Problems

Answers to all problems are at the end of this book. Detailed solutions are available in the *Student Solutions Manual.*

1. **Determining the Subunit Organization of a Protein From Its Molecular Weight and Metal Content** The element molybdenum (atomic weight 95.95) constitutes 0.08% of the weight of nitrate reductase. If the molecular weight of nitrate reductase is 240,000, what is its likely quaternary structure?

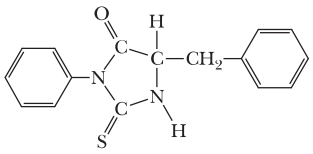
2. **Solving the Sequence of an Oligopeptide from Sequence Analysis Data** Amino acid analysis of an oligopeptide seven residues long gave

   Asp Leu Lys Met Phe Tyr

   The following facts were observed:

   a. Trypsin treatment had no apparent effect.

   b. The phenylthiohydantoin released by Edman degradation was

   c. Brief chymotrypsin treatment yielded several products, including a dipeptide and a tetrapeptide. The amino acid composition of the tetrapeptide was Leu, Lys, and Met.

   d. Cyanogen bromide treatment yielded a dipeptide, a tetrapeptide, and free Lys.

   What is the amino acid sequence of this heptapeptide?

3. **Solving the Sequence of an Oligopeptide from Sequence Analysis Data** Amino acid analysis of a decapeptide revealed the presence of the following products:

   NH_4^+ Asp Glu Tyr Arg
   Met Pro Lys Ser Phe

   The following facts were observed:

   a. Neither carboxypeptidase A nor B treatment of the decapeptide had any effect.

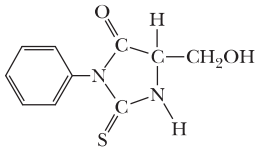
   b. Trypsin treatment yielded two tetrapeptides and free Lys.

   c. Clostripain treatment yielded a tetrapeptide and a hexapeptide.

   d. Cyanogen bromide treatment yielded an octapeptide and a dipeptide of sequence NP (using the one-letter codes).

   e. Chymotrypsin treatment yielded two tripeptides and a tetrapeptide. The N-terminal chymotryptic peptide had a net charge of −1 at neutral pH and a net charge of −3 at pH 12.

   f. One cycle of Edman degradation gave the PTH derivative

   What is the amino acid sequence of this decapeptide?

4. **Getting Insights into a Protein's Properties from Its Behavior during Purification** A protein mixture consisting of proteins A, B, and C was subjected to various protein purification steps. First, the protein mixture was applied to an ion exchange column having anionic groups. When the column was washed with an increasing concentration gradient of chloride ions, the order of elution of the three proteins was B, then C, and finally, A. Next, the protein mixture was chromatographed on a gel filtration column. The order of elution of the three proteins from this column was C, A, B. Finally, the protein mixture was passed over a chromatography column containing a flavin affinity matrix. Only protein A was retained by the column. What can you infer about the relative properties of proteins A, B, and C?

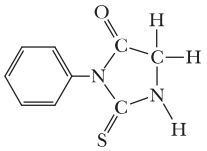
5. **N-Terminal Analysis** A protein was purified and confirmed to consist of only a singlemolecule with a single molecular mass. Yet N-terminal analyses resulted in the identification of two different amino acids. Give a possible explanation.

6. **Solving the Sequence of an Oligopeptide from Sequence Analysis Data** Amino acid analysis of an octapeptide revealed the following composition:

   2 Arg 1 Gly 1 Met 1 Trp 1 Tyr 1 Phe 1 Lys

   The following facts were observed:

   a. Edman degradation gave

   b. CNBr treatment yielded a pentapeptide and a tripeptide containing phenylalanine.

   c. Chymotrypsin treatment yielded a tetrapeptide containing a C-terminal indole amino acid and two dipeptides.

   d. Trypsin treatment yielded a tetrapeptide, a dipeptide, and free Lys and Phe.

   e. Clostripain yielded a pentapeptide, a dipeptide, and free Phe.

   What is the amino acid sequence of this octapeptide?

7. **Solving the Sequence of an Oligopeptide from Sequence Analysis Data** Amino acid analysis of an octapeptide gave the following results:

   1 Ala 1 Arg 1 Asp 1 Gly 3 Ile 1 Val 1 NH_4^+

   The following facts were observed:

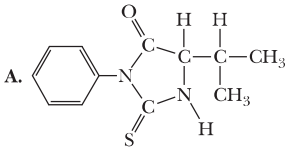
   a. Trypsin treatment yielded a pentapeptide and a tripeptide.

   b. Chemical reduction of the free α-COOH and subsequent acid hydrolysis yielded 2-aminopropanol.

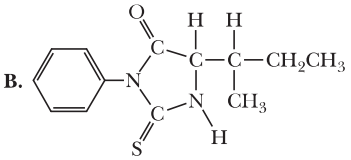
   c. Partial acid hydrolysis of the tryptic pentapeptide yielded, among other products, two dipeptides, each of which contained C-terminal isoleucine. One of these dipeptides migrated as an anionic species upon electrophoresis at neutral pH.

   d. The tryptic tripeptide was degraded in an Edman sequenator, yielding first **A**, then **B**:

   A.

   B.

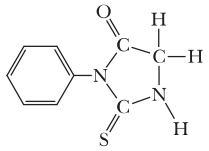
   

   What is an amino acid sequence of the octapeptide? Four sequences are possible, but only one suits the authors. Why?

8. **Solving the Sequence of an Oligopeptide from Sequence Analysis Data** An octapeptide consisting of 2 Gly, 1 Lys, 1 Met, 1 Pro, 1 Arg,

1 Trp, and 1 Tyr was subjected to sequence studies. The following was found:

a. Edman degradation yielded



b. Upon treatment with carboxypeptidases A, B, and Y, only carboxypeptidase Y had any effect.

c. Trypsin treatment gave two tripeptides and a dipeptide.

d. Chymotrypsin treatment gave two tripeptides and a dipeptide. Acid hydrolysis of the dipeptide yielded only Gly.

e. Cyanogen bromide treatment yielded two tetrapeptides.

f. Clostripain treatment gave a pentapeptide and a tripeptide.

What is the amino acid sequence of this octapeptide?

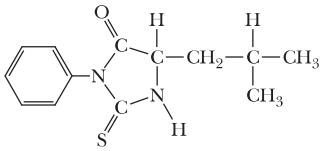
9. Solving the Sequence of an Oligopeptide from Sequence Analysis Data Amino acid analysis of an oligopeptide containing nine residues revealed the presence of the following amino acids:

Arg Cys Gly Leu Met Pro Tyr Val

The following was found:

a. Carboxypeptidase A treatment yielded no free amino acid.

b. Edman analysis of the intact oligopeptide released



c. Neither trypsin nor chymotrypsin treatment of the nonapeptide released smaller fragments. However, combined trypsin and chymotrypsin treatment liberated free Arg.

d. CNBr treatment of the eight-residue fragment left after combined trypsin and chymotrypsin action yielded a six-residue fragment containing Cys, Gly, Pro, Tyr, and Val and a dipeptide.

e. Treatment of the six-residue fragment with β-mercaptoethanol yielded two tripeptides. Brief Edman analysis of the tripeptide mixture yielded only PTH-Cys. (The sequence of each tripeptide, as read from the N-terminal end, is alphabetical if the one-letter designation for amino acids is used.)

What is the amino acid sequence of this nonapeptide?

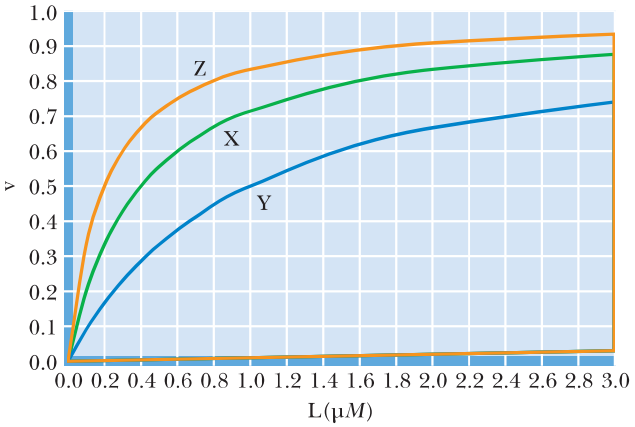
10. Describe the Solid-Phase Chemical Synthesis of a Small Peptide Describe the synthesis of the dipeptide Lys-Ala by Merrifield's solid-phase chemical method of peptide synthesis. What pitfalls might be encountered if you attempted to add a leucine residue to Lys-Ala to make a tripeptide?

11. Identify Proteins Using BLAST Searches of Peptide Fragment Sequences Go to the National Center for Biotechnology Information website at *http://www.ncbi.nlm.nih.gov/*. From the menu of Popular Resources on the right-hand side, click on "BLAST." Under the Basic BLAST heading on the new page that comes up, click on "protein blast." In the Enter Query Sequence box at the top of the page that comes up, enter the following sequence: NQMMKSRNLTKDRCKP. Confirm that the database under "Choose Search Set" is set on "nr" (nonredundant protein sequences), then click the BLAST button at the bottom of the page to see the results of your search. Next, enter this sequence from a different protein: SLQTASAPDVYAIGECA. Identify the proteins from which these sequences were derived.

12. Calculate the Mass of a Protein from Mass Spectrometric *m/z* Values Electrospray ionization mass spectrometry (ESI-MS) of the polypeptide chain of myoglobin yielded a series of *m/z* peaks (similar to those shown in Figure 5.14 for aerolysin K). Two successive peaks had *m/z* values of 1304.7 and 1413.2, respectively. Calculate the mass of the myoglobin polypeptide chain from these data.

13. Phosphorylation of Proteins Introduces New Properties Phosphoproteins are formed when a phosphate group is esterified to an —OH group of a Ser, Thr, or Tyr side chain. At typical cellular pH values, this phosphate group bears two negative charges ($-OPO_3^{2-}$). Compare this side-chain modification with the 20 side chains of the common amino acids found in proteins and comment on the novel properties that it introduces into side-chain possibilities.

14. Binding of Ligands to a Protein A pharmaceutical company studied the binding of three different compounds, X, Y and Z, to a particular protein of interest. For each compound, the fractional saturation (ν; see Section 5.7b) was determined as a function of concentration. The results are shown in the figure. Which of the three compounds has the strongest affinity for the protein? Explain.



15. Using Graphical Analysis to Determine the K_d for the Interaction between a Protein and Its Ligand A quantitative study of the interaction of a protein with its ligand yielded the following results:

Ligand concentration (mM)	1	2	3	4	5	6	9	12
ν (moles of ligand bound per mole of protein)	0.28	0.45	0.56	0.60	0.71	0.75	0.79	0.83

Plot a graph of [L] versus ν. Determine K_D, the dissociation constant for the interaction between the protein and its ligand, from the graph.

16. A protein can bind either of two different ligands, A and B. The protein's K_D for A is 0.3 mM. The protein's K_D for B is 1 mM. Calculate % saturation of the protein with A at concentrations of ligand equal to 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, and 2 mM. Repeat the calculations for ligand B at these concentrations. Describe the relationship between K_D and relative affinity of the protein for ligands A and B.

Biochemistry on the Web

17. Exploring the ExPASy Proteomics Website The human insulin receptor substrate-1 (IRS-1) is designated protein P35568 in the protein knowledge base on the ExPASy Website. Log onto https://www.expasy.org. Type "peptide mass tool" in the search box and click

"Enter" on your computer. In the results page that displays, scroll down and click on the "PeptideMass" resource. Then click on "Browse the resource website." In the empty "Search" box, enter "P35568" as the accession number and click the "perform" button to see the results of trypsin digestion of IRS-1. How many amino acids does IRS-1 have? What is the average molecular mass of IRS-1? What is the amino acid sequence of the tryptic peptide of IRS-1 that has a mass of 1741.9629?

18. **Write a General Chemical Mechanism for Protease Reactions** Proteases such as trypsin and chymotrypsin cleave proteins at different sites, but both use the same reaction mechanism. Based on your knowledge of organic chemistry, suggest a "universal" protease reaction mechanism for hydrolysis of the peptide bond.

19. **Hemoglobin Mutations and Their Effects on Hb Structure and Function** Hundreds of mutations have been described in the genes encoding the α- and β-globin subunits of hemoglobin.
    a. Some of these mutations affect subunit interactions between the subunits. In an examination of the tertiary structure of globin chains, where would you expect to find amino acid changes in mutant globins that affect formation of the hemoglobin $\alpha_2\beta_2$ quaternary structure?
    b. Other mutations, such as the S form of the β-globin chain, increase the tendency of hemoglobin tetramers to polymerize into very large structures. Where might you expect the amino acid substitutions to be in these mutants?

20. **S Values** The ribosomal complex is often subdivided into the so-called 50S and 30S subunits.
    a. What do those "50S" and "30S" terms refer to?
    b. What is the reason for these different "S" values?

Think-Pair-Share Question

Evaluation of Peptide Degradation Consider the peptide shown.

QVDGLMRTSEQMKNSRV

You are asked to apply three digestion methods sequentially using trypsin, chymotrypsin, and staphylococcal protease as the endopeptidases. In between each digestion, you are asked to perform an Edman degradation analysis of the N-terminal amino acids of the fragments generated. You were not told in which order the three endopeptidases are to be applied. There are six possible orders in which the three endopeptidases could be applied.

	First	Second	Third
Order 1	Trypsin	Chymotrypsin	St. protease
Order 2	Trypsin	St. protease	Chymotrypsin
Order 3	Chymotrypsin	St. protease	Trypsin
Order 4	Chymotrypsin	Trypsin	St. protease
Order 5	St. protease	Trypsin	Chymotrypsin
Order 6	St. protease	Chymotrypsin	Trypsin

Select at least three digestion orders from the six shown; another student can select the remaining three. For each three-step digestion, determine the fragments that would be generated and the results one would obtain from the Edman degradation in between each digestion. Discuss whether the order of endopeptidase treatment leads to different end results (the results for the final Edman degradation reaction).

Further Reading

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Buxbaum, E., 2015. *Fundamentals of Protein Structure and Function*. Cham, Switzerland: Springer International Publishing.

Lesk, A. M., 2010. *Introduction to Protein Science: Architecture, Function, and Genomics*, 2nd ed. Oxford: Oxford University Press.

Orengo, C., and Bateman, A., 2014. *Protein Families: Relating Protein Sequence, Structure, and Function*. Hoboken, NJ: John Wiley & Sons, Inc.

Petsko, G. A., and Ringe, D., 2004. *Protein Structure and Function*. Sunderland, MA: Sinauer Associates.

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Karger, B. L., and Hancock, W. S., eds. 1996. High resolution separation and analysis of biological macromolecules. Part A: Fundamentals. In *Methods in Enzymology*, volume 270. New York: Academic Press. Separate sections discussing liquid chromatography, columns and instrumentation, electrophoresis, capillary electrophoresis, and mass spectrometry.

Linn, S., 2009. Strategies and considerations for protein purifications. *Methods in Enzymology* **463**:9–19. Volume 463 of *Methods in Enzymology* covers many aspects of protein purification. This reference and the next are but two of the relevant chapters.

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von Heijne, G., 1987. *Sequence Analysis in Molecular Biology: Treasure Trove or Trivial Pursuit*? San Diego: Academic Press.

Mass Spectrometry

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Burlingame, A. L., ed., 2005. Biological mass spectrometry. In *Methods in Enzymology* **405**. New York: Academic Press.

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Kim, M. S., Pinto, S. M., Getnet, D., et al., 2014. A draft map of the human proteome. *Nature* **509**:575–581. DOI:10.1038/nature13302.

Kinter, M., and Sherman, N. E., 2001. *Protein Sequencing and Identification Using Tandem Mass Spectrometry*. Hoboken, NJ: Wiley-Interscience.

Liebler, D. C., 2002. *Introduction to Proteomics*. Towata, NJ: Humana Press. An excellent primer on proteomics, protein purification methods, sequencing of peptides and proteins by mass spectrometry, and identification of proteins in a complex mixture.

Mann, M., and Wilm, M., 1995. Electrospray mass spectrometry for protein characterization. *Trends in Biochemical Sciences* **20**:219–224.

Beakers E and F:

$$2 \text{ cyto c } (Fe^{2+}) + 2H^+ + \tfrac{1}{2} O_2 \rightleftharpoons 2 \text{ cyto c } (Fe^{3+}) + H_2O$$
$$\Delta \mathscr{E}_0' = 0.816V - (0.254V) = 0.562V$$

c. Overall reaction

$$NADH + H^+ + \tfrac{1}{2} O_2 \rightleftharpoons NAD^+ + H_2O$$
$$\Delta \mathscr{E}_0' = 0.816V - (-0.32V) = 1.136V$$

Chapter 4

1. Structures for glycine, aspartate, leucine, isoleucine, methionine, and threonine are presented in Figure 4.3.
2. Asparagine = Asn = N.

   Arginine = Arg = R.

   Cysteine = Cys = C.

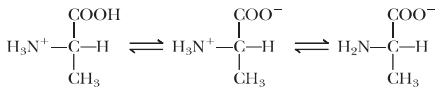
   Lysine = Lys = K.

   Proline = Pro = P.

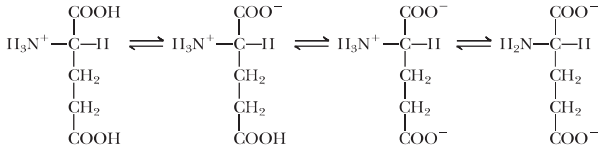
   Tyrosine = Tyr = Y.

   Tryptophan = Trp = W.
3. LDPTY and MHQAT
4. a. The structure is not a zwitterion as it has a surplus of positive charges.

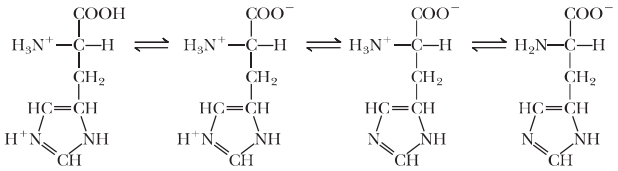
   b. Increasing the pH could remove a proton from one of the N-containing functional groups and balance the positive and negative charges within the structure.
5. **Alanine dissociation:**



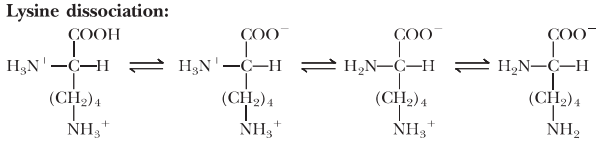
Glutamate dissociation:



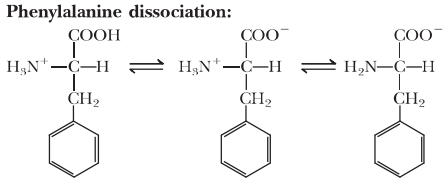
Histidine dissociation:



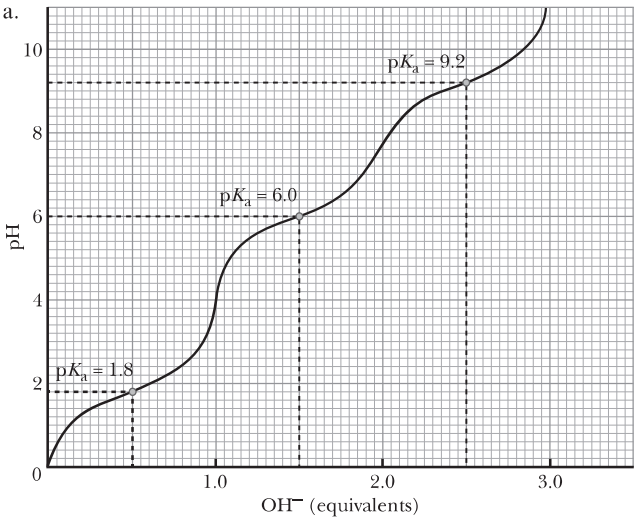
Lysine dissociation:



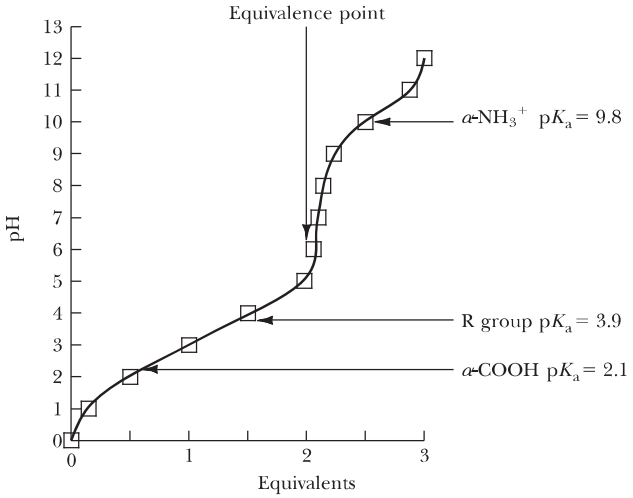
Phenylalanine dissociation:



6. The proximity of the α-carboxyl group lowers the pK_a of the α-amino group.
7. a.



   b. Ratio = 25.1
8.



9. Denoting the four histidine species as His^{2+}, His^+, His^0, and His^-, the concentrations are:

   pH 2: $[His^{2+}] = 0.097\ M$, $[His^+] = 0.153\ M$, $[His^0] = 1.53 \times 10^{-5}\ M$, $[His^-] = 9.6 \times 10^{-13}\ M$.

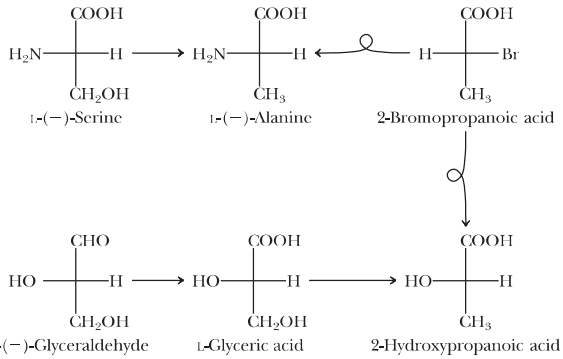
   pH 6.4: $[His^{2+}] = 1.78 \times 10^{-4}\ M$, $[His^+] = 0.071\ M$, $[His^0] = 0.179\ M$, $[His^-] = 2.8 \times 10^{-4}\ M$.

   pH 9.3: $[His^{2+}] = 1.75 \times 10^{-12}\ M$, $[His^+] = 5.5 \times 10^{-5}\ M$, $[His^0] = 0.111\ M$, $[His^-] = 0.139\ M$.
10. pH = pK_a + log (2/1) = 4.3 + 0.3 = 4.6.

    The γ-carboxyl group of glutamic acid is two-thirds dissociated at pH = 4.6.
11. pH = pK_a + log (1/4) = 10.5 + (−0.6) = 9.9.
12. a. The pH of a 0.3 *M* leucine hydrochloride solution is approximately 1.46.

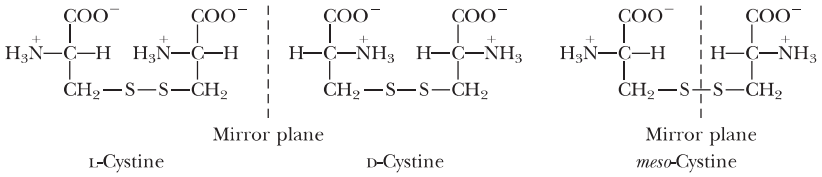
    b. The pH of a 0.3 *M* sodium leucinate solution is approximately 11.5.

    c. The pH of a 0.3 *M* solution of isoelectric leucine is approximately 6.05.
13. The sequence of reactions shown would demonstrate that L(−)-serine is related stereochemically to L(−)-glyceraldehyde:



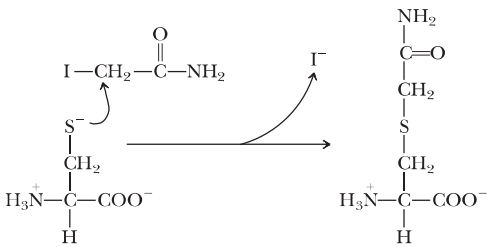
Straight arrows indicate reactions that occur with retention of configuration. Looped arrows indicate inversion of configuration. (*From Kopple, K. D., 1966*. Peptides and Amino Acids. *New York: Benjamin Co.*)

14. Cystine (disulfide-linked cysteine) has two chiral carbons, the two α-carbons of the cysteine moieties. Each chiral center can exist in two forms, so there are four stereoisomers of cystine. However, it is impossible to distinguish the difference between L-cysteine/D-cysteine and D-cysteine/L-cysteine conjugates. So three distinct isomers are formed:



15. 43

16.



17. There are eight Tyr residues in the protein.

18. A water is removed from each amino acid when it is incorporated into a protein, so the "molecular weight" of a residue is lower by 18 units. Also, most proteins have relatively more small side chains (Gly, Ala) and fewer Trp side chains than a statistical average would predict.

19. Aspartame is composed of aspartic acid and phenylalanine, with a carboxymethyl cap. These amino acids are linked by a peptide (amide) bond in aspartame. Heating can cleave amide linkages. For this reason, drinks such as coffee and hot chocolate must be consumed relatively quickly after preparation. Aspartame kept hot for several hours is quite bitter tasting (based on the experience of one of the authors).

20. Phenylketonuria is an autosomal-recessive genetic disease caused by a deficiency or absence of the enzyme phenylalanine hydroxylase (PAH), an essential enzyme that converts phenylalanine to tyrosine. Without sufficient PAH activity, phenylalanine accumulates and is converted to phenylpyruvate (which can be detected in the urine). Without treatment, phenylketonurics eventually experience progressive mental retardation and seizures. Phenylketonuria can be controlled by eliminating phenylalanine from the diet, and phenylketonurics should be advised not to use aspartame.

21. The process for distinguishing *R*- and *S*-configurations of chiral molecules is described in the Critical Developments in Biochemistry box "Rules for Description of Chiral Centers in the (R,S) System" in Section 4.4. Enzymes discriminate between isomers of chiral molecules thanks to the asymmetric arrangement of amino acid residues in the enzyme active site.

22. Appropriate ranges for buffering:
Alanine—1.4–3.4, 8.7–10.7
Histidine—0.8–2.8, 5.0–7.0, 8.2–10.2
Aspartic acid—1.1–4.9, 8.8–10.8
Lysine—1.2–3.2, 8–11.5

23. This exercise is left to the student and will presumably be different for every student.

24. M. A. Rosanoff, an instructor at New York University, proposed the D,L-nomenclature system for chiral molecules.

25. See "A Deeper Look - Why Nature Chose Selenium" on page 82 for a full explanation.

26. This exercise is left to the student and will presumably be different for every student.

27. With pK_a of 8.3, cysteine would make a useful buffer except that cystine, the disulfide of cysteine, can form readily in this pH range. For every cystine formed, two cysteine-SH groups are eliminated, making the buffering capacity of cysteine of limited usefulness. Also, the cysteine sulfhydryl group is the most potent nucleophile among the side chains of the 20 common amino acids.

28. L-threonine is (2*S*, 3*R*)-threonine.
D-threonine is (2*R*, 3*S*)-threonine.
L-allothreonine is (2*S*, 3*S*)-threonine.
D-allothreonine is (2*R*, 3*R*)-threonine.

Think-Pair-Share Question:

PHE	3.82	3.83
ASP	1.08	1.11
PRO	2.78	2.79
MET	3.46	3.47
LYS	4.46	4.47
SER	2.29	2.31
ALA	2.89	2.91
VAL	3.33	3.35
LEU	3.67	3.68