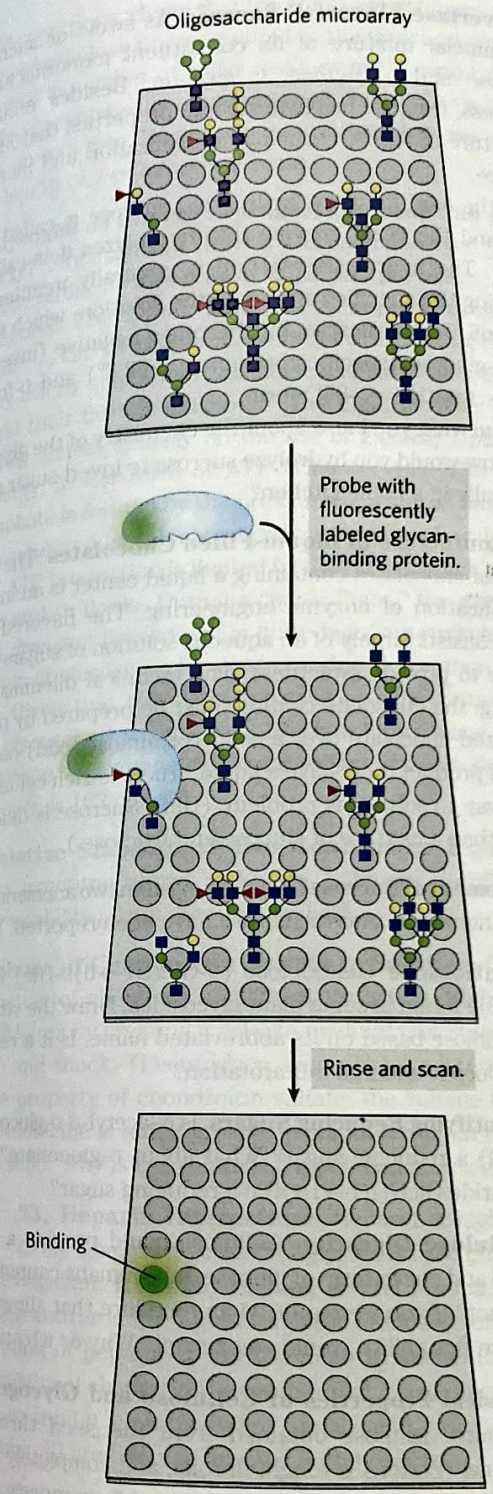


FIGURE 7-40 Oligosaccharide microarrays to determine the specificity and affinity of carbohydrate binding by lectins. Solutions of pure samples of oligosaccharides, synthesized or isolated from nature, are placed in microscopic droplets on a glass slide and attached to the glass through an inert spacer. Each spot represents a different oligosaccharide. The protein sample to be tested for its affinity for oligosaccharides is first conjugated with a fluorescent marker, then the sample is poured over the slide and allowed to equilibrate; any nonadsorbed protein is washed away. Observation of the microarray with a fluorescence microscope shows which spots have adsorbed protein (they glow green), and assessment of the fluorescence intensity gives a rough measure of protein-oligosaccharide binding affinity. [Source: Information from P. H. Seeberger, *Nature Chem. Biol.* 5:368, 2009, Fig. 2a.]

lectin-oligosaccharide interactions and may prove clinically useful.

- Microarrays of pure oligosaccharides are useful in determining the specificity and affinity of lectin binding to specific oligosaccharides.

Key Terms

Terms in bold are defined in the glossary.

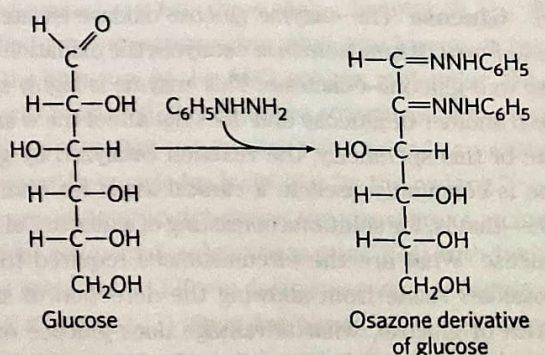
glycoconjugate 241
carbohydrate 241
monosaccharide 241
oligosaccharide 241
disaccharide 241
polysaccharide 241
aldose 242
ketose 242
Fischer projection formulas 242
epimers 242
hemiacetal 243
hemiketal 243
anomers 245
anomeric carbon 245
pyranose 245
furanose 245
Haworth perspective formulas 245
mutarotation 246
hemoglobin glycation 248
reducing sugar 249
***O*-glycosidic bonds** 250
reducing end 250
glycan 252
homopolysaccharide 252
heteropolysaccharide 252
starch 253
glycogen 254
cellulose 254
extracellular matrix (ECM) 258
glycosaminoglycan 258
hyaluronan 259
chondroitin sulfate 259
heparan sulfate 259
proteoglycan 261
glycoprotein 261
glycosphingolipid 261
syndecan 262
glypican 262
glycomics 266
lectin 268
selectins 268
oligosaccharide microarrays 274

Problems

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?

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.

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:



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 10-14. In his paper, Morgan listed several properties of the three antigens, A, B, and O, that were known at that time (p. 314):

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 endoglycosidase or exoglycosidase? (Endoglycosidases cut bonds between interior residues; exoglycosidases remove one residue at a time from the end of a polymer.) Explain your reasoning.

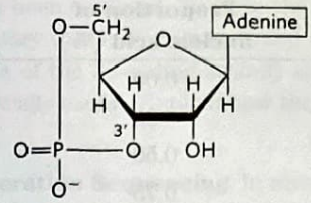
(g) Fucose is also present in the A and B antigens. Based on the structure of these antigens, why does fucose fail to prevent their degradation by the *T. foetus* enzyme? What structure would be produced?

(h) Which of the results in (f) and (g) are consistent with the structures shown in Figure 10-14? Explain your reasoning.

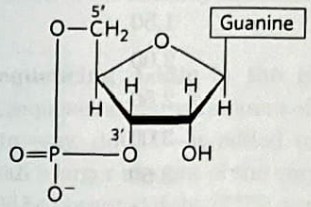
Reference

Morgan, W.T.J. 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.

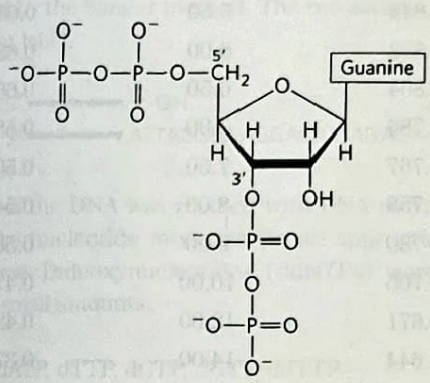
Further Reading is available at www.macmillanlearning.com/LehningerBiochemistry7e.



Adenosine 3′,5′-cyclic monophosphate
(cyclic AMP; cAMP)



Guanosine 3′,5′-cyclic monophosphate
(cyclic GMP; cGMP)



Guanosine 5′-diphosphate,3′-diphosphate
(guanosine tetraphosphate; ppGpp)

FIGURE 8-42 Three regulatory nucleotides.

class of ATP receptors that mediate the sensation of pain is an obvious target for drug development. Extracellular ADP is a signaling molecule that acts through P_{2Y} receptors in sensitive cell types. By preventing ADP from binding the P_{2Y} receptors of platelets, the drug clopidogrel (Plavix) inhibits undesirable blood clotting in patients with cardiac disease. Signaling pathways are discussed in more detail in Chapter 12. ■

SUMMARY 8.4 Other Functions of Nucleotides

- ATP is the central carrier of chemical energy in cells. The presence of an adenosine moiety in a variety of enzyme cofactors may be related to binding-energy requirements.
- Cyclic AMP, formed from ATP in a reaction catalyzed by adenylyl cyclase, is a common second messenger produced in response to hormones and other chemical signals.
- ATP and ADP serve as neurotransmitters in a variety of signaling pathways.

Key Terms

Terms in bold are defined in the glossary.

deoxyribonucleic acid (DNA) 279
ribonucleic acid (RNA) 279
gene 279
ribosomal RNA (rRNA) 279
messenger RNA (mRNA) 279
transfer RNA (tRNA) 279
nucleotide 279
nucleoside 279
pyrimidine 279
purine 279
deoxyribonucleotides 281
ribonucleotide 282
phosphodiester linkage 283
5′ end 283
3′ end 283
oligonucleotide 284
polynucleotide 284
base pair 284
major groove 286
minor groove 286
B-form DNA 288
A-form DNA 288
Z-form DNA 288
palindrome 289
hairpin 289
cruciform 290
triplex DNA 290
G tetraplex 290
transcription 291
monocistronic mRNA 291
polycistronic mRNA 291
mutation 297
polymerase chain reaction (PCR) 301
DNA polymerases 301
Sanger sequencing 302
sequence polymorphisms 304
short tandem repeat (STR) 304
DNA sequencing technologies 306
pyrosequencing 307
reversible terminator sequencing 308
sequencing depth 310
contig 310
ion semiconductor sequencing 310
single-molecule real-time (SMRT) sequencing 310
second messenger 311
adenosine 3′,5′-cyclic monophosphate (cyclic AMP, cAMP) 312

Problems

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?

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?

3. DNA of the Human Body Calculate the weight in grams of a double-helical DNA molecule stretching from 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.

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.

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?

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. Suggest why cells might require a specialized system to repair G–T mismatches.

7. Denaturation of Nucleic Acids A duplex DNA oligonucleotide in which one of the strands has the sequence TAATAC GACTCACTATAGGG has a melting temperature (t_m) of 59 °C. If an RNA duplex oligonucleotide of identical sequence (substituting U for T) is constructed, will its melting temperature be higher or lower?

8. 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. 8-29b) and describe some chemical consequences of base loss.

9. Prediction of Nucleic Acid Structure from Its Sequence A part of a sequenced chromosome has the sequence (on one strand) ATTGCATCCGCGCGTGCGCGCGC-GATCCCGTTACTTTCCG. Which part of this sequence is most likely to take up the Z conformation?

10. Nucleic Acid Structure Explain why the absorption of UV light by double-stranded DNA increases (the hyperchromic effect) when the DNA is denatured.

11. 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 and 260 nm and using the table below, 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

12. 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?

13. Polymerase Chain Reaction One strand of a chromosomal DNA sequence is shown below. An investigator wants to amplify and isolate a DNA fragment defined by the segment shown in red, using the polymerase chain reaction (PCR). Design two PCR primers, each 20 nucleotides long, that can be used to amplify this DNA segment. The final PCR product generated with your primers should include no sequences outside the segment in red.

5′ – – – AATGCCGTCAGCCGATCTGCCTCGAGTCAATCGA
TGCTGGTAACTTGGGGTATAAAGCTTACCCATGGTATCGTAG
TTAGATTGATTGTTAGGTTCTTAGGTTTAGGTTTCTGGTATT
GGTTTAGGGTCTTTGATGCTATTAATTGTTTGGTTTTGATTT
GGTCTTTATATGGTTTATGTTTTAAGCCGGGTTTTGTCTGG-
GATGGTTCGTCTGATGTGCGCGTAGCGTGCGGCG – – – 3′

14. Genomic Sequencing In large-genome sequencing projects, the initial data usually reveal gaps where no sequence

information has been obtained. To close the gaps, DNA primers complementary to the 5′-ending strand (that is, identical to the sequence of the 3′-ending strand) at the end of each contig are especially useful. Explain how these primers might be used.

15. Next-Generation Sequencing In reversible terminator sequencing, how would the sequencing process be affected if the 3′-end-blocking group of each nucleotide were replaced with the 3′-H present in the dideoxynucleotides used in Sanger sequencing?

16. Sanger Sequencing Logic In the Sanger (dideoxy) method for DNA sequencing, a small amount of a dideoxynucleoside 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?

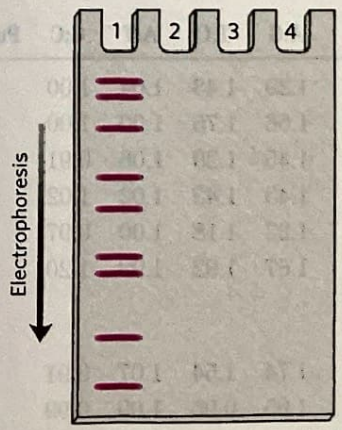
17. DNA Sequencing The following DNA fragment was sequenced by the Sanger method. The red asterisk indicates a fluorescent label.

*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?



18. 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 sequence (5′)GCGCCAUUGC(3′)—OH?

19. 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?

20. Oligonucleotide Synthesis In the scheme of Figure 8-34, 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?

Biochemistry Online

21. 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.pdb.org). Use the PDB identifiers listed below to retrieve the structure summaries for the two forms of DNA. View the 3D structure using JSmol (click the 3D View tab or the JSmol link in the Structure Image window on the summary page). You will need to use both the display menus on the screen and the scripting controls in the JSmol menu (accessed by clicking on the JSmol logo in the lower right corner of the image screen) to complete the following exercises. Refer to the JSmol help links as needed.

(a) Access PDB ID 141D, a highly conserved, repeated DNA sequence from the end of the genome of HIV-1 (the virus

that causes AIDS). Set the Style to Ball and Stick. Then use the scripting controls to color by element (Color > Atoms > By Scheme > Element (CPK)). 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) Access PDB ID 145D, a DNA with the Z conformation. Set the Style to Ball and Stick. Then use the scripting controls to color by element (Main Menu > Color > Atoms > By Scheme > Element (CPK)). 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. From the scripting control Main Menu select Style > Stereographic > Cross-eyed viewing or Wall-eyed viewing. (If you have stereographic glasses available, select the appropriate option.) 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 on 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.)

Data Analysis Problem

22. 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. 286). 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 his 1951 paper, 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

Problems

1. Operational Definition of Lipids How is the definition of "lipid" different from the types of definitions used for other biomolecules, such as amino acids, nucleic acids, and proteins?

2. Structure of an Omega-6 Fatty Acid Draw the structure of the omega-6 fatty acid 16:1.

3. 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?

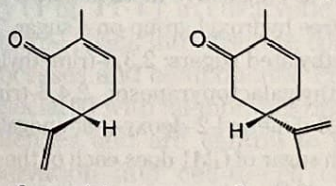
(b) Draw all the possible triacylglycerols that can be constructed from glycerol, palmitic acid, and oleic acid. Rank them in order of increasing melting point.

(c) Branched-chain fatty acids are found in some bacterial membrane lipids. Would their presence increase or decrease the fluidity of the membrane (that is, give the lipids a lower or higher melting point)? Why?

4. 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?

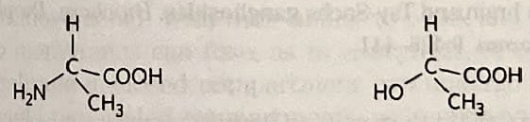
5. Impermeability of Waxes What property of the waxy cuticles that cover plant leaves makes the cuticles impermeable to water?

6. 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

7. 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)

8. 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.

9. 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?

10. 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.

11. Lipids in Blood Group Determination We note in Figure 10-14 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?

12. 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.

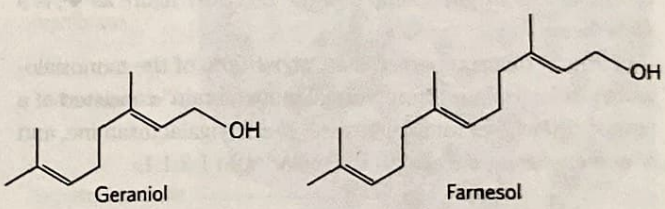
(a) All detergents are amphipathic. What are the hydrophilic and hydrophobic portions of lysolecithin?

(b) The pain and inflammation caused by a snake bite can be treated with certain steroids. What is the basis of this treatment?

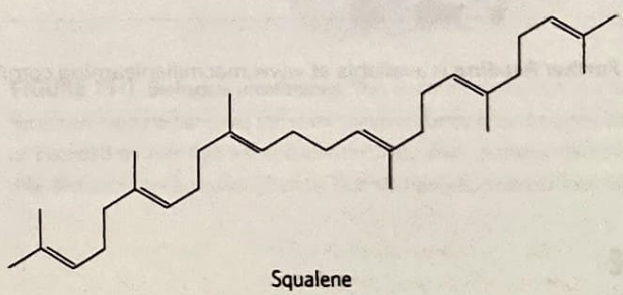
(c) Though the high levels of phospholipase A_2 in venom can be deadly, this enzyme is necessary for a variety of normal metabolic processes. What are these processes?

13. Intracellular Messengers from Phosphatidylinositols When the hormone vasopressin stimulates cleavage of PIP_2 by 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.

14. 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

15. Hydrolysis of Lipids Name the products of mild hydrolysis with dilute NaOH of (a) 1-stearoyl-2,3-dipalmitoylglycerol; (b) 1-palmitoyl-2-oleoylphosphatidylcholine.

16. 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.

17. 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.

18. 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?

19. 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?

Data Analysis Problem

20. Determining the Structure of the Abnormal Lipid in Tay-Sachs Disease Box 10-1, Figure 1, shows the pathway of breakdown of gangliosides in healthy (normal) individuals and in 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 10-1 figure as ◆, is a sialic acid.

Svennerholm reported that "about 90% of the monosialogangliosides 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 10-1, 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 10-1 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 10-1? Explain your reasoning.

(d) What can you conclude about the structure of the Tay-Sachs ganglioside? Is this consistent with the structure in Box 10-1? 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?

Reference

Svennerholm, L. 1962. The chemical structure of normal human brain and Tay-Sachs gangliosides. *Biochem. Biophys. Res. Comm.* 9:436–441.

Further Reading is available at www.macmillanlearning.com/LehningerBiochemistry7e.

directions; examples are the chloride-bicarbonate exchanger of erythrocytes and the ubiquitous Na^+K^+ ATPase.

- In animal cells, Na^+K^+ ATPase maintains the differences in cytosolic and extracellular concentrations of Na^+ and K^+, and the resulting Na^+ gradient is used as the energy source for a variety of secondary active transport processes.
- The Na^+K^+ ATPase of the plasma membrane and the Ca^{2+} transporters of the sarcoplasmic/endoplasmic reticulum (the SERCA pumps) are examples of P-type ATPases; they undergo reversible phosphorylation during their catalytic cycle. F-type ATPase proton pumps (ATP synthases) are central to energy-conserving mechanisms in mitochondria and chloroplasts. V-type ATPases produce gradients of protons across some intracellular membranes, including plant vacuolar membranes.
- ABC transporters carry a variety of substrates (including many drugs) out of cells, using ATP as the energy source.
- Ionophores are lipid-soluble molecules that bind specific ions and carry them passively across membranes, dissipating the energy of electrochemical ion gradients.
- Water moves across membranes through aquaporins. Some aquaporins are regulated; some also transport glycerol or urea.
- Ion channels provide hydrophilic pores through which select ions can diffuse, moving down their electrical or chemical concentration gradients; these channels characteristically are unsaturable, have very high flux rates, and are highly specific for one ion. Most are voltage- or ligand-gated. The neuronal Na^+ channel is voltage-gated, and the acetylcholine receptor ion channel is gated by acetylcholine, which triggers conformational changes that open and close the transmembrane path.

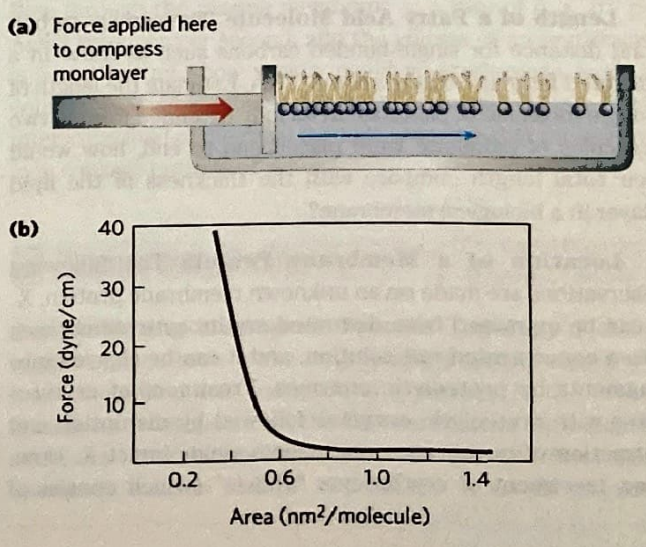
Key Terms

Terms in bold are defined in the glossary.

fluid mosaic model 389
micelle 390
bilayer 390
vesicle 390
integral proteins 391
monotopic 392
polytopic 392
peripheral proteins 392
amphitropic proteins 392
annular lipid 394
hydropathy index 394
β barrel 395
porin 395
positive-inside rule 395
GPI-anchored protein 397
liquid-ordered state (L_o) 397
liquid-disordered state (L_d) 397
flippases 399
floppases 399
scramblases 399
FRAP 400
microdomains 401
rafts 401
caveolin 402
caveolae 402
BAR domain 403
fusion protein 404
v-SNAREs 404
t-SNAREs 404
selectins 405
simple diffusion 406
membrane potential (V_m) 406
electrochemical gradient 406
electrochemical potential 406
transporters 406
passive transport 406
active transport 406
ion channels 407
K_t ($K_{transport}$) 408
electroneutral 412
cotransport 412
antiport 412
symport 412
uniport 412
electrogenic 413
P-type ATPases 413
SERCA pump 414
Na^+K^+ ATPase 415
V-type ATPases 416
F-type ATPases 416
ATP synthase 416
ABC transporters 417
multidrug transporters 418
lactose transporter 418
major facilitator superfamily (MFS) 421
Na^+-glucose symporters 421
ionophore 422
aquaporins (AQPs) 423
ligand-gated channel 425
voltage-gated channel 425
patch-clamping 425
nicotinic acetylcholine receptor 427

Problems

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?



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 table below. 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 E. Gorter and F. Grendel, *J. Exp. Med.* 41:439, 1925.

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.

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 on each other and undergo self-sealing to form vesicles (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?

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?

6. 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?

7. 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 with one transmembrane segment. Suggest biochemical or chemical experiments that might allow you to determine whether the protein is oriented with the amino terminus on the outside or the inside of the cell.

8. 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.

9. 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?

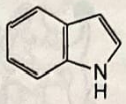
10. Temperature Dependence of Lateral Diffusion The experiment described in Figure 11-16 was performed at 37 °C. If the experiment were carried out at 10 °C, what effect would you expect on the rate of diffusion? Why?

11. 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?

12. Lipid Melting Temperatures Membrane lipids in tissue samples obtained from different parts of a reindeer's leg 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?

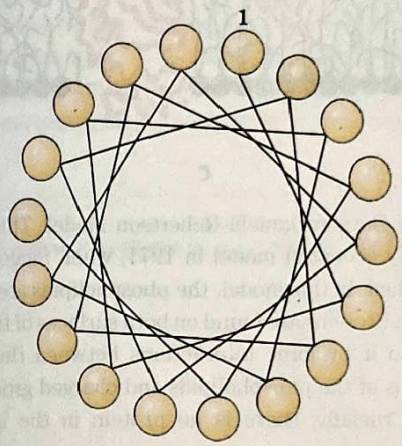
13. Flip-Flop Diffusion The inner leaflet (monolayer) of the human erythrocyte membrane consists predominantly of phosphatidylethanolamine and phosphatidylserine. The outer leaflet consists predominantly of phosphatidylcholine and sphingomyelin. Although the phospholipid components of the membrane can diffuse in the fluid bilayer, this sidedness is preserved at all times. How?

14. 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.

15. 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 shown here 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?

16. 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.

17. 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^+ from the cell into the blood at 37 °C? Assume the concentration of Na^+ inside the cell is 12 mM and in blood plasma it is 145 mM.

18. 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?

19. Energetics of Symport Suppose 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?

20. 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 this Cys-containing transporter polypeptide.

21. Intestinal Uptake of Leucine You are studying the uptake of L-leucine by epithelial cells of the mouse intestine. Measurements of the rates 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 below. 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

22. Effect of an Ionophore on Active Transport Consider the leucine transporter described in Problem 21. Would V_{max} and/or K_t change if you added a Na^+ ionophore to the assay solution containing Na^+? Explain.

23. Water Flow through an Aquaporin A human erythrocyte has about 2×10^5 AQP1 monomers. If water molecules flow through the plasma membrane at a rate of 5×10^8 per AQP1 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.

Biochemistry Online

24. Predicting Membrane Protein Topology I Online bioinformatics tools make hydropathy analysis easy if you know the amino acid sequence of a protein. At the Protein Data Bank (www.pdb.org), the Protein Feature View displays additional information about a protein gleaned from other databases, such as UniProt and SCOP2. A simple graphical

view of a hydropathy plot created using a window of 15 residues shows hydrophobic regions in red and hydrophilic regions in blue.

(a) Looking only at the displayed hydropathy plots in the Protein Feature View, what predictions would you make about the membrane topology of these proteins: glycophorin A (PDB ID 1AFO), myoglobin (PDB ID 1MBO), and aquaporin (PDB ID 2B6O)?

(b) Now, refine your information using the ProtScale tools at the ExPASy bioinformatics resource portal. Each of the PDB Protein Feature Views was created with a UniProt Knowledgebase ID. For glycophorin A, the UniProtKB ID is P02724; for myoglobin, P02185; and for aquaporin, Q6J8I9. Go to the ExPASy portal (http://web.expasy.org/protscale) and select the Kyte & Doolittle hydropathy analysis option, with a window of 7 amino acids. Enter the UniProtKB ID for aquaporin (Q6J8I9, which you can also get from the PDB's Protein Feature View page), then select the option to analyze the complete chain (residues 1 to 263). Use the default values for the other options and click Submit to get a hydropathy plot. Save a GIF image of this plot. Now repeat the analysis using a window of 15 amino acids. Compare the results for the 7-residue and 15-residue window analyses. Which one gives you a better signal-to-noise ratio?

(c) Under what circumstances would it be important to use a narrower window?

25. Predicting Membrane Protein Topology II 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?

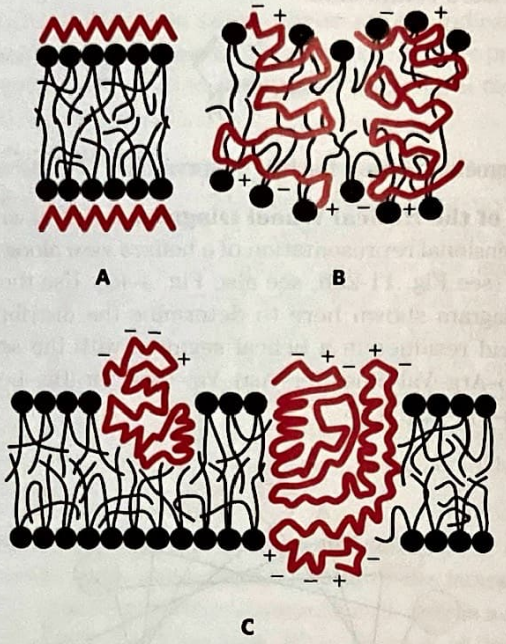
(c) Go to the Protein Data Bank (www.pdb.org). Use the PDB identifier 1DEP to retrieve the data page for a portion of the β-adrenergic receptor (one type of epinephrine receptor) isolated from turkey. Using JSmol to explore the structure, predict whether this portion of the receptor is located within the membrane or at the membrane surface. Explain your answer. Now use the Protein Feature View to see the hydrophobicity analysis of the sequence. Does this support your answer?

(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. 132) for more information.

Data Analysis Problem

26. 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 up to that time:



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 of 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 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, 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) Singer wrote in his article: "The average amino acid composition of membrane proteins is not distinguishable from

in these genes are genetically recessive but can lead to tumor formation.

- Cancer is generally the result of an accumulation of mutations in oncogenes and tumor suppressor genes.
- When stability genes, which encode proteins necessary for the repair of genetic damage, are mutated, other mutations go unrepaired, including mutations in proto-oncogenes and tumor suppressor genes that can lead to cancer.
- Apoptosis is programmed and controlled cell death that functions during normal development and adulthood to get rid of unnecessary, damaged, or infected cells. Apoptosis can be triggered by extracellular signals such as TNF, acting through plasma membrane receptors.

Key Terms

Terms in bold are defined in the glossary.

signal transduction 437
specificity 437
cooperativity 438
amplification 438
enzyme cascade 438
modularity 438
scaffold proteins 438
desensitization 439
integration 439
response localization 439
G protein–coupled receptors (GPCRs) 440
guanosine nucleotide–binding proteins 440
G proteins 440
second messenger 440
agonist 441
antagonist 441
β-adrenergic receptors 441
heptahelical receptors 441
stimulatory G protein (G_s) 441
adenylyl cyclase 442
cAMP-dependent protein kinase (protein kinase A; PKA) 442
P loop 444
GTPase activator protein (GAP) 445
regulator of G protein signaling (RGS) 445
guanosine nucleotide–exchange factor (GEF) 445
consensus sequence 446
β-arrestin (βarr; arrestin 2) 449
G protein–coupled receptor kinases (GRKs) 449
cAMP response element binding protein (CREB) 450
inhibitory G protein (G_i) 450
adaptor proteins 450
AKAPs (A kinase anchoring proteins) 450
phospholipase C (PLC) 451
inositol 1,4,5-trisphosphate (IP_3) 451
protein kinase C (PKC) 451
green fluorescent protein (GFP) 452
fluorescence resonance energy transfer (FRET) 452
calmodulin (CaM) 453
Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases) 454
rhodopsin 456
opsin 456
rhodopsin kinase 457
receptor potential 459
gustducin 459
receptor Tyr kinase (RTK) 461
autophosphorylation 461
SH2 domain 461
Ras 461
small G proteins 461
MAPKs 462
guanosine 3′,5′-cyclic monophosphate (cyclic GMP; cGMP) 466
cGMP-dependent protein kinase (protein kinase G; PKG) 466
atrial natriuretic factor (ANF) 466
NO synthase 467
PTB domains 468
voltage-gated ion channels 472
ionotropic 473
metabotropic 473
hormone response element (HRE) 474
two-component signaling systems 475
receptor histidine kinase 475
response regulator 475
cyclin 477
cyclin-dependent protein kinase (CDK) 477
ubiquitin 478
proteasome 478
growth factors 478
retinoblastoma protein (pRb) 480
oncogene 481
proto-oncogene 481
tumor suppressor gene 484
programmed cell death 485
apoptosis 485

Problems

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.

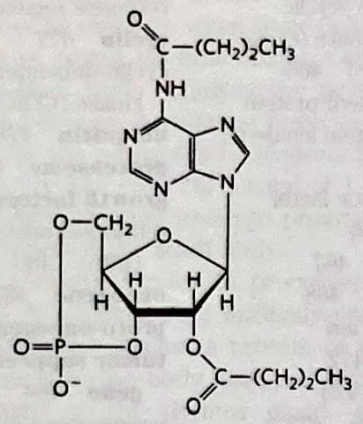
(a) Addition of epinephrine to a homogenate of normal liver resulted in an increase in the activity of glycogen phosphorylase. However, when the homogenate was first centrifuged at a high speed and epinephrine or glucagon was added to the clear supernatant fraction that contains phosphorylase, no increase 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.)

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)

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.

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.

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 [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? If so, how might one design a better drug that does not have these effects?

6. Termination of Hormonal Signals Signals carried by hormones must eventually be terminated. Describe several different mechanisms for signal termination.

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 12-2) to demonstrate this interaction in living cells? Which proteins would you fuse? Which wavelengths would you use to illuminate the cells, and which wavelengths 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?

8. EGTA Injection EGTA (ethylene glycol-bis(β-amino ethyl 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?

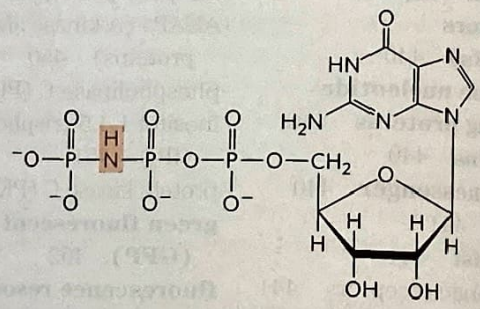
9. Amplification of Hormonal Signals Describe all the sources of amplification in the insulin receptor system.

10. Mutations in *ras* How would a mutation in *ras* that leads to formation of a Ras protein with no GTPase activity affect a cell's response to insulin?

11. Differences among G Proteins Compare the G protein G_s, which acts in transducing the signal from β-adrenergic receptors, and the G protein Ras. What properties do they share? How do they differ? What is the functional difference between G_s and G_i?

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.

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.



Gpp(NH)p
(β,γ-imidoguanosine 5′-triphosphate)

Predict the effect of microinjection of Gpp(NH)p into a myocyte on the cell's response to β-adrenergic stimulation.

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 acetylcholine receptor (AChR; an integral membrane protein) and prevents its 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.

15. 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. The signal released by one neuron can be either excitatory or inhibitory to the following, postsynaptic neuron.)

16. Visual Desensitization Oguchi 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 disease. Explain in molecular terms how this defect would account for night blindness.

17. 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?

18. 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 channel proteins 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 of 32 nM—a property known as EC_{50}. 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 mM). What sensation would you expect from contact with low levels of menthol? With high levels?

19. 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. 12-37.)

(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.)

20. 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.

21. 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.

22. 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.

Data Analysis Problem

23. Exploring Taste Sensation in Mice 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 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 non-overlapping sets of taste receptor cells. Which model does this support? Explain your reasoning.