosol, and [PP_i] is therefore kept too low for PP_i to be a useful phosphoryl group donor.

15. Regulation of Starch and Sucrose Synthesis Sucrose synthesis occurs in the cytosol and starch synthesis in the chloroplast stroma, yet the two processes are intricately balanced. What factors shift the reactions in favor of **(a)** starch synthesis and **(b)** sucrose synthesis?

Answer

(a) Low levels of P_i in the cytosol and high levels of triose phosphate in the chloroplast favor formation of starch.

(b) High levels of triose phosphate in the cytosol favor formation of sucrose.

16. Regulation of Sucrose Synthesis In the regulation of sucrose synthesis from the triose phosphates produced during photosynthesis, 3-phosphoglycerate and P_i play critical roles (see Fig. 20–26). Explain why the concentrations of these two regulators reflect the rate of photosynthesis.

Answer 3-Phosphoglycerate is the primary product of photosynthesis; [P_i] rises when light-driven synthesis of ATP from ADP and P_i slows. The concentrations of these two metabolites thus provide clues to the energetic state of the leaf cell. When photosynthesis is occurring at a high rate, [P_i] drops and [3-phosphoglycerate] rises; in the dark, [P_i] rises and [3-phosphoglycerate] falls. The rate-limiting step in sucrose synthesis is the formation of ADP-glucose from glucose 1-phosphate and ATP, and this step is inhibited by P_i and activated by 3-phosphoglycerate (see Fig. 20–28). Sucrose synthesis therefore occurs at a high rate when photosynthesis is occurring, [P_i] is low, and [3-phosphoglycerate] is high.

17. Sucrose and Dental Caries The most prevalent infection in humans worldwide is dental caries, which stems from the colonization and destruction of tooth enamel by a variety of acidifying microorganisms. These organisms synthesize and live within a water-insoluble network of dextrans, called dental plaque, composed of ($\alpha 1 \rightarrow 6$)-linked polymers of glucose with many ($\alpha 1 \rightarrow 3$) branch points. Polymerization of dextran requires dietary sucrose, and the reaction is catalyzed by a bacterial enzyme, dextran-sucrose glucosyltransferase.

(a) Write the overall reaction for dextran polymerization.

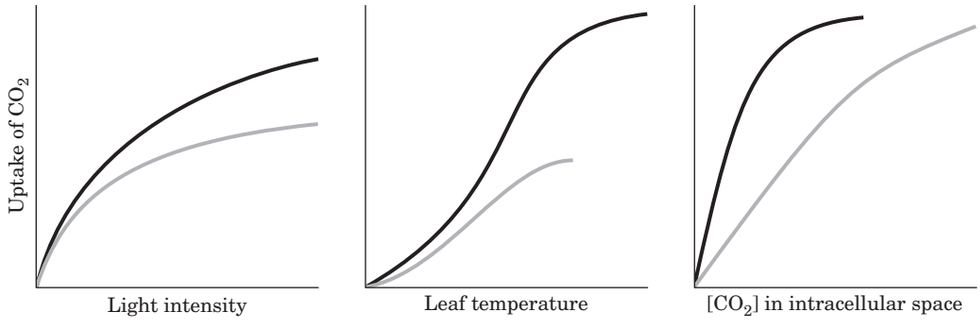
(b) In addition to providing a substrate for the formation of dental plaque, how does dietary sucrose also provide oral bacteria with an abundant source of metabolic energy?

Answer

(a) $$\text{Sucrose} + \underset{\text{dextran}_n}{(\text{glucose})_n} \longrightarrow \underset{\text{dextran}_{n+1}}{(\text{glucose})_{n+1}} + \text{fructose}$$

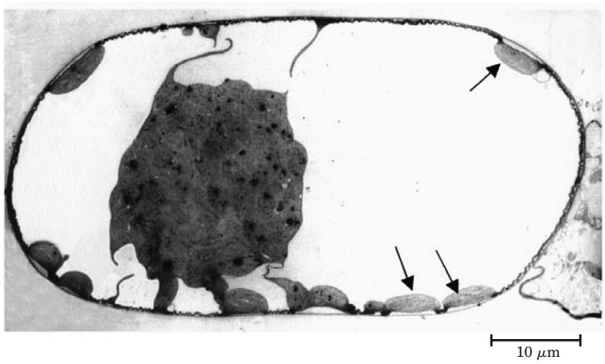
(b) Fructose generated in the synthesis of dextran is readily taken up by the bacteria and metabolized to acidic compounds.

18. Differences between C_3 and C_4 Plants The plant genus *Atriplex* includes some C_3 and some C_4 species. From the data in the plots below (species 1, upper curve; species 2, lower curve), identify which is a C_3 plant and which is a C_4 plant. Justify your answer in molecular terms that account for the data in all three plots.



Answer Species 1 is the C_4 variety; species 2, the C_3 variety. The "mistaken" use of O_2 instead of CO_2 by rubisco is greater at higher light intensities (higher photosynthetic rates) and at higher temperatures, so under these conditions the C_4 plant has the advantage. In the rightmost panel, the fixation of CO_2 is measured as a function of $[CO_2]$, and again the C_4 plant has the advantage. The lower K_m for CO_2 of PEP carboxylase (relative to rubisco) gives the C_4 plant the advantage at lower CO_2 concentrations.

19. C_4 Pathway in a Single Cell In typical C_4 plants, the initial capture of CO_2 occurs in one cell type, and the Calvin cycle reactions occur in another (see Fig. 20–23). Voznesenskaya and colleagues have described a plant, *Bienertia cycloptera*—which grows in salty depressions of semidesert in Central Asia—that shows the biochemical properties of a C_4 plant but unlike typical C_4 plants does not segregate the reactions of CO_2 fixation into two cell types. PEP carboxylase and rubisco are present in the same cell. However, the cells have two types of chloroplasts, which are localized differently, as shown in the micrograph. One type, relatively poor in grana (thylakoids), is confined to the periphery; the more typical chloroplasts are clustered in the center of the cell, separated from the peripheral chloroplasts by large vacuoles. Thin cytosolic bridges pass through the vacuoles, connecting the peripheral and central cytosol. [Voznesenskaya, E.V., Fraceschi, V.R., Kiirats, O., Artyusheva, E.G., Freitag, H., & Edwards, G.E. (2002) Proof of C_4 photosynthesis without Kranz anatomy in *Bienertia cycloptera* (Chenopodiaceae). *Plant J.* **31,** 649–662.]



In this plant, where would you expect to find **(a)** PEP carboxylase, **(b)** rubisco, and **(c)** starch granules? Explain your answers with a model for CO_2 fixation in these C_4 cells.

Answer In this organism, the initial capture of CO_2 and the final CO_2 fixation by rubisco take place not in different cell types but in two different regions of the same cell. The enzymes for initial fixation of CO_2 into oxaloacetate are at the periphery, where the concentrations of CO_2 and O_2 are relatively high; rubisco is in the cluster of chloroplasts at the center of the cell, where the O_2 concentration is low, limited by diffusion into the cell.

(a) Periphery of the cell.

(b) Chloroplasts at center of the cell.

(c) Chloroplasts at center of the cell.

Data Analysis Problem

20. Rubisco of Bacterial Endosymbionts of Hydrothermal Vent Animals Undersea hydrothermal vents support remarkable ecosystems. At these extreme depths there is no light to support photosynthesis, yet thriving vent communities are found. Much of their primary productivity occurs through chemosynthesis carried out by bacterial symbionts that live in specialized organs (trophosomes) of certain vent animals.

Chemosynthesis in these bacteria involves a process that is virtually identical to photosynthesis. Carbon dioxide is fixed by rubisco and reduced to glucose, and the necessary ATP and NADPH are produced by electron-transfer processes similar to those of the light-dependent reactions of photosynthesis. The key difference is that in chemosynthesis, the energy driving electron transfer comes from a highly exergonic chemical reaction rather than from light. Different chemosynthetic bacteria use different reactions for this purpose. The bacteria found in hydrothermal vent animals typically use the oxidation of H_2S (abundant in the vent water) by O_2, producing elemental sulfur. These bacteria also use the conversion of H_2S to sulfur as a source of electrons for chemosynthetic CO_2 reduction.

(a) What is the overall reaction for chemosynthesis in these bacteria? You do not need to write a balanced equation; just give the starting materials and products.

(b) Ultimately, these endosymbiotic bacteria obtain their energy from sunlight. Explain how this occurs.

Robinson and colleagues (2003) explored the properties of rubisco from the bacterial endosymbiont of the giant tube worm *Riftia pachyptila*. Rubisco, from any source, catalyzes the reaction of either CO_2 (Fig. 20–7) or O_2 (Fig. 20–20) with ribulose 1,5-bisphosphate. In general, rubisco reacts more readily with CO_2 than O_2. The degree of selectivity (Ω) can be expressed as

$$\frac{V_{\text{carboxylation}}}{V_{\text{oxygenation}}} = \Omega\,\frac{[CO_2]}{[O_2]}$$

where V is the reaction velocity.

Robinson and coworkers measured the Ω value for the rubisco of the bacterial endosymbionts. They purified rubisco from tube-worm trophosomes, reacted it with mixtures of different ratios of O_2 and CO_2 in the presence of [1-^{3}H]ribulose 1,5-bisphosphate, and measured the ratio of [^{3}H]phosphoglycerate to [^{3}H]phosphoglycolate.

(c) The measured ratio of [^{3}H]phosphoglycerate to [^{3}H]phosphoglycolate is equal to the ratio $V_{\text{carboxylation}}/V_{\text{oxygenation}}$. Explain why.

(d) Why would [5-^{3}H]ribulose 1,5-bisphosphate not be a suitable substrate for this assay?

The Ω for the endosymbiont rubisco had a value of 8.6 ± 0.9.

(e) The atmospheric (molar) concentration of O_2 is 20% and that of CO_2 is about 380 parts per million. If the endosymbiont were to carry out chemosynthesis under these atmospheric conditions, what would be the value of $V_{\text{carboxylation}}/V_{\text{oxygenation}}$?

(f) Based on your answer to **(e),** would you expect Ω for the rubisco of a terrestrial plant to be higher than, the same as, or lower than 8.6? Explain your reasoning.

Two stable isotopes of carbon are commonly found in the environment: the more abundant ^{12}C and the rare ^{13}C. All rubisco enzymes catalyze the fixation of $^{12}CO_2$ faster than that of $^{13}CO_2$. As a result, the carbon in glucose is slightly enriched in ^{12}C compared with the isotopic composition of CO_2 in the environment. Several factors are involved in this "preferential" use of $^{12}CO_2$, but one factor is the fundamental physics of gases. The temperature of a gas is related to the kinetic energy of its molecules. Kinetic energy is given by $\frac{1}{2}mv^2$, where m is molecular mass and v is velocity. Thus, at the same temperature (same kinetic energy), the molecules of a lighter gas will be moving faster than those of a heavier gas.

(g) How could this contribute to rubisco's "preference" for $^{12}CO_2$ over $^{13}CO_2$?

Some of the first convincing evidence that the tube-worm hosts were obtaining their fixed carbon from the endosymbionts was that the $^{13}C/^{12}C$ ratio in the animals was much closer to that of the bacteria than that of nonvent marine animals.

(h) Why is this more convincing evidence for a symbiotic relationship than earlier studies that simply showed the presence of rubisco in the bacteria found in trophosomes?

Answer

(a) By analogy to the oxygenic photosynthesis carried out by plants ($H_2O + CO_2 \rightarrow$ glucose $+ O_2$), the reaction would be $H_2S + O_2 + CO_2 \rightarrow$ glucose $+ H_2O + S$. This is the sum of the reduction of CO_2 by H_2S ($H_2S + CO_2 \rightarrow$ glucose $+ S$) and the energy input ($H_2S + O_2 \rightarrow S + H_2O$).

(b) The H_2S and CO_2 are produced chemically in deep-sea sediments, but the O_2, like the vast majority of O_2 on Earth, is produced by photosynthesis, which is driven by light energy.

(c) In the assay used by Robinson et al., ^3H labels the C-1 of ribulose 1,5-bisphosphate, so reaction with CO_2 yields one molecule of [^{3}H]3-phosphoglycerate and one molecule of unlabeled 3-phosphoglycerate; reaction with O_2 produces one molecule of [^{3}H]2-phosphoglycolate and one molecule of unlabeled 3-phosphoglycerate. Thus the ratio of [^{3}H]3-phosphoglycerate to [^{3}H]2-phosphoglycolate equals the ratio of carboxylation to oxygenation.

(d) If the ^3H labeled C-5, *both* oxygenation and carboxylation would yield [^{3}H]3-phosphoglycerate and it would be impossible to distinguish which reaction had produced the labeled product; the reaction could not be used to measure Ω.

(e) Substituting $\frac{[CO_2]}{[O_2]} = \frac{0.00038}{0.2} = 0.0019$ into $\frac{V_{\text{carboxylation}}}{V_{\text{oxygenation}}} = \Omega \frac{[CO_2]}{[O_2]}$ gives

$$\frac{V_{\text{carboxylation}}}{V_{\text{oxygenation}}} = (8.6)(0.0019) = 0.016$$

Therefore, the rate of oxygenation would be roughly 60 times the rate of carboxylation!

(f) If terrestrial plants had $\Omega = 8.6$, carboxylation would occur at a much lower rate than oxygenation. This would be extremely inefficient, so one would expect the rubisco of terrestrial plants to have an Ω substantially higher than 8.6. In fact, Ω values for land plants vary between 10 and 250. Even with these values, the expected rate of the oxygenation reaction is still very high.

(g) The rubisco reaction occurs with CO_2 as a gas. At the same temperature, $^{13}CO_2$ molecules diffuse more slowly than the lighter $^{12}CO_2$ molecules, and thus $^{13}CO_2$ will enter the active site (and become incorporated into substrate) more slowly than $^{12}CO_2$.

(h) For the relationship to be truly symbiotic, the tube worms must be getting a substantial amount of their carbon from the bacteria. The presence of rubisco in the endosymbionts simply shows that they are capable of chemosynthesis, not that they are supplying the host with a significant fraction of its carbon. On the other hand, showing that the $^{13}C : {}^{12}C$ ratio in the host is more similar to that in the endosymbiont than that in other marine animals strongly suggests that the tube worms are getting the majority of their carbon from the bacteria.

Reference

Robinson, J.J., Scott, K.M., Swanson, S.T., O'Leary, M.H., Horken, K., Tabita, F.R., & Cavanaugh, C.M. (2003) Kinetic isotope effect and characterization of form II RubisCO from the chemoautotrophic endosymbionts of the hydrothermal vent tubeworm *Riftia pachyptila*. *Limnol. Oceanogr.* **48,** 48–54.