 Chapter 2

Key Terms

Terms in bold are defined in the glossary.

hydrogen bond 48
bond energy 48
hydrophilic 50
hydrophobic 50
amphipathic 52
micelle 52
hydrophobic effect 52
hydrophobic interactions 53
van der Waals interactions 53
osmolarity 55
osmosis 56
isotonic 56
hypertonic 56
hypotonic 56
equilibrium constant (K_{eq}) 58
ion product of water (K_w) 59
pH 60
acidosis 61
alkalosis 61
conjugate acid-base pair 61
acid dissociation constant (K_a) 62
pK_a 62
titration curve 62
buffer 64
buffering region 64
Henderson-Hasselbalch equation 65
condensation 69
hydrolysis 69

Problems

1. Effect of Local Environment on Ionic Bond Strength If the ATP-binding site of an enzyme is buried in the interior of the enzyme, in a hydrophobic environment, is the ionic interaction between enzyme and substrate stronger or weaker than that same interaction would be on the surface of the enzyme, exposed to water? Why?

2. Biological Advantage of Weak Interactions The interactions between biomolecules are often stabilized by weak interactions such as hydrogen bonds. How might this be an advantage to the organism?

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

4. 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?

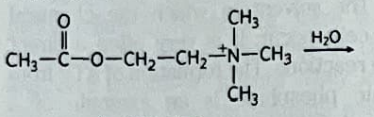
5. Calculation of Hydrogen Ion Concentration from pH What is the H^+ concentration of a solution with pH of (a) 3.82; (b) 6.52; (c) 11.11?

6. 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?

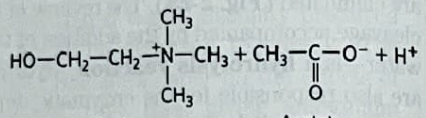
7. Calculation of the pH of a Strong Acid or Base (a) Write out the acid dissociation reaction for hydrochloric acid. (b) Calculate the pH of a solution of 5.0×10^{-4} M HCl. (c) Write out the acid dissociation reaction for sodium hydroxide. (d) Calculate the pH of a solution of 7.0×10^{-5} M NaOH.

8. 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.

9. 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 converted to choline and acetic acid, which dissociates to yield acetate and a hydrogen ion:



Acetylcholine



Choline Acetate

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 there was no buffer in the assay mixture, determine the number of moles of acetylcholine in the 15 mL sample.

10. 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)?

11. Meanings of K_a and pK_a (a) Does a strong acid have a greater or lesser tendency to lose its proton than a weak acid? (b) Does the strong acid have a higher or lower K_a than the weak acid? (c) Does the strong acid have a higher or lower pK_a than the weak acid?

12. 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. 2-15) 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. 2-16).

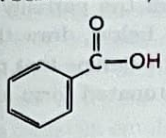
13. Identifying the Conjugate Base Which is the conjugate base in each of the pairs below?

(a) RCOOH, $RCOO^-$
(b) RNH_2, RNH_3^+
(c) $H_2PO_4^-$, H_3PO_4
(d) H_2CO_3, HCO_3^-

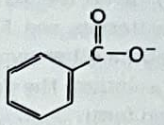
14. Calculation of the pH of a Mixture of a Weak Acid and Its Conjugate Base Calculate the pH of a dilute solution that contains a molar ratio of potassium acetate to acetic acid (pK_a = 4.76) of (a) 2:1; (b) 1:3; (c) 5:1; (d) 1:1; (e) 1:10.

15. 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 any 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.

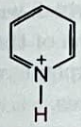


Benzoic acid
$pK_a \approx 5$



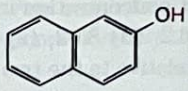
Benzoate ion

Are the following compounds more soluble in an aqueous solution of 0.1 M NaOH or 0.1 M HCl? (The dissociable protons are shown in red.)



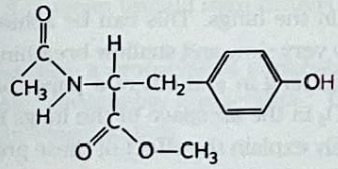
Pyridine ion
$pK_a \approx 5$

(a)



β-Naphthol
$pK_a \approx 10$

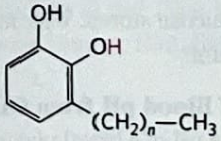
(b)



N-Acetyltyrosine methyl ester
$pK_a \approx 10$

(c)

16. 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.

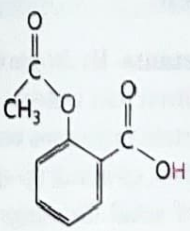


$pK_a \approx 8$

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).

17. pH and Drug Absorption Aspirin is a weak acid with a pK_a of 3.5 (the ionizable H is shown in red):



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.

18. 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)?

19. 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?

20. 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?

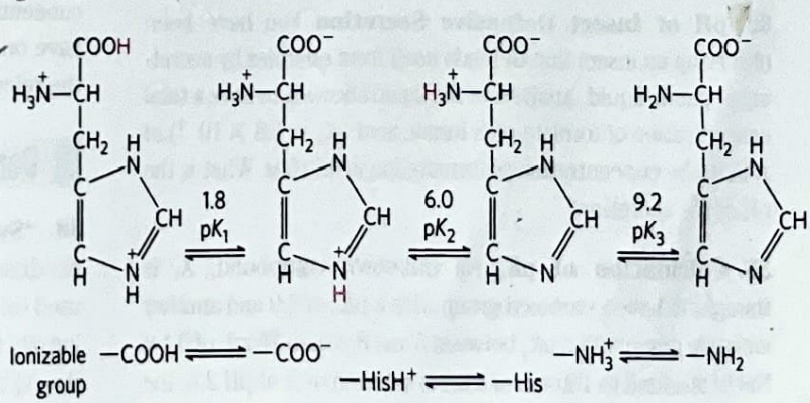
(b) In a 0.1 M solution of glycine at pH 9.0, what fraction of glycine has its amino group in the $—NH_3^+$ form?

(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?

21. Calculation of the pK_a of an Ionizable Group by Titration The pK_a values of a compound with two ionizable groups are $pK_1 = 4.10$ and pK_2 between 7 and 10. A biochemist has 10 mL of a 1.0 M solution of this compound at a pH of 8.00. She adds 10.0 mL of 1.00 M HCl, which changes the pH to 3.20. What is pK_2?

22. Calculation of the pH of a Solution of a Polyprotic Acid Histidine has ionizable groups with pK_a values of 1.8, 6.0, and 9.2, as shown below (His = imidazole group). A biochemist makes up 100 mL of a 0.100 M solution of histidine at a pH of 5.40. She then adds 40 mL of 0.10 M HCl. What is the pH of the resulting solution?



23. Calculation of Original pH from Final pH after Titration A biochemist has 100 mL of a 0.10 M solution of a weak acid with a pK_a of 6.3. She adds 6.0 mL of 1.0 M HCl, which changes the pH to 5.7. What was the pH of the original solution?

24. 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 three pK_a values: 2.14, 6.86, and 12.4. Hint: Only one of the pK_a values is relevant here.

25. 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. 2-16). See Problem 24 for the pK_a values of phosphoric acid.

26. 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.

27. 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.

28. 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.

29. 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?

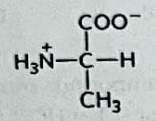
30. Use of Molar Concentrations to Calculate pH What is the pH of a solution that contains 0.20 M sodium acetate and 0.60 M acetic acid ($pK_a = 4.76$)?

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

32. 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?

33. 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.

34. 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.

35. Control of Blood pH by Respiratory 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 ($CH_3CH(OH)COOH$; $K_a = 1.38 \times 10^{-4}$ M) from their glucose stores. Why might hyperventilation before a dash be useful?

36. 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 67 that the relevant pK_a of carbonic acid is 6.1.

37. 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.

Data Analysis Problem

38. "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.

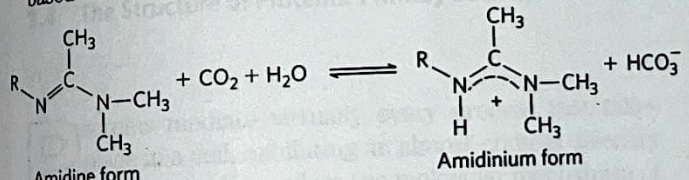
Surfactants are a class of amphipathic compounds that includes soaps, detergents, and emulsifiers. With the use of surfactants, hydrophobic compounds can be suspended in aqueous solution by forming micelles (see Fig. 2-7). A micelle has a hydrophobic core consisting of the hydrophobic compound and the 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:



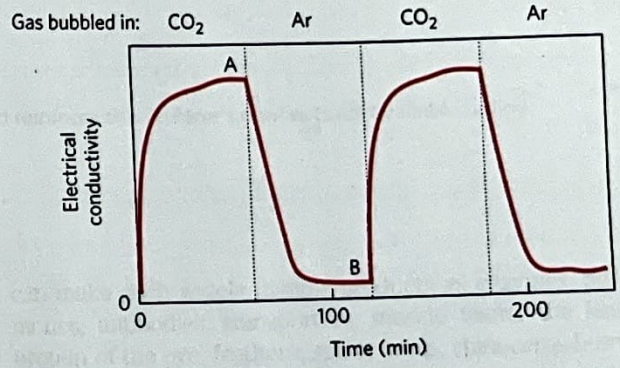
Amidine form — Amidinium form

(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.

Reference

Y. Liu, P.G. Jessop, M. Cunningham, C.A. Eckert, and C.L. Liotta. 2006. Switchable surfactants. *Science* 313:958–960.

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

promises to be among the most vibrant of the scientific frontiers in the twenty-first century.

SUMMARY 3.4 The Structure of Proteins: Primary Structure

■ Differences in protein function result from differences in amino acid composition and sequence. Some variations in sequence may occur in a particular protein, with little or no effect on its function.

■ Amino acid sequences are deduced by fragmenting polypeptides into smaller peptides with reagents known to cleave specific peptide bonds, determining the amino acid sequence of each fragment by the automated Edman degradation procedure, and then ordering the peptide fragments by finding sequence overlaps between fragments generated by different reagents. A protein sequence can also be deduced from the nucleotide sequence of its corresponding gene in DNA or by mass spectrometry.

■ Short proteins and peptides (up to about 100 residues) can be chemically synthesized. The peptide is built up, one amino acid residue at a time, while tethered to a solid support.

■ Protein sequences are a rich source of information about protein structure and function, as well as the evolution of life on Earth. Sophisticated methods are being developed to trace evolution by analyzing the slow changes in amino acid sequences of homologous proteins.

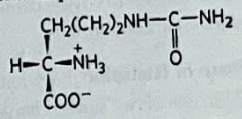
Key Terms

Terms in bold are defined in the glossary.

amino acids 75
residue 75
R group 76
chiral center 76
enantiomers 76
absolute configuration 78
D, L system 78
polarity 78
absorbance, *A* 80
zwitterion 81
isoelectric pH (isoelectric point, pI) 84
peptide 85
protein 85
peptide bond 85
oligopeptide 86
polypeptide 86
oligomeric protein 87
protomer 87
conjugated protein 89
prosthetic group 89
crude extract 89
fraction 89
fractionation 89
dialysis 90
column chromatography 90
ion-exchange chromatography 90
size-exclusion chromatography 92
affinity chromatography 92
high-performance liquid chromatography (HPLC) 92
electrophoresis 92
sodium dodecyl sulfate (SDS) 94
isoelectric focusing 94
specific activity 95
primary structure 97
secondary structure 97
tertiary structure 97
quaternary structure 97
Edman degradation 98
proteases 99
MALDI MS 101
ESI MS 101
consensus sequence 104
bioinformatics 105
horizontal gene transfer 106
homologous proteins 106
homologs 106
paralogs 106
orthologs 106
signature sequence 107

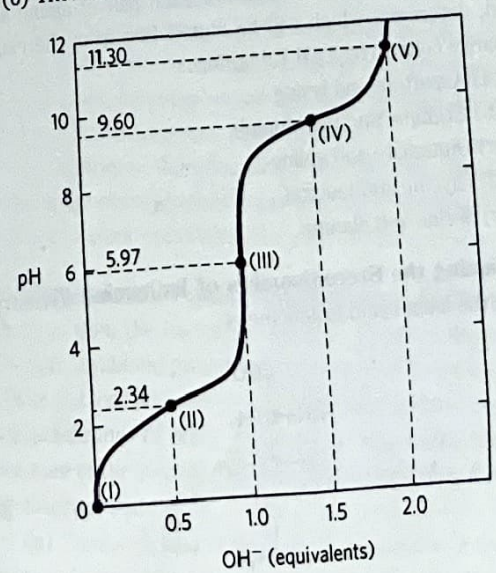
Problems

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

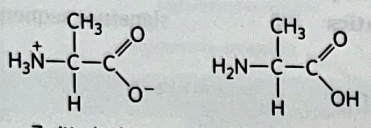


2. Relationship between the Titration Curve and the Acid-Base Properties of Glycine A 100 mL solution of 0.1 M glycine at pH 1.72 was titrated with 2 M NaOH solution. The pH was monitored and the results were plotted as shown in the graph. The key points in the titration are designated I to V. For each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

(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.



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.

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?

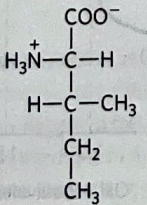
(b) Draw the structures of the predominant ionization state of histidine at pH 1, 4, 8, and 12. Note that the ionization state can be approximated by treating each ionizable group independently.

(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?

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) aggregation of nonpolar amino acid side chains with the hydrophobic backbone of the polystyrene resin. For each pair of amino acids listed, determine which will be eluted first from the cation-exchange column by a pH 7.0 buffer.

(a) Aspartate and lysine
(b) Arginine and methionine
(c) Glutamate and valine
(d) Glycine and leucine
(e) Serine and alanine

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.

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 oligopeptide?

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

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

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., assume there is only one Trp residue per protein molecule).

(b) Size-exclusion chromatography of BSA gives a molecular weight estimate of 70,000. How many Trp residues are present in a molecule of serum albumin?

10. Subunit Composition of a Protein A protein has a molecular mass of 400 kDa when measured by size-exclusion chromatography. 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.

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.

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?

13. 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?

14. Solubility of Polypeptides One method for separating polypeptides makes use of their different solubilities. The solubility of large polypeptides in water depends on 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 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

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?

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?

17. Peptide Purification At pH 7.0, in what order would the following three peptides (described by their amino acid composition) be eluted from a column filled with a cation-exchange polymer?

Peptide 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%.

Peptide 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%.

Peptide 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%.

18. Sequence Determination of the Brain Peptide Leucine Enkephalin A group of peptides that influence nerve transmission in certain parts of the brain have 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.

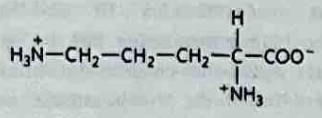
(b) Treatment of the peptide with 1-fluoro-2,4-dinitrobenzene followed by complete hydrolysis and chromatography indicated the presence of the 2,4-dinitrophenyl derivative of tyrosine. No free tyrosine could be found.

(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.

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

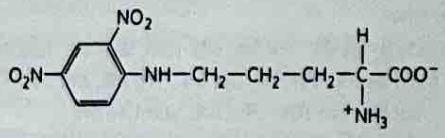
present in proteins but present in some peptides. It has the structure



(b) The molecular weight of the peptide was estimated as ~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: 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.

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.

21. Sequence Comparisons Proteins called molecular chaperones (described in Chapter 4) assist in the process of protein folding. One class of chaperones found in organisms from bacteria to mammals is heat shock protein 90 (Hsp90). All Hsp90 chaperones contain a 10 amino acid "signature sequence" that allows 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?

22. Chromatographic Methods Three polypeptides, the sequences of which are represented below using the one-letter code for their amino acids, are present in a mixture:

1. ATKNRASCLVPKHGALMFWRHKQLVSDPIL QKRQHILVCRNAAG
2. GPYFGDEPLDVHDEPEEG
3. PHLLSAWKGMEGVGKSQSFAALIVILA

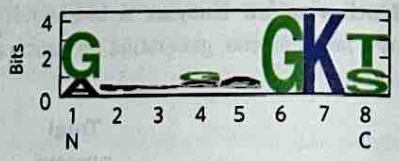
Of the three, which one would migrate most slowly during chromatography through:

(a) an ion-exchange resin, beads coated with positively charged groups?

(b) an ion-exchange resin, beads coated with negatively charged groups?

(c) a size-exclusion (gel-filtration) column designed to separate small peptides such as these?

(d) Which peptide contains the ATP-binding motif shown in the following sequence logo?

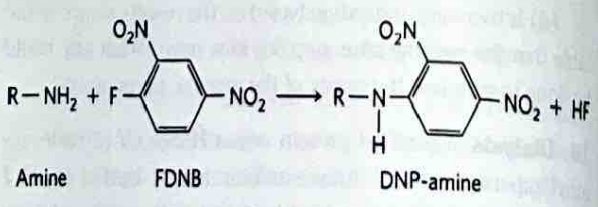


Data Analysis Problem

23. Determining the Amino Acid Sequence of Insulin Figure 3-24 shows the amino acid sequence of bovine 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's team 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 amide or guanidinium) 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 (see Fig. 3-28).

Paper Chromatography. This more primitive version of thin-layer chromatography (see Fig. 10-25) 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 bovine insulin (see 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 bovine 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:

$$\overset{1}{\text{Gly}}\text{–Ile–Val–Glx–Glx–Cys–Cys–Ala–Ser–}\overset{10}{\text{Val}}\text{–}$$
$$\text{Cys–Ser–Leu–Tyr–}\overset{15}{\text{Glx}}\text{–Leu–Glx–Asx–Tyr–}\overset{20}{\text{Cys}}\text{–Asx}$$

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.

References

Sanger, F. 1945. The free amino groups of insulin. *Biochem. J.* 39:507–515.

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

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

sheets (Fig. 2). The interaction of PrP^{Sc} with PrP^{C} converts the latter to PrP^{Sc}, initiating a domino effect in which more and more of the brain protein converts to the disease-causing form. The mechanism by which the presence of PrP^{Sc} leads to spongiform encephalopathy is not understood.

In inherited forms of prion diseases, a mutation in the gene encoding PrP produces a change in one amino acid residue that is believed to make the conversion of PrP^{C} to PrP^{Sc} more likely. A complete understanding of prion diseases awaits new information on how prion protein affects brain function. Structural information about PrP is beginning to provide insights into the molecular process that allows the prion proteins to interact so as to alter their conformation (Fig. 2). The significance of prions may extend well beyond spongiform encephalopathies. Evidence is building that prionlike proteins may be responsible for additional neurodegenerative diseases such as multiple system atrophy (MSA), a disease that resembles Parkinson disease.

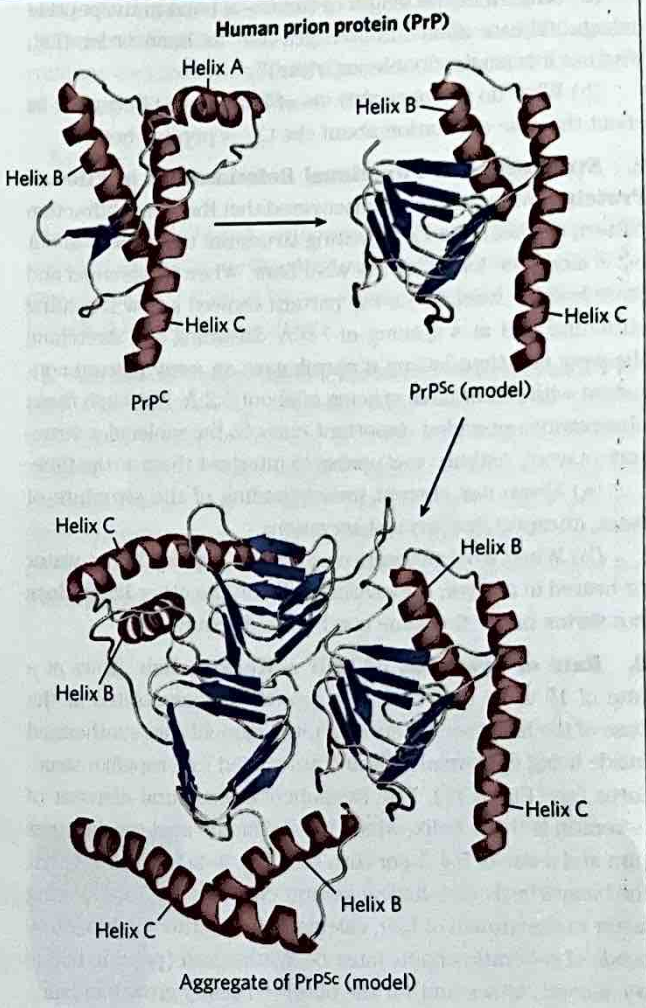


FIGURE 2 Structure of the globular domain of human PrP and models of the misfolded, disease-causing conformation PrP^{Sc}, and an aggregate of PrP^{Sc}. The α helices are labeled to help illustrate the conformational change. Helix A is incorporated into the β-sheet structure of the misfolded conformation. [Sources: Human PrP from PDB ID 1QLX, R. Zahn et al., *Proc. Natl. Acad. Sci. USA* 97:145, 2000. Models from C. Govaerts et al., *Proc. Natl. Acad. Sci. USA* 101:8342, 2004.]

- For many proteins, folding is facilitated by Hsp70 chaperones and by chaperonins. Disulfide-bond formation and the cis-trans isomerization of Pro peptide bonds are catalyzed by specific enzymes.
- Protein misfolding is the molecular basis of a wide range of human diseases, including the amyloidoses.

Key Terms

Terms in bold are defined in the glossary.

conformation 116
native conformation 116
hydrophobic effect 116
solvation layer 116
peptide group 118
Ramachandran plot 119
secondary structure 119
α helix 120
β conformation 123
β sheet 123
β turn 123
circular dichroism (CD) spectroscopy 125
tertiary structure 125
quaternary structure 125
fibrous proteins 125
globular proteins 125
α-keratin 126
collagen 127
silk fibroin 130
Protein Data Bank (PDB) 132
motif 133
fold 133
domain 137
intrinsically disordered proteins 138
topology diagram 140
protein family 141
multimer 141
oligomer 141
protomer 141
proteostasis 142
denaturation 143
renaturation 144
chaperone 146
Hsp70 146
chaperonin 146
protein disulfide isomerase (PDI) 147
peptide prolyl cis-trans isomerase (PPI) 147
amyloid 147
amyloidoses 147
autophagy 148
prion 150

Problems

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?

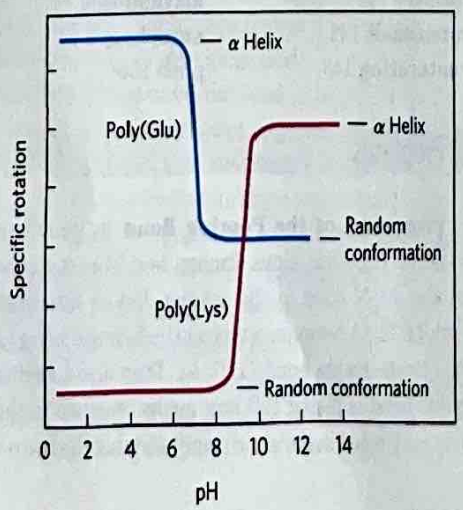
2. Structural and Functional Relationships in Fibrous Proteins William Astbury discovered that the x-ray diffraction pattern of wool shows a repeating structural unit spaced about 5.2 Å along the length of the wool fiber. When he steamed and stretched the wool, the x-ray pattern showed a new repeating 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.

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. 4-11). 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.

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 circularly polarized light. Polyglutamate, a polypeptide made up of only L-Glu residues, has the α-helix 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.



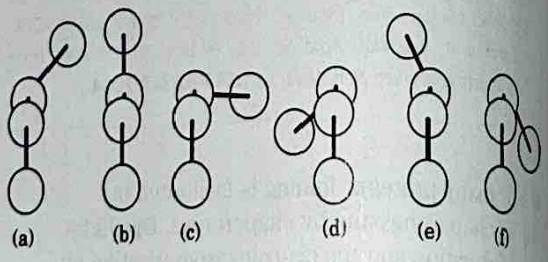
What is the explanation for the effect of the pH changes on the conformations of poly(Glu) and poly(Lys)? Why does the transition occur over such a narrow range of pH?

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?

6. Dihedral Angles A series of torsion angles, ϕ and ψ, that might be taken up by the peptide backbone is shown below. Which of these closely correspond to ϕ and ψ for an idealized collagen triple helix? Refer to Figure 4-9 as a guide.



7. 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.

```
 1   2   3   4   5   6   7   8   9  10
Ile-Ala-His-Thr-Tyr-Gly-Pro-Phe-Glu-Ala-

11  12  13  14  15  16  17  18  19  20
Ala-Met-Cys-Lys-Trp-Glu-Ala-Gln-Pro-Asp-

21  22  23  24  25  26  27  28
Gly-Met-Glu-Cys-Ala-Phe-His-Arg
```

(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 (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.)

8. Bacteriorhodopsin in Purple Membrane Proteins Under the proper environmental conditions, the salt-loving archaeon *Halobacterium halobium* synthesizes a membrane protein (M_r 26,000) known as bacteriorhodopsin, which is purple because it contains retinal (see Fig. 10-20). 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 acid residues 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.)

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

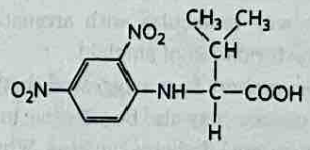
10. Interpreting Ramachandran Plots Examine the two proteins labeled (a) and (b) below. Which of the two Ramachandran plots, labeled (c) and (d), is more likely to be derived from which protein? Why? [Sources: (a) PDB ID 1GWY, J. M. Mancheno et al., *Structure* 11:1319, 2003. (b) PDB ID 1A6M, J. Vojtechovsky et al., *Biophys. J.* 77:2153, 1999.]

11. 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 in red:

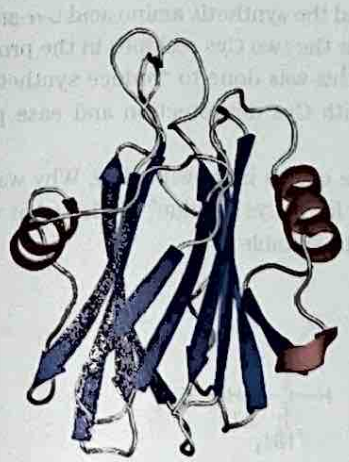
$$-\text{X}-\text{Gly}-\text{Pro}-\text{Y}- \xrightarrow{H_2O} -\text{X}-\text{COO}^- + \text{H}_3\overset{+}{\text{N}}-\text{Gly}-\text{Pro}-\text{Y}-$$

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?

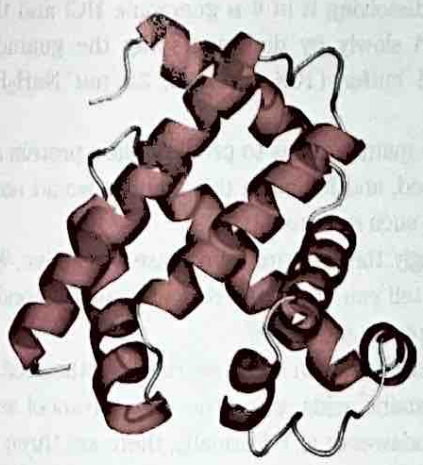
12. Number of Polypeptide Chains in a Multisubunit Protein A sample (660 mg) of an oligomeric protein of M_r 132,000 was treated with an excess of 1-fluoro-2,4-dinitrobenzene (Sanger's reagent) under slightly alkaline conditions until the chemical reaction was complete. The peptide bonds of the protein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



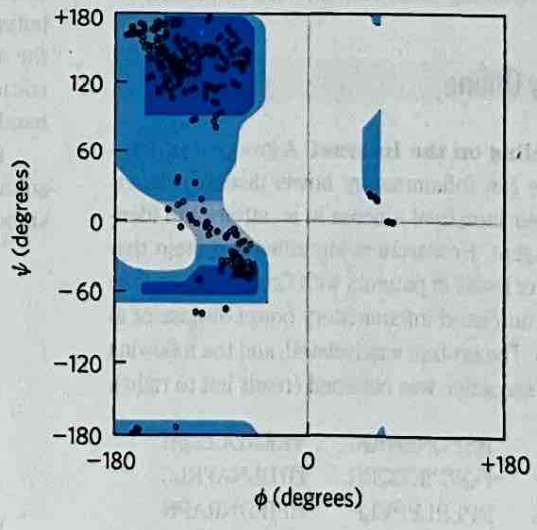
(a)



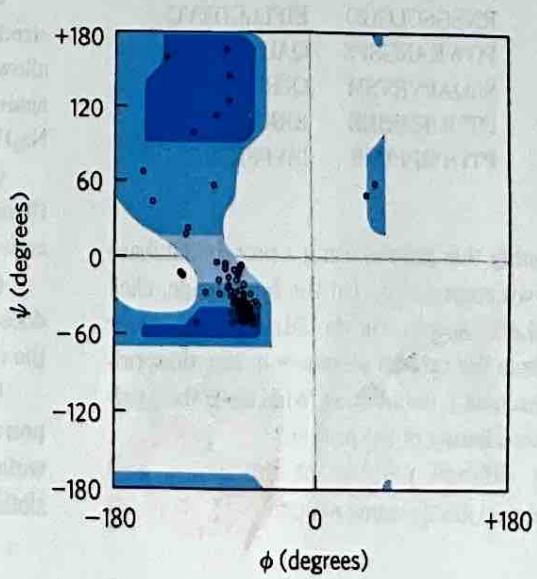
(b)



(c)



(d)



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 analytic technique could you employ to determine whether the polypeptide chains in this protein are similar or different?

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

(a) LKAENDEAARAMSEA

(b) CRAGGFPWDQPGTSN

14. Amyloid Fibers in Disease Several small aromatic molecules, such as phenol red (used as a nontoxic 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 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 disease may also be effective in treating type 2 (non-insulin-dependent) diabetes mellitus. Why might a single drug be effective in treating these two different conditions?

Biochemistry Online

15. Protein Modeling on the Internet A group of patients with Crohn 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 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 such as UniProt (www.uniprot.org). On the home page, click on the link for a BLAST search. On the BLAST page, 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.pdb.org) or Structural Classification of Proteins (SCOP2; http://scop2.mrc-lmb.cam.ac.uk).

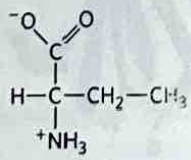
(d) In the course of your Web searches, what did you learn about the cellular function of the protein?

Data Analysis Problem

16. 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 preproteins 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 3-32. 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 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 NaH_2PO_4/Na_2HPO_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 be (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.

	Substrate hydrolysis		Inhibition		
			Peptide inhibitor		
HIV Protease	D-substrate	L-substrate	D-inhibitor	L-inhibitor	Evans blue (achiral)
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.

References

Milton, R.C., S.C. Milton, and S.B. Kent. 1992. Total chemical synthesis of a D-enzyme: the enantiomers of HIV-1 protease show demonstration of reciprocal chiral substrate specificity. *Science* 256:1445–1448.

Wlodawer, A., M. Miller, M. Jaskólski, B.K. Sathyanarayana, E. Baldwin, I.T. Weber, L.M. Selk, L. Clawson, J. Schneider, and S.B. Kent. 1989. Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease. *Science* 245:616–621.

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

Thin filaments also contain a large protein called **nebulin** (~7,000 amino acid residues), thought to be structured as an α helix that is long enough to span the length of the filament. The M line similarly organizes the thick filaments. It contains the proteins **paramyosin**, **C-protein**, and **M-protein**. Another class of proteins called **titins**, the largest single polypeptide chains discovered thus far (the titin of human cardiac muscle has 26,926 amino acid residues), link the thick filaments to the Z disk, providing additional organization to the overall structure. Among their structural functions, the proteins nebulin and titin are believed to act as "molecular rulers," regulating the length of the thin and thick filaments, respectively. Titin extends from the Z disk to the M line, regulating the length of the sarcomere itself and preventing overextension of the muscle. The characteristic sarcomere length varies from one muscle tissue to the next in a vertebrate, largely due to the different titin variants in the tissues.

Myosin Thick Filaments Slide along Actin Thin Filaments

The interaction between actin and myosin, like that between all proteins and ligands, involves weak bonds. When ATP is not bound to myosin, a face on the myosin head group binds tightly to actin **(Fig. 5-31)**. When ATP binds to myosin and is hydrolyzed to ADP and phosphate, a coordinated and cyclic series of conformational changes occurs in which myosin releases the F-actin subunit and binds another subunit farther along the thin filament.

The cycle has four major steps (Fig. 5-31). In step ❶, ATP binds to myosin and a cleft in the myosin molecule opens, disrupting the actin-myosin interaction so that the bound actin is released. ATP is then hydrolyzed in step ❷, causing a conformational change in the protein to a "high-energy" state that moves the myosin head and changes its orientation in relation to the actin thin filament. Myosin then binds weakly to an F-actin subunit closer to the Z disk than the one just released. As the phosphate product of ATP hydrolysis is released from myosin in step ❸, another conformational change occurs in which the myosin cleft closes, strengthening the myosin-actin binding. This is followed quickly by step ❹, a "power stroke" during which the conformation of the myosin head returns to the original resting state, its orientation relative to the bound actin changing so as to pull the tail of the myosin toward the Z disk. ADP is then released to complete the cycle. Each cycle generates about 3 to 4 pN (piconewtons) of force and moves the thick filament 5 to 10 nm relative to the thin filament.

Because there are many myosin heads in a thick filament, at any given moment some (probably 1% to 3%) are bound to thin filaments. This prevents thick filaments from slipping backward when an individual myosin head releases the actin subunit to which it was bound. The thick filament thus actively slides forward past the adjacent thin filaments. This process, coordinated among the many sarcomeres in a muscle fiber, brings about muscle contraction.

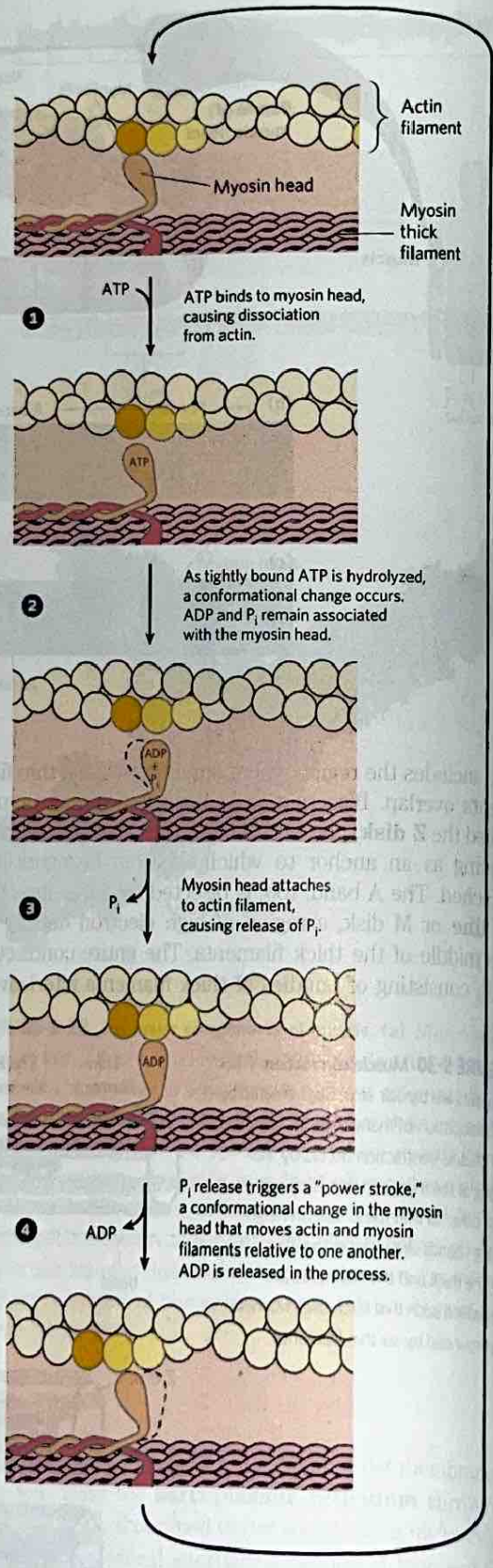


FIGURE 5-31 Molecular mechanism of muscle contraction. Conformational changes in the myosin head that are coupled to stages in the ATP hydrolytic cycle cause myosin to successively dissociate from one actin subunit, then associate with another farther along the actin filament. In this way, the myosin heads slide along the thin filaments, drawing the thick filament array into the thin filament array (see Fig. 5-30).

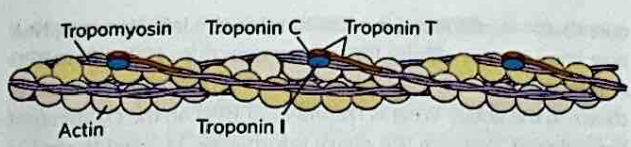


FIGURE 5-32 Regulation of muscle contraction by tropomyosin and troponin. Tropomyosin and troponin are bound to F-actin in the thin filaments. In the relaxed muscle, these two proteins are arranged around the actin filaments so as to block the binding sites for myosin. Tropomyosin is a two-stranded coiled coil of α helices, the same structural motif as in α-keratin (see Fig. 4-11). It forms head-to-tail polymers twisting around the two actin chains. Troponin is attached to the actin-tropomyosin complex at regular intervals of 38.5 nm. Troponin consists of three different subunits: I, C, and T. Troponin I prevents binding of the myosin head to actin; troponin C has a binding site for Ca^{2+}; and troponin T links the entire troponin complex to tropomyosin. When the muscle receives a neural signal to initiate contraction, Ca^{2+} is released from the sarcoplasmic reticulum (see Fig. 5-29a) and binds to troponin C. This causes a conformational change in troponin C, which alters the positions of troponin I and tropomyosin so as to relieve the inhibition by troponin I and allow muscle contraction.

The interaction between actin and myosin must be regulated so that contraction occurs only in response to appropriate signals from the nervous system. The regulation is mediated by a complex of two proteins, **tropomyosin** and **troponin (Fig. 5-32)**. Tropomyosin binds to the thin filament, blocking the attachment sites for the myosin head groups. Troponin is a Ca^{2+}-binding protein. A nerve impulse causes release of Ca^{2+} ions from the sarcoplasmic reticulum. The released Ca^{2+} binds to troponin (another protein-ligand interaction) and causes a conformational change in the tropomyosin-troponin complexes, exposing the myosin-binding sites on the thin filaments. Contraction follows.

Working skeletal muscle requires two types of molecular functions that are common in proteins—binding and catalysis. The actin-myosin interaction, a protein-ligand interaction like that of immunoglobulins with antigens, is reversible and leaves the participants unchanged. When ATP binds myosin, however, it is hydrolyzed to ADP and P_i. Myosin is not only an actin-binding protein, it is also an ATPase—an enzyme. The function of enzymes in catalyzing chemical transformations is the topic of the next chapter.

SUMMARY 5.3 Protein Interactions Modulated by Chemical Energy: Actin, Myosin, and Molecular Motors

■ Protein-ligand interactions achieve a special degree of spatial and temporal organization in motor proteins. Muscle contraction results from choreographed interactions between myosin and actin, coupled to the hydrolysis of ATP by myosin.

■ Myosin consists of two heavy and four light chains, forming a fibrous coiled coil (tail) domain and a globular (head) domain. Myosin molecules are organized into thick filaments, which slide past thin filaments composed largely of actin. ATP hydrolysis in myosin is coupled to a series of conformational changes in the myosin head, leading to dissociation of myosin from one F-actin subunit and its eventual reassociation with another, farther along the thin filament. The myosin thus slides along the actin filaments.

■ Muscle contraction is stimulated by the release of Ca^{2+} from the sarcoplasmic reticulum. The Ca^{2+} binds to the protein troponin, leading to a conformational change in a troponin-tropomyosin complex that triggers the cycle of actin-myosin interactions.

Key Terms

Terms in bold are defined in the glossary.

ligand 157
binding site 157
induced fit 157
hemoglobin 158
heme 158
porphyrin 158
heme protein 158
globins 159
equilibrium expression 160
association constant, K_a 160
dissociation constant, K_d 160
allosteric protein 166
modulator 166
Hill equation 167
Bohr effect 170
immune response 174
lymphocytes 174
antibody 174
immunoglobulin 174
B lymphocyte or **B cell** 174
T lymphocyte or **T cell** 174
antigen 175
epitope 175
hapten 175
immunoglobulin fold 176
polyclonal antibodies 177
monoclonal antibodies 177
ELISA 178
immunoblotting 178
Western blotting 178
myosin 179
actin 179
sarcomere 179

Problems

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.

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.

3. Hemoglobin's Affinity for Oxygen 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 breathing). (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 part per million (ppm) in a normal indoor atmosphere to 30 ppm in a home that has a malfunctioning or leaking furnace.

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

5. Reversible Ligand Binding II A binding protein binds to a ligand L with a K_d of 400 nM. What is the concentration of ligand when Y is (a) 0.25, (b) 0.6, (c) 0.95?

6. Reversible Ligand Binding III Three membrane receptor proteins bind tightly to a hormone. Based on the data in the table below, (a) what is the K_d for hormone binding by protein 2? (Include appropriate units.) (b) Which of these proteins binds *most* tightly to this hormone?

Hormone concentration (nM)	Y		
	Protein 1	Protein 2	Protein 3
0.2	0.048	0.29	0.17
0.5	0.11	0.5	0.33
1	0.2	0.67	0.5
4	0.5	0.89	0.8
10	0.71	0.95	0.91
20	0.83	0.97	0.95
50	0.93	0.99	0.98

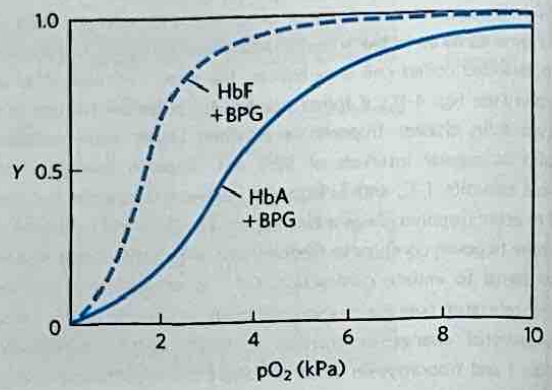
7. Cooperativity in Hemoglobin Under appropriate conditions, hemoglobin dissociates into its four subunits. The isolated α subunit binds oxygen, but the O_2-saturation curve is hyperbolic rather than sigmoid. In addition, the binding of oxygen to the isolated α subunit is not affected by the presence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

8. 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?



9. 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.

10. 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.

11. 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 Y be (a) 0.2, (b) 0.5, (c) 0.6, (d) 0.8?

12. 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?

13. 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 person, 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.

14. How We Become a "Stiff" When a vertebrate dies, its muscles stiffen as they are deprived of ATP, a state called rigor mortis. Using your knowledge of the catalytic cycle of myosin in muscle contraction, explain the molecular basis of the rigor state.

15. 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).

Biochemistry Online

16. 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 such as JSmol, a free and user-friendly molecular viewer that is compatible with most browsers and operating systems.

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.pdb.org), and view the structure using JSmol or a similar viewer.

(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?

17. Exploring Antibodies in the Protein Data Bank Use the PDB Molecule of the Month article at www.rcsb.org/pdb/101/motm.do?momID=21 to complete the following exercises.

(a) How many specific antigen-binding sites are there on the first immunoglobulin image on the Web page (image derived from PDB ID 1IGT)?

(b) When a virus enters your lungs, how long does it take for you to produce one or more antibodies that bind to it?

(c) Approximately how many types of different antibodies are present in your blood?

(d) Explore the structure of the immunoglobulin molecule (PDB ID 1IGT) on the Web page by clicking the link in the article or by going directly to www.rcsb.org/pdb/explore/explore.do?structureId=1igt. Use one of the structure viewers provided on the PDB site to create a ribbon structure for this immunoglobulin. Identify the two light chains and two heavy chains, and give them different colors.

Data Analysis Problem

18. 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 bound to actin and hydrolyzed 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 were performed but provided no conclusive evidence. Then, 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 long 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.

(a) In an experiment, [X] = 6 mM, and V_0 = 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 [X] 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 (a), but a different [X], an amount of Z is added that reduces V_0 to 240 nM min^{-1}. What is the [X] 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.

13. 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)
2.5×10^{-6}	28
4.0×10^{-6}	40
1×10^{-5}	70
2×10^{-5}	95
4×10^{-5}	112
1×10^{-4}	128
2×10^{-3}	139
1×10^{-2}	140

14. 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^{-1})	Rate of formation of PGG_2 with 10 mg/mL ibuprofen (mM min^{-1})
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.

15. 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:

$$\text{Glycylglycine} + H_2O \longrightarrow 2\,\text{glycine}$$

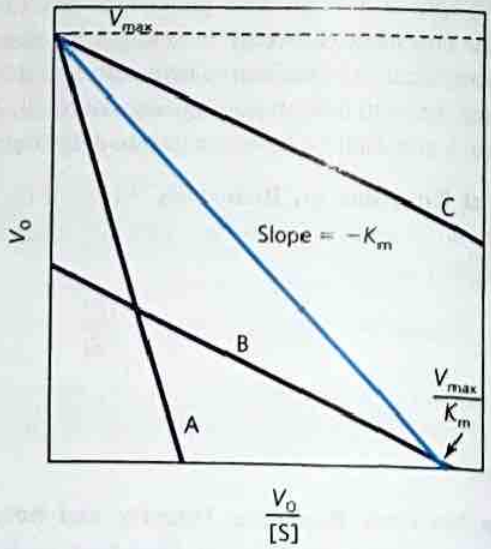
[S] (mM)	Product formed (μmol/min^{-1})
1.5	0.21
2.0	0.24
3.0	0.28
4.0	0.33
8.0	0.40
16.0	0.45

Use graphical analysis (see Box 6-1) to determine the V_{max} and K_m for this enzyme preparation and substrate.

16. The Eadie-Hofstee Equation There are several ways to transform the Michaelis-Menten equation so as to plot data and derive kinetic parameters, each with different advantages depending on the data set being analyzed. 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 versus $V_0/[S]$ for an enzyme-catalyzed reaction is shown below. The blue curve was obtained in the absence of inhibitor. Which of the other curves (A, B, or C) shows the enzyme activity when a competitive inhibitor was added to the reaction mixture? Hint: See Equation 6-30.



17. The Turnover Number of Carbonic Anhydrase Carbonic anhydrase of erythrocytes (M_r 30,000) has one of the

highest turnover numbers known. 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})?

18. 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

$$[E_t] = [E] + [ES] + [EI]$$

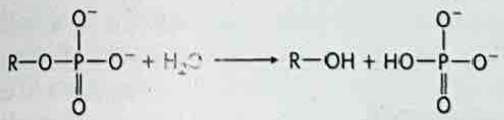
and the definitions of α and K_I provided in the text, derive the rate equation above. Use the derivation of the Michaelis-Menten equation as a guide.

19. 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:

$$\text{Enz-SH} + Ag^+ \longrightarrow \text{Enz–S–Ag} + H^+$$

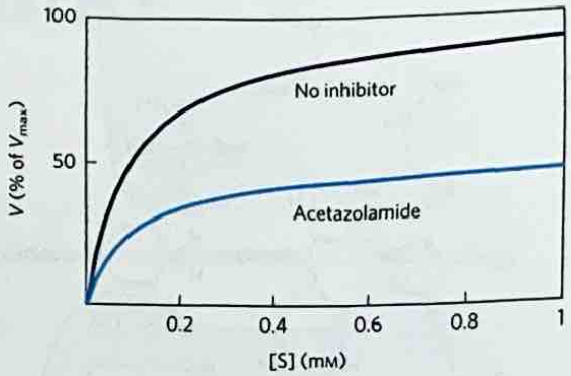
The affinity of Ag^+ for sulfhydryl groups is so great that Ag^+ can be used to titrate —SH groups quantitatively. To 10.0 mL of a solution containing 1.0 mg/mL of a pure enzyme, an investigator added just enough $AgNO_3$ to completely inactivate the enzyme. A total of 0.342 μmol of $AgNO_3$ was required. Calculate the minimum molecular weight of the enzyme. Why does the value obtained in this way give only the *minimum* molecular weight?

20. 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):



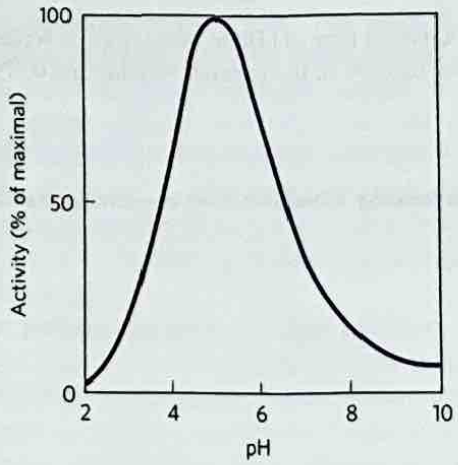
Acid phosphatases are produced by erythrocytes and by the liver, kidney, spleen, and prostate gland. The enzyme of the prostate gland is clinically important, because its increased activity in the blood can be an indication of prostate cancer. The phosphatase from the prostate gland is strongly inhibited 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?

21. 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.



22. 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 $V_0 = V_{max}/2\alpha'$.

23. 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?



Data Analysis Problem

24. Exploring and Engineering Lactate Dehydrogenase Examining the structure of an enzyme can lead to 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