Chapter 3 Amino Acids, Peptides, and Proteins

Multiple Choice Questions

1. Amino acids
Page: 76 Difficulty: 1 Ans: C
The chirality of an amino acid results from the fact that its α carbon:

A) has no net charge.
B) is a carboxylic acid.
C) is bonded to four different chemical groups.
D) is in the L absolute configuration in naturally occurring proteins.
E) is symmetric.

2. Amino acids
Page: 76 Difficulty: 2 Ans: B
Of the 20 standard amino acids, only _____ is not optically active. The reason is that its side chain _____.

A) alanine; is a simple methyl group
B) glycine; is a hydrogen atom
C) glycine; is unbranched
D) lysine; contains only nitrogen
E) proline; forms a covalent bond with the amino group

3. Amino acids
Page: 79 Difficulty: 1 Ans: C
Two amino acids of the standard 20 contain sulfur atoms. They are:

A) cysteine and serine.
B) cysteine and threonine.
C) methionine and cysteine
D) methionine and serine
E) threonine and serine.

4. Amino acids
Page: 79 Difficulty: 1 Ans: A
All of the amino acids that are found in proteins, except for proline, contain a(n):

A) amino group.
B) carbonyl group.
C) carboxyl group.
D) ester group.
E) thiol group.

5. Amino acids
Pages: 79–80 Difficulty: 3 Ans: C
Which of the following statements about aromatic amino acids is correct?

A) All are strongly hydrophilic.
B) Histidine's ring structure results in its being categorized as aromatic or basic, depending on pH.
C) On a molar basis, tryptophan absorbs more ultraviolet light than tyrosine.
D) The major contribution to the characteristic absorption of light at 280 nm by proteins is the phenylalanine R group.
E) The presence of a ring structure in its R group determines whether or not an amino acid is aromatic.

6. Amino acids
Page: 80 Difficulty: 2 Ans: A
Which of the following statements about *cystine* is correct?

A) Cystine forms when the —CH_2—SH R group is oxidized to form a —CH_2—S—S—CH_2— disulfide bridge between two cysteines.
B) Cystine is an example of a nonstandard amino acid, derived by linking two standard amino acids.
C) Cystine is formed by the oxidation of the carboxylic acid group on cysteine.
D) Cystine is formed through a peptide linkage between two cysteines.
E) Two cystines are released when a —CH_2—S—S—CH_2— disulfide bridge is reduced to —CH_2—SH.

7. Amino acids
Page: 80 Difficulty: 2 Ans: A
The uncommon amino acid selenocysteine has an R group with the structure —CH_2—SeH (p$K_a \approx 5$). In an aqueous solution, pH = 7.0, selenocysteine would:

A) be a fully ionized zwitterion with no net charge.
B) be found in proteins as D-selenocysteine.
C) never be found in a protein.
D) be nonionic.
E) not be optically active.

8. Amino acids
Page: 81 Difficulty: 1 Ans: A
Amino acids are ampholytes because they can function as either a(n):

A) acid or a base.
B) neutral molecule or an ion.
C) polar or a nonpolar molecule.
D) standard or a nonstandard monomer in proteins.
E) transparent or a light-absorbing compound.

9. Amino acids
Pages: 82–83 Difficulty: 2 Ans: D
Titration of valine by a strong base, for example NaOH, reveals two p*K*'s. The titration reaction occurring at pK_2 (pK_2 = 9.62) is:

A) —COOH + OH^- → —COO^- + H_2O.
B) —COOH + —NH_2 → —COO^- + —NH_2^+.
C) —COO^- + —NH_2^+ → —COOH + —NH_2.
D) —NH_3^+ + OH^- → —NH_2 + H_2O.
E) —NH_2 + OH^- → —NH^- + H_2O.

10. Amino acids
Pages: 82–83 Difficulty: 1 Ans: C
In a highly basic solution, pH = 13, the dominant form of glycine is:

A) NH_2—CH_2—COOH.
B) NH_2—CH_2—COO^-.
C) NH_2—CH_3^+—COO^-.
D) NH_3^+—CH_2—COOH.
E) NH_3^+—CH_2—COO^-.

11. Amino acids
Page: 84 Difficulty: 2 Ans: B
For amino acids with neutral R groups, at any pH below the pI of the amino acid, the population of amino acids in solution will have:

A) a net negative charge.
B) a net positive charge.
C) no charged groups.
D) no net charge.
E) positive and negative charges in equal concentration.

12. Amino acids
Page: 84 Difficulty: 3 Ans: B
What is the approximate charge difference between glutamic acid and α-ketoglutarate at pH 9.5?

A) 0
B) ½
C) 1
D) 1½
E) 2

13. Peptides and proteins
Page: 85 Difficulty: 1 Ans: B
The formation of a peptide bond between two amino acids is an example of a(n) _____ reaction.

A) cleavage
B) condensation
C) group transfer
D) isomerization
E) oxidation reduction

14. Peptides and proteins
Page: 86 Difficulty: 1 Ans: C
The peptide alanylglutamylglycylalanylleucine has:

A) a disulfide bridge.
B) five peptide bonds.
C) four peptide bonds.
D) no free carboxyl group.
E) two free amino groups.

15. Peptides and proteins
Page: 86 Difficulty: 1 Ans: C
An octapeptide composed of four repeating glycylalanyl units has:

A) one free amino group on an alanyl residue.
B) one free amino group on an alanyl residue and one free carboxyl group on a glycyl residue.
C) one free amino group on a glycyl residue and one free carboxyl group on an alanyl residue.
D) two free amino and two free carboxyl groups.
E) two free carboxyl groups, both on glycyl residues.

16. Peptides and proteins
Page: 86 Difficulty: 1 Ans: C
At the isoelectric pH of a tetrapeptide:

A) only the amino and carboxyl termini contribute charge.
B) the amino and carboxyl termini are not charged.
C) the total net charge is zero.
D) there are four ionic charges.
E) two internal amino acids of the tetrapeptide cannot have ionizable R groups.

17. Peptides and proteins
Pages: 87–88 Difficulty: 2 Ans: C
Which of the following is correct with respect to the amino acid composition of proteins?

A) Larger proteins have a more uniform distribution of amino acids than smaller proteins.
B) Proteins contain at least one each of the 20 different standard amino acids.
C) Proteins with different functions usually differ significantly in their amino acid composition.
D) Proteins with the same molecular weight have the same amino acid composition.
E) The average molecular weight of an amino acid in a protein increases with the size of the protein.

18. Peptides and proteins
Page: 87 Difficulty: 2 Ans: B
The average molecular weight of the 20 standard amino acids is 138, but biochemists use 110 when estimating the number of amino acids in a protein of known molecular weight. Why?

A) The number 110 is based on the fact that the average molecular weight of a protein is 110,000 with an average of 1,000 amino acids.
B) The number 110 reflects the higher proportion of small amino acids in proteins, as well as the loss of water when the peptide bond forms.
C) The number 110 reflects the number of amino acids found in the typical small protein, and only small proteins have their molecular weight estimated this way.
D) The number 110 takes into account the relatively small size of nonstandard amino acids.
E) The number 138 represents the molecular weight of conjugated amino acids.

19. Peptides and proteins
Page: 88 Difficulty: 1 Ans: C
In a conjugated protein, a prosthetic group is:

A) a fibrous region of a globular protein.
B) a nonidentical subunit of a protein with many identical subunits.
C) a part of the protein that is not composed of amino acids.
D) a subunit of an oligomeric protein.
E) synonymous with "protomer."

20. Peptides and proteins
Page: 88 Difficulty: 1 Ans: A
Prosthetic groups in the class of proteins known as glycoproteins are composed of:

A) carbohydrates.
B) flavin nucleotides.
C) lipids.
D) metals .
E) phosphates.

21. Peptides and proteins
Page: 88 Difficulty: 1 Ans: B
Which of the following refers to particularly stable arrangements of amino acid residues in a protein that give rise to recurring patterns?

A) Primary structure
B) Secondary structure
C) Tertiary structure
D) Quaternary structure
E) None of the above

22. Peptides and proteins
Page: 88 Difficulty: 1 Ans: D
Which of the following describes the overall three-dimensional folding of a polypeptide?

A) Primary structure
B) Secondary structure
C) Tertiary structure
D) Quaternary structure
E) None of the above

23. Working with proteins
Page: 89 Difficulty: 1 Ans: E
For the study of a protein in detail, an effort is usually made to first:

A) conjugate the protein to a known molecule.
B) determine its amino acid composition.
C) determine its amino acid sequence.
D) determine its molecular weight.
E) purify the protein.

24. Working with proteins
Page: 91 Difficulty: 2 Ans: B
In a mixture of the five proteins listed below, which should elute second in size-exclusion (gel-filtration) chromatography?

A) cytochrome *c* $M_r = 13{,}000$
B) immunoglobulin G $M_r = 145{,}000$
C) ribonuclease A $M_r = 13{,}700$
D) RNA polymerase $M_r = 450{,}000$
E) serum albumin $M_r = 68{,}500$

25. Working with proteins
Page: 92 Difficulty: 2 Ans: E
By adding SDS (sodium dodecyl sulfate) during the electrophoresis of proteins, it is possible to:

A) determine a protein's isoelectric point.
B) determine an enzyme's specific activity.
C) determine the amino acid composition of the protein.
D) preserve a protein's native structure and biological activity.
E) separate proteins exclusively on the basis of molecular weight.

26. Working with proteins
Page: 93 Difficulty: 2 Ans: B
To determine the isoelectric point of a protein, first establish that a gel:

A) contains a denaturing detergent that can distribute uniform negative charges over the protein's surface.

B) exhibits a stable pH gradient when ampholytes become distributed in an electric field.
C) is washed with an antibody specific to the protein of interest.

D) neutralizes all ionic groups on a protein by titrating them with strong bases.
E) relates the unknown protein to a series of protein markers with known molecular weights, M_r.

27. Working with proteins
Pages: 93–94 Difficulty: 3 Ans: A
The first step in two-dimensional gel electrophoresis generates a series of protein bands by isoelectric focusing. In a second step, a strip of this gel is turned 90 degrees, placed on another gel containing SDS, and electric current is again applied. In this second step:

A) proteins with similar isoelectric points become further separated according to their molecular weights.
B) the individual bands become stained so that the isoelectric focus pattern can be visualized.
C) the individual bands become visualized by interacting with protein-specific antibodies in the second gel.
D) the individual bands undergo a second, more intense isoelectric focusing.
E) the proteins in the bands separate more completely because the second electric current is in the opposite polarity to the first current.

28. Working with proteins
Pages: 94–95 Difficulty: 1 Ans: B
The term *specific activity* differs from the term *activity* in that specific activity:

A) is measured only under optimal conditions.
B) is the activity (enzyme units) in a milligram of protein.
C) is the activity (enzyme units) of a specific protein.
D) refers only to a purified protein.
E) refers to proteins other than enzymes.

29. The covalent structure of proteins
Page: 96 Difficulty: 1 Ans: B
The functional differences, as well as differences in three-dimensional structures, between two different enzymes from *E. coli* result directly from their different:

A) affinities for ATP.
B) amino acid sequences.
C) roles in DNA metabolism.
D) roles in the metabolism of *E. coli*.
E) secondary structures.

30. The covalent structure of proteins
Page: 99 Difficulty: 2 Ans: C
One method used to prevent disulfide bond interference with protein sequencing procedures is:

A) cleaving proteins with proteases that specifically recognize disulfide bonds.
B) protecting the disulfide bridge against spontaneous reduction to cysteinyl sulfhydryl groups.
C) reducing disulfide bridges and preventing their re-formation by further modifying the —SH groups.
D) removing cystines from protein sequences by proteolytic cleavage.
E) sequencing proteins that do not contain cysteinyl residues.

31. The covalent structure of proteins
Page: 100 Difficulty: 3 Ans: C
A nonapeptide was determined to have the following amino acid composition: $(Lys)_2$, $(Gly)_2$, $(Phe)_2$, His, Leu, Met. The native peptide was incubated with 1-fluoro-2,4-dinitrobenzene (FDNB) and then hydrolyzed; 2,4-dinitrophenylhistidine was identified by HPLC. When the native peptide was exposed to cyanogen bromide (CNBr), an octapeptide and free glycine were recovered. Incubation of the native peptide with trypsin gave a pentapeptide, a tripeptide, and free Lys. 2,4-Dinitrophenyl-histidine was recovered from the pentapeptide, and 2,4-dinitrophenylphenylalanine was recovered from the tripeptide. Digestion with the enzyme pepsin produced a dipeptide, a tripeptide, and a tetrapeptide. The tetrapeptide was composed of $(Lys)_2$, Phe, and Gly. The native sequence was determined to be:

A) Gly–Phe–Lys–Lys–Gly–Leu–Met–Phe–His.
B) His–Leu–Gly–Lys–Lys–Phe–Phe–Gly–Met.
C) His–Leu–Phe–Gly–Lys–Lys–Phe–Met–Gly.
D) His–Phe–Leu–Gly–Lys–Lys–Phe–Met–Gly.
E) Met–Leu–Phe–Lys–Phe–Gly–Gly–Lys–His.

32. The covalent structure of proteins
Page: 100 Difficulty: 1 Ans: C
Even when a gene is available and its sequence of nucleotides is known, chemical studies of the protein are still required to determine:

A) molecular weight of the protein.
B) the amino-terminal amino acid.
C) the location of disulfide bonds.
D) the number of amino acids in the protein.
E) whether the protein has the amino acid methionine in its sequence.

33. The covalent structure of proteins
Page: 101 Difficulty: 1 Ans: C
The term "proteome" has been used to describe:

A) regions (domains) within proteins.
B) regularities in protein structures.
C) the complement of proteins encoded by an organism's DNA.
D) the structure of a protein-synthesizing ribosome.
E) the tertiary structure of a protein.

34. The covalent structure of proteins
Pages: 102–103 Difficulty: 2 Ans: C
A major advance in the application of mass spectrometry to macromolecules came with the development of techniques to overcome which of the following problems?

A) Macromolecules were insoluble in the solvents used in mass spectrometry.
B) Mass spectrometric analyses of macromolecules were too complex to interpret.
C) Mass spectrometric analysis involved molecules in the gas phase.
D) Most macromolecules could not be purified to the degree required for mass spectrometric

analysis.

E) The specialized instruments required were prohibitively expensive.

35. Protein sequences and evolution
Pages: 107–109 Difficulty: 3 Ans: A
Compare the following sequences taken from four different proteins, and select the answer that best characterizes their relationships.

	A	B	C
1	DVEKGKKIDIMKCS	HTVEKGGKHKTGPNLH	GLFGRKTGQAPGYSYT
2	DVQRALKIDNNLGQ	HTVEKGAKHKTAPNVH	GLADRIAYQAKATNEE
3	LVTRPLYIFPNEGQ	HTLEKAAKHKTGPNLH	ALKSSKDLMFTVINDD
4	FFMNEDALVARSSN	HQFAASSIHKNAPQFH	NLKDSKTYLKPVISET

A) Based only on sequences in column B, protein 4 reveals the greatest evolutionary divergence.
B) Comparing proteins 1 and 2 in column A reveals that these two proteins have diverged the most throughout evolution.
C) Protein 4 is the protein that shows the greatest overall homology to protein 1.
D) Proteins 2 and 3 show a greater evolutionary distance than proteins 1 and 4.
E) The portions of amino acid sequence shown suggest that these proteins are completely unrelated.

Short Answer Questions

36. Amino acids
Page: 76 Difficulty: 1
What are the structural characteristics common to all amino acids found in naturally occurring proteins?

Ans: All amino acids found in naturally occurring proteins have an α carbon to which are attached a carboxylic acid, an amine, a hydrogen, and a variable side chain. All the amino acids are also in the L configuration.

37. Amino acids
Page: 79 Difficulty: 1
Only one of the common amino acids has no free α-amino group. Name this amino acid and draw its structure.

Ans: The amino acid L-proline has no free α-amino group, but rather has an imino group formed by cyclization of the R-group aliphatic chain with the amino group (see Fig. 3-5, p. 79).

38. Amino acids
Pages: 78–79 Difficulty: 2
Briefly describe the five major groupings of amino acids.

Ans: Amino acids may be categorized by the chemistry of their R groups: (1) nonpolar aliphatics; (2) polar, uncharged; (3) aromatic; (4) positively charged; (5) negatively charged. (See Fig. 3-5, p. 79.)

39. Amino acids
Pages: 78–79 Difficulty: 2

A	B	C	D	E
Tyr-Lys-Met	Gly-Pro-Arg	Asp-Trp-Tyr	Asp-His-Glu	Leu-Val-Phe

Which one of the above tripeptides:
____(a) is most negatively charged at pH 7?
____(b) will yield DNP-tyrosine when reacted with l-fluoro-2,4-dinitrobenzene and hydrolyzed in acid?
____(c) contains the largest number of nonpolar R groups?
____(d) contains sulfur?
____(e) will have the greatest light absorbance at 280 nm?

Ans: (a) D; (b) A; (c) E; (d) A; (e) C

40. Amino acids
Page: 79 Difficulty: 2
Draw the structures of the amino acids phenylalanine and aspartate in the ionization state you would expect at pH 7.0. Why is aspartate very soluble in water, whereas phenylalanine is much less soluble?

Ans: Aspartate has a polar (hydrophilic) side chain, which forms hydrogen bonds with water. In contrast, phenylalanine has a nonpolar (hydrophobic) side chain. (See Fig. 3-5, p. 79 for structures.)

41. Amino acids
Page: 80 Difficulty: 3
Name two uncommon amino acids that occur in proteins. By what route do they get into proteins?

Ans: Some examples are 4-hydroxyproline, 5-hydroxylysine, γ-carboxyglutamate, *N*-methyllysine, desmosine, and selenocysteine. Uncommon amino acids in proteins (other than selenocysteine) usually result from chemical modifications of standard amino acid R groups after a protein has been synthesized.

42. Amino acids
Page: 81 Difficulty: 1
Why do amino acids, when dissolved in water, become zwitterions?

Ans: Near pH = 7, the carboxylic acid group (—COOH) will dissociate to become a negatively charged —COO^- group, and the —NH_2 amino group will attract a proton to become a positively charged —NH_3^+ group.

43. Amino acids
Page: 82 Difficulty: 1
As more OH^- equivalents (base) are added to an amino acid solution, what titration reaction will occur around pH = 9.5?

Ans: Around pH = 9.5, the —NH_3^+ group will be titrated according to the reaction: —NH_3^+ + OH^- → —NH_2 + H_2O.

44. Amino acids
Page: 83 Difficulty: 3
In the amino acid glycine, what effect does the positively charged —NH_3^+ group have on the pK_a of an amino acid's —COOH group?

Ans: The positively charged amino group stabilizes the negatively charged ionized form of the carboxyl group, —COO^-, and repels the departing H^+ thereby promoting deprotonation. The effect is to lower the pK_a of the carboxyl group (see Fig. 3-11, p. 83).

45. Amino acids
Page: 83 Difficulty: 3
How does the shape of a titration curve confirm the fact that the pH region of greatest buffering power for an amino acid solution is around its p*K*'s?

Ans: In a certain range around the pK_a's of an amino acid, the titration curve levels off. This indicates that for a solution with pH ≈ p*K*, any given addition of base or acid equivalents will result in the smallest change in pH—which is the definition of a buffer.

46. Amino acids
Page: 83 Difficulty: 2
Leucine has two dissociable protons: one with a pK_a of 2.3, the other with a pK_a of 9.7. Sketch a properly labeled titration curve for leucine titrated with NaOH; indicate where the pH = p*K* and the region(s) in which buffering occurs.

Ans: See the titration curve for glycine in Fig. 3-10, p. 83.

47. Amino acids
Page: 84 Difficulty: 2
What is the pI, and how is it determined for amino acids that have nonionizable R groups?

Ans: The pI is the isoelectric point. It occurs at a characteristic pH when a molecule has an equal number of positive and negative charges, or no *net* charge. For amino acids with nonionizable R groups, pI is the arithmetic mean of a molecule's two pK_a values:

pI = 1/2 (pK_1 + pK_2)

48. Amino acids
Page: 84 Difficulty: 2
The amino acid histidine has a side chain for which the pK_a is 6.0. Calculate what fraction of the histidine side chains will carry a positive charge at pH 5.4. Be sure to show your work.

Ans: $$\text{pH} = \text{p}K_a + \log \frac{[\text{conjugate base}]}{[\text{acid}]}$$

$$\text{p}K_a - \text{pH} = \log \frac{[\text{acid}]}{[\text{conjugate base}]}$$

$$\text{antilog}\,(\text{p}K_a - \text{pH}) = \frac{[\text{acid}]}{[\text{conjugate base}]}$$

$$\text{antilog}\,(6.0 - 5.4) = \frac{[\text{acid}]}{[\text{conjugate base}]}$$

4 = [acid]/[conjugate base], or
4[conjugate base] = [acid]

Therefore, at pH 5.4, 4/5 (80%) of the histidine will be in the protonated form.

49. Amino acids
Page: 84 Difficulty: 2
The amino acid histidine has three ionizable groups, with pK_a values of 1.8, 6.0, and 9.2. (a) Which pK_a corresponds to the histidine side chain? (b) In a solution at pH 5.4, what percentage of the histidine side chains will carry a positive charge?

Ans: (a) 6.0; (b) 80%. (See the previous problem for expanded solution to this problem.)

50. Amino acids
Page: 85 Difficulty: 2
What is the uniquely important acid-base characteristic of the histidine R group?

Ans: Only the imidazole ring of the histidine R group has a pK_a near physiological pH (pK_a = 6.0), which suggests that histidine may provide buffering power in intercellular and intracellular fluids.

51. Peptides and proteins
Page: 86 Difficulty: 1
How can a polypeptide have only one free amino group and one free carboxyl group?

Ans: This is possible only if the peptide has no side chains with carboxyl or amino groups. Then, with the exception of the single amino-terminal amino acid and the single carboxyl-terminal amino acid, all the other α-amino and carboxyl groups are covalently condensed into peptide bonds.

52. Peptides and proteins
Page: 86 Difficulty: 1
Hydrolysis of peptide bonds is an exergonic reaction. Why, then, are peptide bonds quite stable?

Ans: Peptide bonds are stable because hydrolysis of peptide (or amide) bonds has a high activation energy and as a result occurs very slowly.

53. Peptides and proteins
Page: 86 Difficulty: 2
Draw the structure of Gly–Ala–Glu in the ionic form that predominates at pH 7.

Ans: The peptide must have an amino-terminal Gly residue, a carboxyl-terminal Glu residue, and ionized amino and carboxyl groups.

54. Peptides and proteins
Page: 86 Difficulty: 2
The artificial sweetener NutraSweet®, also called aspartame, is a simple dipeptide, aspartylphenylalanine methyl ester, on which the free carboxyl of the dipeptide is esterified to methyl alcohol. Draw the structure of aspartame, showing the ionizable groups in the form they have at pH 7. (The ionizable group in the side chain of aspartate has a pK_a of 3.96.)

Ans: See the structure on p. 86.

55. Peptides and proteins
Page: 87 Difficulty: 1
If the average molecular weight of the 20 standard amino acids is 138, why do biochemists divide a protein's molecular weight by 110 to estimate its number of amino acid residues?

Ans: For each peptide bond formed, a molecule of water is lost, bringing the average molecular weight down to 120. To reflect the preponderance of low-molecular-weight amino acids, the average molecular weight is lowered further to 110.

56. Peptides and proteins
Page: 87 Difficulty: 2
Lys residues make up 10.5% of the weight of ribonuclease. The ribonuclease molecule contains 10 Lys residues. Calculate the molecular weight of ribonuclease.

Ans: From the structure of lysine, we can calculate its molecular weight (146); when it condenses (loses H_2O, $M_r = 18$) to form a peptide bond, the resulting residue contributes 146 – 18 = 128 to the protein's molecular weight. If 10 Lys residues contribute 10.5% of the protein's molecular weight, each Lys residue is 1.05%. To calculate the total molecular weight, divide 128 by 1.05% (0.0105); the result is 12,190. (The actual value is 13,700.)

57. Peptides and proteins
Page: 88 Difficulty: 1
Define the primary structure of a protein.

Ans: The primary structure of a protein is its unique sequence of amino acids and any disulfide bridges present in the native structure, that is, its covalent bond structure.

58. Working with proteins
Pages: 90-91 Difficulty: 2
Why do smaller molecules elute after large molecules when a mixture of proteins is passed through a size-exclusion (gel filtration) column?

Ans: The column matrix is composed of cross-linked polymers with pores of selected sizes. Smaller molecules can enter pores in the polymer beads from which larger molecules would be excluded. Smaller molecules therefore have a larger three-dimensional space in which to diffuse, making their path through the column longer. Larger molecules migrate faster because they pass directly through the column, unhindered by the bead pores.

59. Working with proteins
Pages: 90-91 Difficulty: 2
For each of these methods of separating proteins, describe the principle of the method, and tell what property of proteins allows their separation by this technique.
(a) ion-exchange chromatography
(b) size-exclusion (gel filtration) chromatography
(c) affinity chromatography

Ans: (a) Ion-exchange chromatography separates proteins on the basis of their charges. (b) Size-exclusion or gel filtration chromatography separates on the basis of size (c) Affinity chromatography separates proteins with specific, high affinity for some ligand (attached to an inert support) from other proteins with no such affinity. (See Fig. 3-18, p. 91.)

60. Working with proteins
Pages: 90-93 Difficulty: 2
A biochemist is attempting to separate a DNA-binding protein (protein X) from other proteins in a solution. Only three other proteins (A, B, and C) are present. The proteins have the following properties:

	pI (isoelectric point)	Size M_r	Bind to DNA?
protein A	7.4	82,000	yes
protein B	3.8	21,500	yes
protein C	7.9	23,000	no
protein X	7.8	22,000	yes

What type of protein separation techniques might she use to separate
(a) protein X from protein A?
(b) protein X from protein B?
(c) protein X from protein C?

Ans: (a) size-exclusion (gel filtration) chromatography to separate on the basis of size; (b) ion-exchange chromatography or isoelectric focusing to separate on the basis of charge; (c) specific affinity chromatography, using immobilized DNA.

61. Working with proteins
Page: 92 Difficulty: 2
What factors would make it difficult to interpret the results of a gel electrophoresis of proteins in the absence of sodium dodecyl sulfate (SDS)?

Ans: Without SDS, protein migration through a gel would be influenced by the protein's intrinsic net charge—which could be positive or negative—and its unique three-dimensional shape, in addition to its molecular weight. Thus, it would be difficult to ascertain the difference between proteins based upon a comparison of their mobilities in gel electrophoresis.

62. Working with proteins
Pages: 93-95 Difficulty: 2
How can isoelectric focusing be used in conjunction with SDS gel electrophoresis?

Ans: Isoelectric focusing can separate proteins of the same molecular weight on the basis of differing isoelectric points. SDS gel electrophoresis can then separate proteins with the same isoelectric points on the basis of differing molecular weights. When combined in two-dimensional electrophoresis, a great resolution of large numbers of proteins can be achieved.

63. Working with proteins
Pages: 94-95 Difficulty: 3
You are given a solution containing an enzyme that converts B into A. Describe what you would do to determine the specific activity of this enzyme solution.

Ans: First, add a known volume of the enzyme solution (say, 0.01 mL) to a solution of its substrate B and measure the initial rate at which product A is formed, expressed as μmol/mL of enzyme solution/min. Then measure the total protein concentration, expressed as mg/mL. Finally, divide the enzyme activity (μmol/min/mL) by the protein concentration (mg/mL); the quotient is the specific activity.

64. Working with proteins
Pages: 94-95 Difficulty: 2
As a protein is purified, both the amount of total protein and the activity of the purified protein decrease. Why, then, does the *specific activity* of the purified protein increase?

Ans: Specific activity is the units of enzyme activity (μmol of product/min) divided by the amount of protein (mg). As the protein is purified, some of it is lost in each step, resulting in a drop in activity. However, other contaminating proteins are lost to a much greater extent. Therefore, with each purification step, the purified protein constitutes a greater proportion of the total, resulting in an increase in specific activity. (See also Table 3-5, p. 92.)

65. The covalent structure of proteins
Pages: 97-100 Difficulty: 2
In one or two sentences, describe the usefulness of each of the following reagents or reactions in the analysis of protein structure:

(a) Edman reagent (phenylisothiocyanate)
(b) Sanger reagent (1-fluoro-2,4-dinitrobenzene, FDNB)
(c) trypsin

Ans: (a) used in determination of the amino acid sequence of a peptide, starting at its amino terminus; (b) used in determination of amino-terminal amino acid of a polypeptide; (c) used to produce specific peptide fragments from a polypeptide.

66. The covalent structure of proteins
Pages: 98-100 Difficulty: 2
A polypeptide is hydrolyzed, and it is determined that there are 3 Lys residues and 2 Arg residues (as well as other residues). How many peptide fragments can be expected when the native polypeptide is incubated with the proteolytic enzyme trypsin?

Ans: Six fragments would be expected, unless the carboxyl-terminal residue is Lys or Arg; in which case there would be five.

67. The covalent structure of proteins
Pages: 98-100 Difficulty: 2
The following reagents are often used in protein chemistry. Match the reagent with the purpose for which it is best suited. Some answers may be used more than once or not at all; more than one reagent may be suitable for a given purpose.

(a) CNBr (cyanogen bromide)
(b) Edman reagent (phenylisothiocyanate)
(c) FDNB
(d) dithiothreitol
(e) performic acid
(f) chymotrypsin
(g) trypsin

___ hydrolysis of peptide bonds on the carboxyl side of Lys and Arg
___ cleavage of peptide bonds on the carboxyl side of Met
___ breakage of disulfide (—S—S—) bonds
___ determination of the amino acid sequence of a peptide
___ determining the amino-terminal amino acid in a polypeptide

Ans: g; a; d and e; b; c

68. The covalent structure of proteins
Pages: 98-100 Difficulty: 2
A biochemist wishes to determine the sequence of a protein that contains 123 amino acid residues. After breaking all of the disulfide bonds, the protein is treated with cyanogen bromide (CNBr), and it is determined that that this treatment breaks up the protein into seven conveniently sized peptides, which are separated from each other. It is your turn to take over. Outline the steps you would take to determine, unambiguously, the sequence of amino acid residues in the original protein.

Ans: (1) Use Edman degradation to determine the sequence of each peptide. (2) Create a second set of peptides by treatment of the protein with a specific protease (e.g., trypsin), and determine the sequence of each of these. (3) Place the peptides in order by their overlaps. (4) Finally, by a similar analysis of the original protein without first breaking disulfide bonds, determine the number and location of —S—S— bridges.

69. The covalent structure of proteins
Pages: 98-101 Difficulty: 3
You are trying to determine the sequence of a protein that you know is pure. Give the most likely explanation for each of the following experimental observations. You may use a simple diagram for your answer.

(a) The Sanger reagent (FDNB, fluorodinitrobenzene) identifies Ala and Leu as amino-terminal residues, in roughly equal amounts.
(b) Your protein has an apparent M_r of 80,000, as determined by SDS-polyacrylamide gel electrophoresis. After treatment of the protein with performic acid, the same technique reveals two proteins of M_r 35,000 and 45,000.
(c) Size-exclusion chromatography (gel filtration) experiments indicate the native protein has an apparent M_r of 160,000.

Ans: (a) The protein has some multiple of two subunits, with Ala and Leu as the amino-terminal residues. (b) The protein has two subunits (M_r 35,000 and 45,000), joined by one or more disulfide bonds. (c) The native protein (M_r 160,000) has two M_r 35,000 subunits and two M_r 40,000 subunits.

70. Protein sequences and evolution
Page: 107 Difficulty: 2
Distinguish between homologs, paralogs, and orthologs as classes of related proteins.

Ans: Homologs are any members of a particular protein family, paralogs are two homologs present in the same species, and orthologs are are two homologs present in different species.

71. Protein sequences and evolution
Page: 109 Difficulty: 2
How are "signature sequences" useful in analyzing groups of functionally related proteins?

Ans: Such sequences are often present in one taxonomic group or shared by closely related taxonomic groups, but are absent in evolutionarily more distant groups. They thus aid in constructing more elaborate evolutionary trees based on protein sequences.