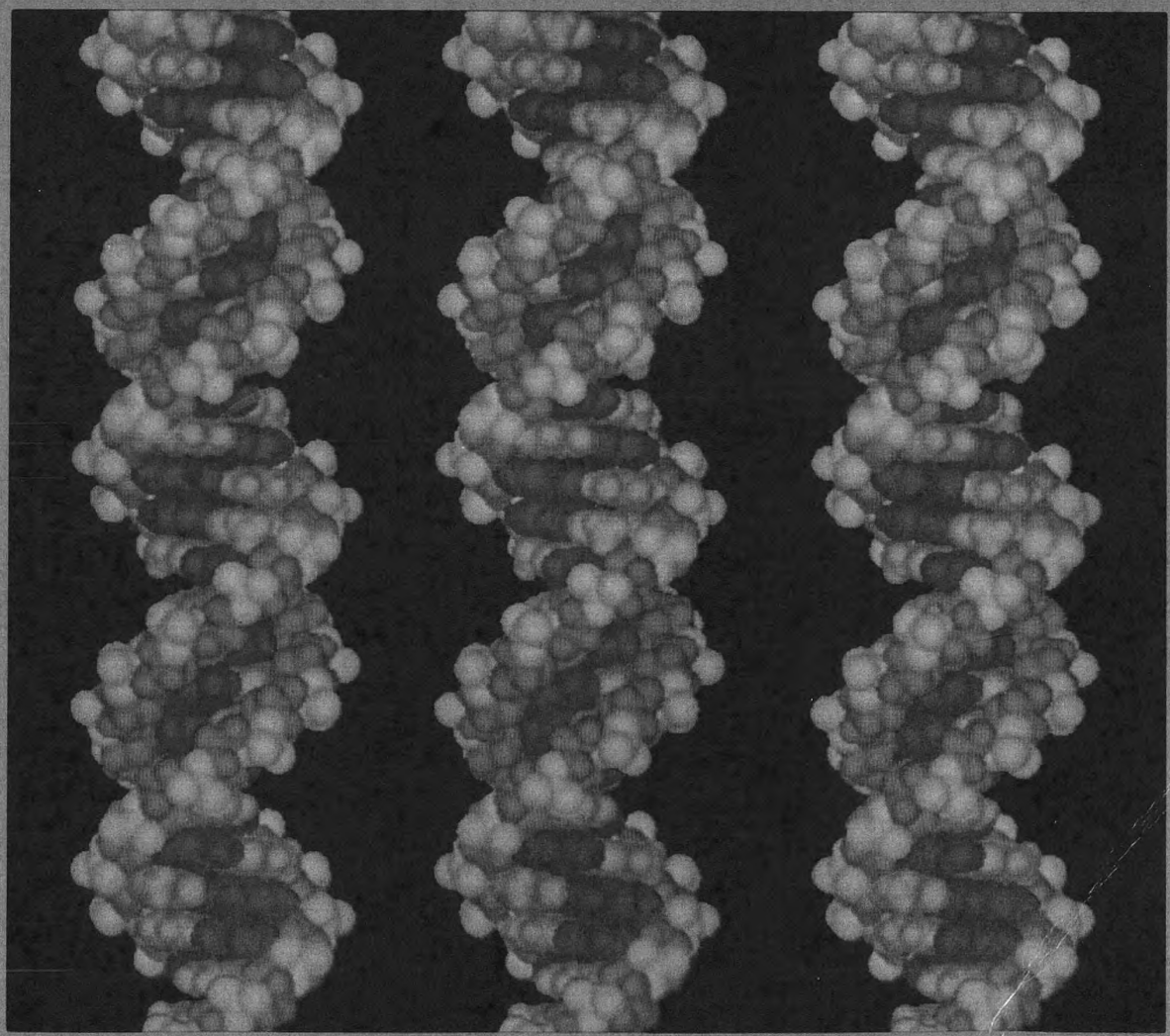


FERGUSON - MILLER

BIOCHEMISTRY

A PROBLEMS APPROACH

SECOND EDITION



WOOD • WILSON • BENBOW • HOOD

SECOND EDITION

Biochemistry

A Problems Approach

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2 Amino Acids

Proteins serve as the catalysts for many important reactions in living systems and as structural elements that dictate much of biological architecture. The properties of proteins are determined by the physical and chemical properties of their monomer units, the amino acids. This chapter considers the important chemical characteristics of the 20 common amino acids.

Concepts

2.1 Amino acids form zwitterions.

The general structure for an amino acid is shown in its nonionized and zwitterionic forms in Figure 2.1. The amino group ($-\text{NH}_2$), the carboxyl group ($-\text{COOH}$), a hydrogen atom (H), and the side chain (R group) are attached to a carbon atom (C) known as the alpha (α) carbon.

In neutral solution ($\text{pH} = 7$), both the α -amino and α -carboxyl groups are ionized. The resulting charged form is called a **zwitterion**. The structures of the 20 common amino acids in neutral solution, their names, abbreviations, pK_a and pI values (Concept 2.2) are given in Table 2.1. The amino acids are grouped in the table according to the chemical properties of their side chains.

2.2 Amino acids behave as acids and bases.

- A. The pH of a solution is defined as $-\log [\text{H}^+]$, in which $[\text{H}^+]$ is the hydrogen-ion concentration.
- B. Similarly, the pK_a of a general acid, HA, is defined as $-\log K_a$, in which K_a is the equilibrium constant for the dissociation $\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^-$:

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \quad (2.1)$$

All amino acids have at least two dissociation constants, one for the carboxyl group (K_{a1}),

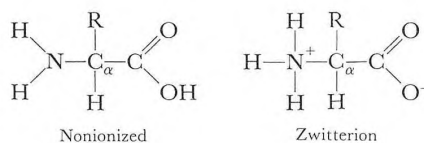
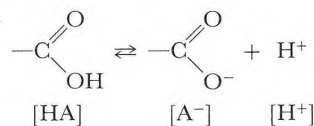


Figure 2.1
Two ionization states of a general amino acid.

Table 2.1
Structures, pK_a Values, and pI Values of the Twenty
Common Amino Acids

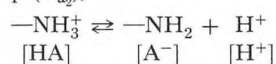
Structure	Name	Abbreviation ^a	pK_{a_1} (α -Carboxyl)	pK_{a_2} (α -Amino)	pK_{a_3} (side chain)	pI
Nonpolar side chain						
	Glycine	Gly (G)	2.3	9.6		6.0
	Alanine	Ala (A)	2.3	9.7		6.0
	Valine	Val (V)	2.3	9.6		6.0
	Leucine	Leu (L)	2.4	9.6		6.0
	Isoleucine	Ilu (I)	2.4	9.7		6.1
	Methionine	Met (M)	2.3	9.2		5.8
	Phenylalanine	Phe (F)	1.8	9.1		5.5
	Proline	Pro (P)	2.0	10.6		6.3
Neutral polar side chain						
	Serine	Ser (S)	2.2	9.2		5.7
	Threonine	Thr (T)	2.6	10.4		6.5
	Cysteine	Cys (C)	1.7	10.8	8.3 (Sulfhydryl)	5.0
	Asparagine	Asn (N)	2.0	8.8		5.4
	Glutamine	Gln (Q)	2.2	9.1		5.7

Table 2.1 (Continued)

Structure	Name	Abbreviation ^a	pK _{a1} (α-Carboxyl)	pK _{a2} (α-Amino)	pK _{a3} (side chain)	pI
$\begin{array}{c} \text{H} \\ \\ \text{---OOC---C---CH}_2\text{---} \text{C}_6\text{H}_4\text{---OH} \\ \\ \text{NH}_3^+ \end{array}$	Tyrosine	Tyr (Y)	2.2	9.1	10.1 (Phenolic hydroxyl)	5.7
$\begin{array}{c} \text{H} \\ \\ \text{---OOC---C---CH}_2\text{---} \text{C} \begin{array}{l} \diagup \text{C}_6\text{H}_5 \\ \diagdown \text{HC---N} \\ \quad \quad \quad \\ \quad \quad \quad \text{H} \end{array} \end{array}$	Tryptophan	Trp (W)	2.4	9.4		5.9
Charged polar side chain						
$\begin{array}{c} \text{H} \\ \\ \text{---OOC---C---CH}_2\text{---C} \begin{array}{l} \diagup \text{O}^- \\ \diagdown \text{O} \end{array} \\ \\ \text{NH}_3^+ \end{array}$	Aspartate	Asp (D)	2.1	9.8	3.9 (β-Carboxyl)	3.0
$\begin{array}{c} \text{H} \\ \\ \text{---OOC---C---CH}_2\text{---CH}_2\text{---C} \begin{array}{l} \diagup \text{O}^- \\ \diagdown \text{O} \end{array} \\ \\ \text{NH}_3^+ \end{array}$	Glutamate	Glu (E)	2.2	9.7	4.3 (γ-Carboxyl)	3.2
$\begin{array}{c} \text{H} \\ \\ \text{---OOC---C---CH}_2\text{---C} \begin{array}{l} \diagup \text{CH} \\ \diagdown \text{HN} \quad \text{C} \quad \text{NH}^+ \\ \quad \quad \quad \\ \quad \quad \quad \text{H} \end{array} \\ \\ \text{NH}_3^+ \end{array}$	Histidine	His (H)	1.8	9.2	6.0 (Imidazole)	7.6
$\begin{array}{c} \text{H} \\ \\ \text{---OOC---C---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---NH}_3^+ \\ \\ \text{NH}_3^+ \end{array}$	Lysine	Lys (K)	2.2	9.0	10.5 (ε-Amino)	9.8
$\begin{array}{c} \text{H} \\ \\ \text{---OOC---C---CH}_2\text{---CH}_2\text{---CH}_2\text{---NH---C} \begin{array}{l} \diagup \text{NH}_2 \\ \diagdown \text{NH}_2^+ \end{array} \\ \\ \text{NH}_3^+ \end{array}$	Arginine	Arg (R)	2.2	9.0	12.5 (Guanidino)	10.8

^a The standard three-letter abbreviation is followed, in parentheses, by the alternative one-letter abbreviation preferred for computer representation of amino acid sequences.

and one for the amino group (K_{a2}),



In addition, several amino acids have an ionizable side chain with a third dissociation constant (K_{a3}). For example, Asp and Glu have a second carboxyl group and Lys has a second amino group. The pK_a values for all ionizable groups of the 20 common amino acids are listed in Table 2.1.

- C. The pH of a solution and the dissociation constant, K_a , of an ionizable group in the solution are related by the Henderson-Hasselbalch equation,

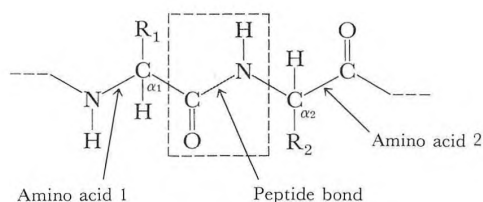
$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad (2.2)$$

This equation can be used to determine the fraction of ionizable groups found in each of the possible ionization states in solution at a known pH. Note that at $\text{pH} = \text{p}K_a$, half of the ionizable groups are dissociated.

- D. The pI , or isoelectric point, of an amino acid is the pH at which it carries no net charge. For monoamino, monocarboxylic acids, pI is defined by the simple relationship

$$\text{pI} = \frac{1}{2}(\text{p}K_{a1} + \text{p}K_{a2}) \quad (2.3)$$

Figure 2.2
The peptide bond joining adjacent amino acids in a protein.



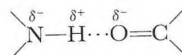
- E. The pK_a values of the ionizable groups of amino acids are affected by neighboring chemical groups on the molecule, principally by electron-withdrawing or electron-donating inductive effects. For example, the amino group, the peptide bond (see Figure 2.2) and the carboxyl group attract electrons. Aliphatic groups [e.g., $-\text{CH}_3$, $-(\text{CH}_2)_n-$] act as weak electron donors.

2.3 In proteins amino acids are linked by peptide bonds.

- A. A **peptide bond** is formed when the carboxyl group of one amino acid combines with the amino group of another amino acid, with elimination of water. The structure of the peptide bond is shown in Figure 2.2. A polymer of amino acids joined by peptide bonds is called a **polypeptide**. A protein consists of one or more polypeptides, usually folded into a specific three-dimensional conformation.
- B. The peptide bond has partial double-bond character. As a result, the six atoms, C_{α_1} , $\text{C}=\text{O}$, $\text{N}-\text{H}$, and C_{α_2} , lie in the same plane. Successive C_{α} atoms are always in the *trans* configuration across the peptide bond between them.

2.4 The characteristics of amino acids determine the properties of polypeptides.

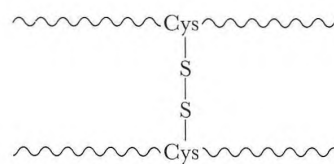
- A. The planar groups of atoms involved in two adjacent peptide bonds can rotate relative to one another about the $\text{C}_{\alpha}-\text{N}$ and $\text{C}_{\alpha}-\text{C}$ bonds (Figure 2.2). Steric features of the side chains limit rotation to a few favorable configurations. The restrictions that these factors introduce into polypeptide structure are described in Chapter 4.
- B. Both the nitrogen and the oxygen atoms of the peptide bond can participate in hydrogen-bond formation (Concept 1.2):



A repeating pattern of H-bonding between these atoms leads to ordered polypeptide structures (Chapter 4).

- C. The amino acid side chains determine the specific chemical properties and the conformation of a polypeptide. The most important feature of side chains is their polar (hydrophilic) or nonpolar (hydrophobic) character. Polar side chains contain the electronegative atoms N, O, or S. Five of the polar side chains are charged (ionized) at $\text{pH} = 7$. The remainder are neutral, but readily form H-bonds. Hence both classes are soluble in water (hydrophilic). Nonpolar side chains consist only of C and H atoms (except for the S atom in Met). These chains do not form hydrogen bonds and therefore are relatively insoluble in water (hydrophobic).

Cys side chains in proteins usually are linked covalently by disulfide bonds, which form cross-links between two segments of polypeptide:



2.5 Amino acids can be separated by paper electrophoresis and paper chromatography.

Paper electrophoresis separates compounds primarily on the basis of their net electrical charge, which can be predicted from the pK_a values of the ionizable groups and the pH of the medium, using the Henderson-Hasselbalch equation. Paper chromatography separates compounds on the basis of their partition coefficients, that is, their relative solubility in polar and nonpolar solvents. Polar molecules are more soluble in polar solvents, whereas nonpolar molecules are more soluble in nonpolar solvents. In paper chromatography the polar phase is stationary (water bound to the cellulose fibers of the paper), and the organic phase (e.g., butanol) is mobile.

Paper electrophoresis and paper chromatography can be used individually or together to separate and analyze mixtures of amino acids, as shown in Figure 2.3. The combination of these two procedures employs paper chromatography in one dimension and (after rotating the paper 90°) paper electrophoresis in the other dimension. This technique, called **fingerprinting**, separates on the basis of both charge and polar character.

2.6 Additional concepts and techniques are presented in the Problems section.

- A. Cation-exchange chromatography. Problem 2.8.
- B. Amino acids as buffers. Problems 2.13, 2.16, and 2.17.

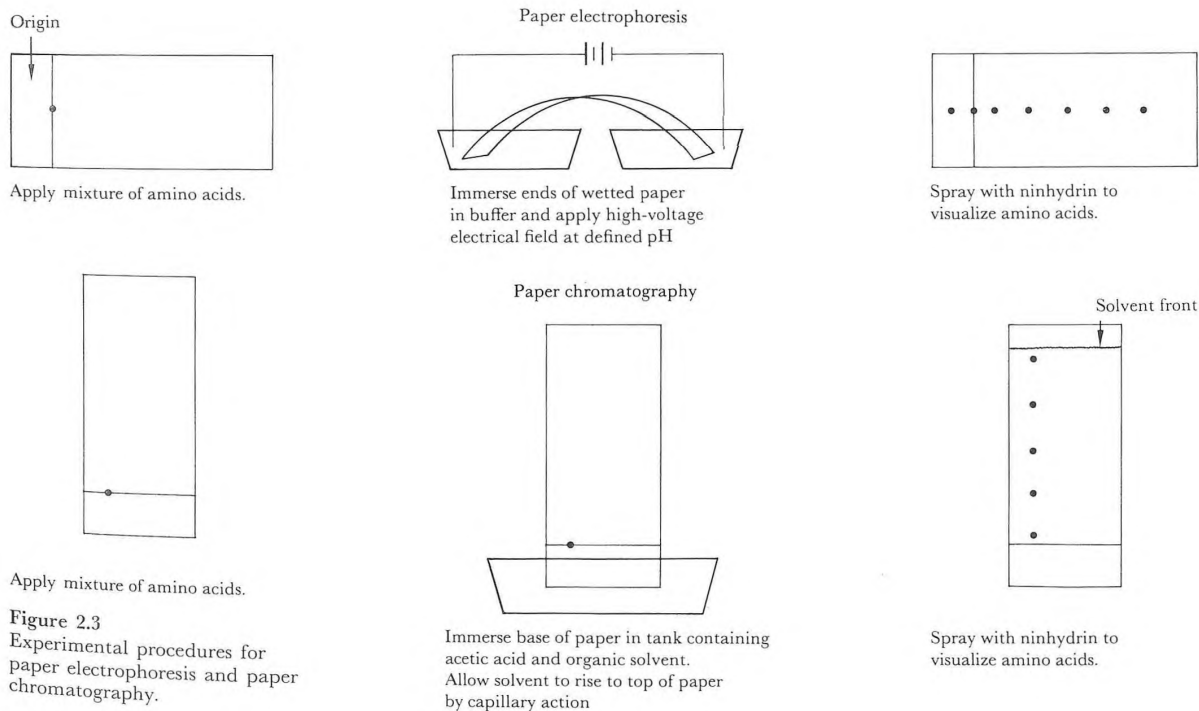


Figure 2.3
Experimental procedures for paper electrophoresis and paper chromatography.

References

COMPREHENSIVE TEXTS

Lehninger: Chapter 4

Metzler: Chapter 2

Stryer: Chapter 2

White et al.: Chapter 4

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R. E. Dickerson and I. Geis, *The Structure and Action of Proteins*, Benjamin/Cummings, Menlo Park, Calif., 1969. Chapter 1.

Problems

- 2.1 Answer the following with true or false. If false, explain why.
- Only at very low or very high pH does the nonionized form of an amino acid predominate.
 - Leu is more nonpolar than Ala.
 - At a pH greater than the pK_a of an ionizable group, more than half of such groups are dissociated.
 - The pI of the tripeptide Lys-Lys-Lys must be greater than any of its individual pK_a values.
 - There is free rotation about a peptide bond.
 - Paper electrophoresis separates amino acids on the basis of their polar character.
- 2.2 By inspection of their structures (Table 2.1), decide which amino acids fit the following descriptions:
- _____ alters polypeptide folding because it is not a true amino acid. In the polypeptides of myoglobin and hemoglobin every bend does not have a _____, but every _____ produces a bend. (All three blanks represent the same residue, which is unique in the manner that it forms peptide bonds.)
 - _____ has a nonpolar side chain and interacts with other aromatic rings. (Aromatic rings have a tendency to stack with other aromatic rings.)
 - _____ is small, and does not contain sulfur. It can form internal H-bonds in a folded protein.
 - The lone electron pair at one of the ring nitrogens in _____ makes it, like Met, a potential ligand, important in binding the iron atoms in hemoglobin.
 - _____ plays a crucial role in stabilizing the structure of many proteins by virtue of the ability of two such residues on different (or the same) polypeptides to form a covalent linkage between their side chains.
 - The carbons in an amino acid are labeled with Greek letters beginning with the α -carbon and extending out the side chain: β , γ , δ , ϵ . If two or three groups other than hydrogen atoms are attached to the β carbon, the molecule is said to branch at the β carbon. Two adjacent residues that branch at the β carbon make the polypeptide structure known as the α helix unstable. By this criterion _____, _____, and _____ should be α -helix breakers when adjacent.
 - In proteins under normal physiological conditions (near $pH = 7$), _____ and _____ side chains are almost entirely positively charged, but _____ side chains are only partially positively charged.
- 2.3 Proteins are long polymers of amino acids joined by peptide bonds. In aqueous solution at $pH = 7$ most proteins are folded so that the nonpolar amino acid side chains are inside in a nonpolar environment, whereas most of the polar side chains are outside, in contact with water. By inspection of the structures in Table 2.1, answer the following questions:
- Are the following amino acids likely to have their side chains on the outside or on the inside of a globular protein in solution: Val, Pro, Phe, Asp, Lys, Ile, and His?
 - Why might Gly and Ala be found either inside or outside?
 - How might Ser, Thr, Asn, and Gln be accommodated inside, even though they are polar?
 - Where might Cys be found and why?

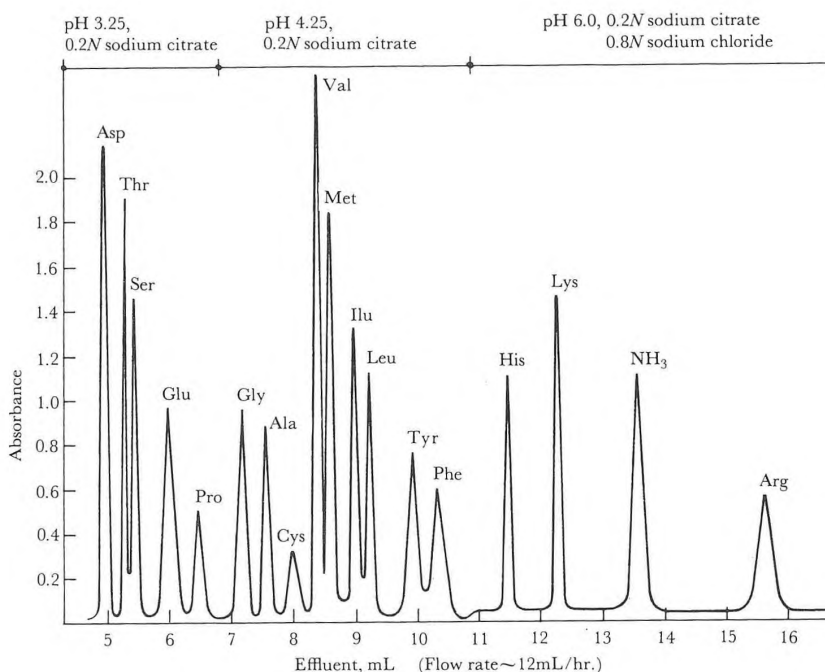
- 2.4 Draw the following amino acid or oligopeptide structures and, if possible, build these structures with space-filling molecular models. By convention, the first-named residue is the N-terminal amino acid (i.e., has a free α -amino group).
- The dipeptide Ala-His
 - The tripeptide Glu-Pro-Cys
 - Show how an oligopeptide of Leu and Lys can be either a branched-chain or a straight-chain structure. (Only straight-chain structures occur in most natural proteins.)
- 2.5 Each of the ionizable groups in an amino acid has two possible ionization states: charged and uncharged (e.g., $-\text{COOH}$, $-\text{COO}^-$). Therefore, a molecule with n such groups has 2^n possible ionization states.
- Draw the four possible ionization states of the amino acid Ser.
 - By inspection, indicate which ionization state predominates at $\text{pH} = 1$, $\text{pH} = 3$, $\text{pH} = 7$, and $\text{pH} = 11$.
- 2.6
- A mixture of the following amino acids is subjected to paper electrophoresis at $\text{pH} = 3.9$: Ala, Ser, Phe, Leu, Arg, Asp, and His. Which will go toward the anode (+)? Toward the cathode (−)?
 - Amino acids with identical charges often separate slightly during paper electrophoresis; for example, Gly separates from Leu. Can you suggest an explanation?
 - Assume that you have a mixture of Ala, Val, Glu, Lys, and Thr at $\text{pH} = 6.0$. Draw the pattern that will be obtained by ninhydrin staining of the amino acids following paper electrophoresis. Indicate the anode (+), cathode (−), origin, and any unresolved amino acids. (Ninhydrin makes the amino acids visible by reacting with free α -amino groups to form colored spots.)
- 2.7
- Table 2.2 shows the pK_a values of several peptides. Indicate the direction of migration of the following peptides at $\text{pH} = 3$ and $\text{pH} = 10$ using the pK_a values deduced from a comparison of Tables 2.1 and 2.2: Phe-Ilu; Lys-Lys-Lys; Arg-Asp.

Table 2.2
 pK_a Values for Some
Peptides (Problem 2.7)

	α -Carboxyl	β -Carboxyl	α -Amino	ϵ -Amino
Gly-Gly	3.1		8.1	
Gly-Gly-Gly	3.3		7.9	
Ala-Ala	3.2		8.4	
Ala-Ala-Ala-Ala	3.4		7.9	
Ala-Gly-Gly	3.2		8.2	
Gly-Ala	3.2		8.2	
Ala-Gly	3.2		8.2	
Lys-Ala	3.2		7.6	10.7
Ala-Lys-Ala	3.2		7.7	10.4
Ala-Lys-Ala-Ala	3.6		8.0	10.6
Ala-Ala-Lys-Ala	3.6		8.0	10.6
Asp-Gly	2.1	4.5	9.1	

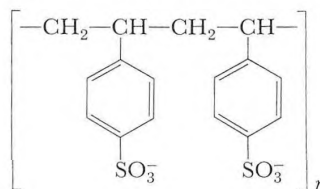
- What conclusions can you draw from a comparison of the pK_a 's of amino acids and peptides?
- 2.8 An amino acid analyzer is an instrument that automatically separates amino acids by chromatography on a column of cation-exchange resin and determines their molar ratios (Figure 2.4). The resin contains polystyrene with sulfonic acid groups covalently bonded to the benzene rings as shown in Figure 2.5. Two major kinds of effects act to separate the amino acids: electrostatic interactions with the negatively charged sulfonic acid groups, and hydrophobic interactions with the nonpolar benzene rings.
- Explain the elution order shown in Figure 2.4 for the following sets of amino acids (i.e., why does set 1 elute before set 2, etc.):
- | | |
|-----------------------|-------------|
| 1. Asp | 5. Tyr, Phe |
| 2. Ser, Thr | 6. Lys, His |
| 3. Gly, Ala | 7. Arg |
| 4. Val, Met, Leu, Ilu | |

Figure 2.4
Separation of amino acids by cation-exchange chromatography (Problem 2.8). The areas under the peaks are proportional to the amounts of amino acids in the mixture. Three buffers of successively higher pH are used to elute the amino acids from the column.



- b. Explain the elution order shown in Figure 2.4 for amino acids within sets 3 and 5. Explain why Asp elutes before Glu.
- 2.9 How many different polypeptides of 61 residues can be made from the 20 naturally occurring amino acids?
- 2.10 Indicate the relative mobilities of amino acids in the following mixtures during paper chromatography in a butanol:acetic acid:water system. Assume a pH of 4.5 in the aqueous phase.
 - a. Ile, Lys
 - b. Phe, Ser
 - c. Ala, Val, Leu
 - d. Pro, Val
 - e. Glu, Asp
 - f. Tyr, Ala, Ser, His
- 2.11 Indicate which of the following pairs of peptides (or amino acids) could be separated, and which could not, using the conditions of Problem 2.10.
 - a. Lys-Lys; Lys
 - b. Leu-Leu-Leu; Leu
 - c. Trp-Ile-Phe; Arg-Ile-Phe
 - d. Cys-Cys-Ser; Ala-Ala-Ser
- 2.12 Which of the following polypeptides is more soluble in water?
 - a. Phe₂₀ or Gly₂₀
 - b. Asp₂₀ or Glu₂₀ at pH = 6.0
 - c. Phe₂₀ or Tyr₂₀
- 2.13 Amino acids occasionally are used as buffers. A buffer is a solution that resists changes

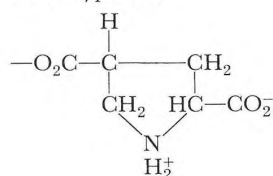
Figure 2.5
Structure of the cation-exchange resin Dowex 50 (Problem 2.8).



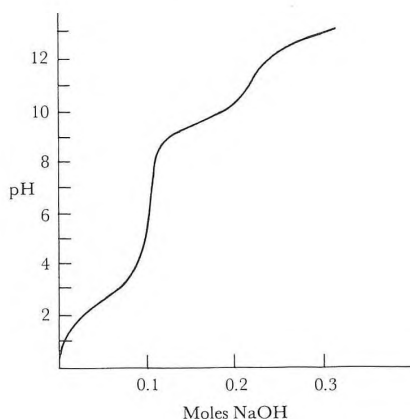
in pH when acid or base is added. The pH range over which a buffer is effective is called the buffering range, usually defined as $pK_a + 1$ to $pK_a - 1$.

- Indicate the buffering range (or ranges) of Gly, His, Asp, and Lys.
 - Choose an amino acid to buffer at pH = 4, pH = 6, pH = 9, and pH = 12.
 - Would you expect a protein containing 100 amino acids to be as good a buffer as its constituent free amino acids at equivalent molar amino acid concentrations? Explain.
- 2.14 The ϵ -amino group of Lys has a pK_a of 10.5.
- What fraction of these groups will be protonated (i.e., $-\text{NH}_3^+$ rather than $-\text{NH}_2$) in a dilute solution of Lys at pH = 9.5?
 - At pH = 11.0?
 - Explain why the pK_a of the ϵ -amino group is higher than that of the α -amino group.
- 2.15 The γ -carboxyl group of Glu has a pK_a of 4.3.
- What fraction of these groups will be unprotonated (i.e., $-\text{COO}^-$ rather than $-\text{COOH}$) in a dilute solution of Glu at pH = 5.0?
 - At pH = 3.8?
 - Explain why this pK_a is higher than the pK_a of the α -carboxyl group.
- 2.16
- Assume you have a solution containing 0.1 mol of Ala adjusted to pH = 0.5 with hydrochloric acid. You begin adding 1.0M NaOH (a strong base). Sketch the resulting titration curve showing all inflection points. Show your calculations for a few points on the curve. (Pick the pH values to simplify the calculations, rather than simply using pH = 1, = 2, = 3, and so on.) State all your assumptions.
 - Sketch a similar titration curve for a solution containing 0.1 mol of His at pH = 0.5, making the assumptions stated in part a but performing no calculations.
- 2.17
- Using the pK_a values in Table 2.1, identify the amino acid that has the titration curve shown in Figure 2.6a.
 - Identify the amino acid that has the titration curve shown in Figure 2.6b. How does this curve differ from the ones you constructed for Problem 2.16?
 - What are the similarities and differences among the four titration curves examined in Problems 2.16 and 2.17a and b?
- 2.18 From kangaroo-tail collagen you have isolated a series of previously uncharacterized γ -substituted prolines. From their titration curves you determine the pK_a 's of the ionizable groups to be as follows:

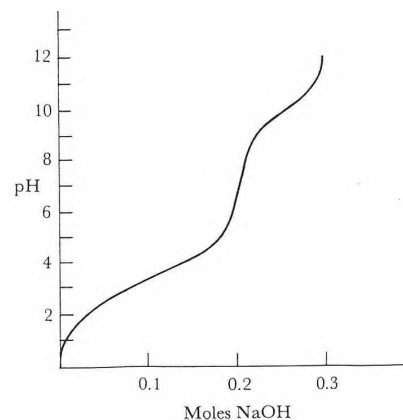
- a. Carboxypoline



α -Carboxyl	pK_a 2.0
α -Amino	10.5
γ -Carboxyl	3.8



(a)



(b)

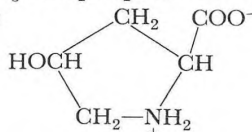
Figure 2.6
Titration curves of two amino
acids (Problem 2.17).

b. Aminoproline	α -Carboxyl	1.9
	α -Amino	9.8
	γ -Amino	10.0
c. Sulfhydrylproline	α -Carboxyl	1.9
	α -Amino	9.9
	γ -Sulfhydryl	9.9

Using these pK_a values, calculate the isoelectric point, pI , for each amino acid.

- ☆ 2.19 Figure 2.4 shows the elution pattern of 16 common amino acids separated by cation-exchange chromatography. If the following amino acids were present in such an experiment, where would you expect them to elute in relation to the 16 amino acids shown in the figure? Briefly explain your answers.

- Norleucine: side chain $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$
- Cysteic acid: side chain $-\text{CH}_2\text{SO}_3^{2-}$
- Phosphoserine: side chain $-\text{CH}_2\text{OPO}_3^{2-}$
- Ethanolamine: $\text{H}_3^+\text{NCH}_2\text{CH}_2\text{OH}$
- Hydroxyproline:



- ☆ 2.20 Figure 2.7 is a diagram of the fingerprint obtained by subjecting a mixture of 18 amino acids (all but Asn and Gln) to electrophoresis at $\text{pH} = 6.5$ in one dimension and chromatography in the other (butanol:acetic acid:pyridine). Identify the numbered spots. They correspond to the amino acids Arg, Asp, Cys, Pro, Lys, Glu, Ile, Leu, and His.

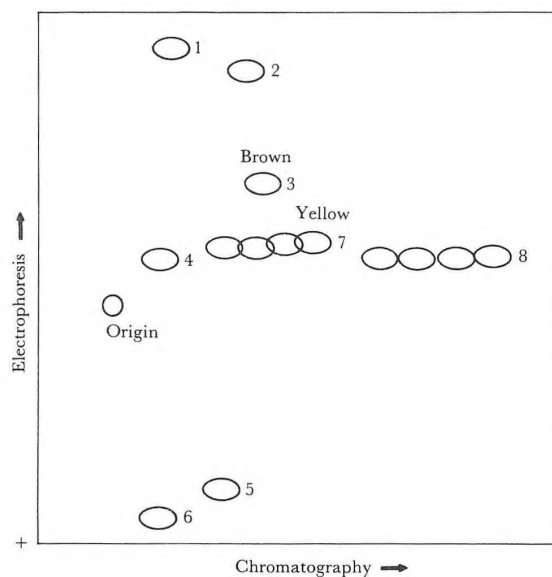


Figure 2.7
Fingerprint of 18 amino acids
(Problem 2.20).

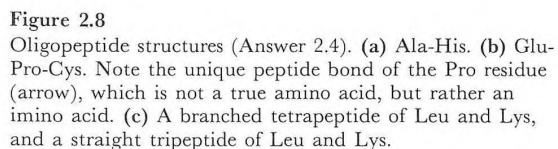
Answers

- 2.1 a. False. The nonionized form of an amino acid never predominates in aqueous solution.
 b. True
 c. True
 d. True. At its pI the tripeptide will have one full negative charge ($\alpha\text{-COO}^-$), one essentially nonionized amino group ($\alpha\text{-NH}_2$), and three partially charged amino groups ($\epsilon\text{-NH}_3^+$). Because the total of the positive charges must equal that of the negative charges at pI , each of the three ϵ -amino groups will be about one-third ionized. The pH at which they will be one-third ionized is higher than their pK_a 's.
 e. False. Peptide bonds have partial double-bond character and consequently no free rotation.
 f. False. Paper electrophoresis separates on the basis of net charge.
- 2.2 a. Pro. Pro is an imino acid; see Figure 2.8b.
 b. Phe
 c. Ser (or Thr)
 d. His
 e. Cys. The bridge is a disulfide bond, $\text{—CH}_2\text{—S—S—CH}_2\text{—}$.
 f. Thr, Val, Ile
 g. Arg, Lys, His
- 2.3 a. Inside: Val, Pro, Phe, Ile; outside: Asp, Lys, His.
 b. Gly (—H) and Ala (—CH_3) have small side chains and accordingly do not have enough atoms to be strongly hydrophobic. Thus they can be accommodated inside or outside.
 c. Ser, Thr, Asn, and Gln have uncharged polar side chains at $pH = 7$. They can participate in the formation of internal H-bonds, which neutralize their polar character.
 d. Inside, because two Cys residues often form a —S—S— disulfide bond, which neutralizes the polar character of both residues.
- 2.4 The complete structures are shown in Figure 2.8.
- 2.5 a. See Figure 2.9.
 b. By comparing the pH with the pK_{a_1} and pK_{a_2} values of Ser, it is easy to determine which state will predominate. Consider $pH = 1$. The pK_{a_1} of 2.2 for the reaction $\text{—COOH} \rightleftharpoons \text{—COO}^- + \text{H}^+$ indicates that at $pH = 2.2$ exactly half of the carboxyl groups are in the —COOH state, and half are in the —COO^- state. Below $pH = 2.2$ the —COOH state predominates. For the amino group, the pK_{a_2} of 9.2 for the reaction $\text{—NH}_3^+ \rightleftharpoons \text{—NH}_2 + \text{H}^+$ indicates that the —NH_3^+ state predominates at any pH below 9.2. At $pH = 1$ the NH_3^+ , COOH state, or state 4, predominates. (The fraction of molecules in each state could be calculated precisely from equation 2.2.)

The answers to part b are

pH	Predominant state
1	4
3	3
pI	3
7	3
11	2

- 2.6 a. The pI 's of Ala, Ser, Phe, and Leu are near $pH = 6$. Because the pI is the pH at which the net charge is 0, at $pH = 3.9$ these molecules have a net positive charge (i.e., some of their carboxyl groups are in the —COOH state, whereas virtually all of their amino groups are in the —NH_3^+ state). Ala, Ser, Phe, and Leu would move toward the cathode and would not separate. His and Arg with pI 's of 7.6 and 10.8, respectively, would move toward the cathode, and Asp with a pI of 3.0 would move toward the anode. His and Arg would separate from the other amino acids that move toward the cathode.



- A larger molecule will move more slowly than a smaller one with the same charge during electrophoresis, because the charge-to-mass ratio is smaller and accordingly the force causing migration is smaller per unit of mass.
- See Figure 2.10. A comparison of pI 's indicates that Glu, with a net negative charge, will move toward the anode; Lys, with a net positive charge, will move toward the

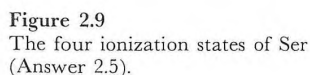
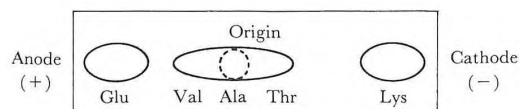


Figure 2.10
Electrophoretic separation of
Ala, Glu, Lys, Thr, and Val at
pH = 6.0 (Answer 2.6).



cathode. At pH = 6, Val, Ala, and Thr are near their isoelectric points. Though Thr might be expected to separate from Val and Ala, it does not do so cleanly in practice.

2.7 a.

	pH = 3	pH = 10
Phe-Ilu	Cathode	Anode
Lys-Lys-Lys	Cathode	Cathode
Arg-Asp	Cathode	Anode

b. The pK_{a_1} 's (α -carboxyl) for peptides are higher than for amino acids (≈ 3.2 versus ≈ 2.2). The pK_{a_2} 's (α -amino) are lower for peptides than for amino acids (≈ 8 versus ≈ 9.5). The pK_{a_3} 's are approximately the same regardless of whether the side chain is attached to an amino acid or to a peptide.

- 2.8 a. 1. At pH = 3.3 Asp has a net negative charge and accordingly has little charge attraction for the negatively charged sulfonic acid resin. Therefore, it passes most rapidly through the column.
2. Ser and Thr have a slight positive charge and are retained slightly through charge attraction by the column. The hydroxyl groups are polar and accordingly do not interact with the nonpolar polystyrene matrix.
3. Gly and Ala are somewhat positively charged; thus they are retained on the column. In addition, they are more hydrophobic than Ser and Thr and therefore elute somewhat later. At pH = 4.3 Gly and Ala are eluted from the column because their net positive charge is reduced.
4. Val, Met, Leu, and Ilu also are somewhat positively charged. Their very nonpolar side chains cause them to be retained by the nonpolar polystyrene longer than Gly and Ala.
5. Tyr and Phe have properties similar to Val, Met, Leu, and Ilu, and in addition have aromatic rings that interact by "stacking" with the aromatic rings of polystyrene.
6. The remaining amino acids are eluted only after a second pH increase, because their net positive charges require even more titration than any of the previous amino acids.
7. Arg is the most strongly positively charged of the amino acids; thus, it is retained longest.
- b. Ala is more hydrophobic than Gly. Tyr is less hydrophobic than Phe. Glu is more hydrophobic and less charged than Asp.

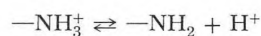
2.9 $20^{61} \approx 2 \times 10^{79}$. This figure is greater than the estimated number of atoms in the universe!

- 2.10 The $\alpha\text{-NH}_3^+$ and the $\alpha\text{-COO}^-$ charges more or less cancel one another at a slightly acidic pH and separation is on the basis of nonpolar character.
- a. Ilu is very hydrophobic and Lys is charged. Therefore, Lys would move very slowly and Ilu would move rapidly.
- b. Phe is less polar than Ser and moves considerably faster.
- c. $\text{Leu} > \text{Val} > \text{Ala}$. In other words, Leu moves faster than Val, which moves faster than Ala, because Leu is the most hydrophobic and Ala is the least hydrophobic of the three.

- d. Val > Pro
 e. Glu moves faster than Asp because more of Glu is in the nonionized state (necessary for solubility in the nonpolar solvent) and because the side chain of Glu is more hydrophobic than that of Asp.
 f. Tyr > Ala > Ser $\simeq$ His
- 2.11 The relative mobilities are
 a. Lys-Lys $\simeq$ Lys
 b. Leu-Leu-Leu > Leu
 c. Trp-Ilu-Phe > Arg-Ilu-Phe
 d. Ala-Ala-Ser > Cys-Cys-Ser
- 2.12 a. Gly₂₀ > Phe₂₀
 b. Asp₂₀ $\simeq$ Glu₂₀ at pH = 6.0
 c. Tyr₂₀ > Phe₂₀
- 2.13 a. As calculated by the Henderson-Hasselbalch equation (equation 2.2), a buffering range of $pK_a \pm 1$ encompasses 82% of the total buffering capacity of an ionizable group. From this definition the buffering ranges are

Gly	1.3 to 3.3	8.6 to 10.6	
His	0.8 to 2.8	8.2 to 10.2	5.0 to 7.0
Asp	1.1 to 3.1	8.8 to 10.8	2.9 to 4.9
Lys	1.2 to 3.2	8.0 to 10.0	9.5 to 11.5

- b. Asp or Glu at pH = 4
 His at pH = 6
 Almost any amino acid at pH = 9 (α -amino group)
 Arg at pH = 12
- c. Only ionizable groups have buffering capacity. At an equivalent concentration of amino acid residues the protein will be a much poorer buffer, because the α -amino and α -carboxyl groups of all but the terminal amino acids are in peptide linkages and are not charged.
- 2.14 a. This problem is a simple application of the Henderson-Hasselbalch equation (equation 2.2). The reaction being studied is



Therefore,

$$[-\text{NH}_3^+] = [\text{HA}] \quad \text{and} \quad [-\text{NH}_2] = [\text{A}^-]$$

At pH = 9.5,

$$9.5 = 10.5 + \log \frac{[-\text{NH}_2]}{[-\text{NH}_3^+]}$$

$$-1 = \log \frac{[-\text{NH}_2]}{[-\text{NH}_3^+]}$$

$$10^{-1} = \frac{[-\text{NH}_2]}{[-\text{NH}_3^+]} \quad \text{or} \quad \frac{[-\text{NH}_2]}{[-\text{NH}_3^+]} = \frac{1}{10}$$

Thus, the fraction of protonated molecules is

$$\frac{10}{10 + 1} = \frac{10}{11} \simeq 91\% \text{ at pH} = 9.5$$

- b. At pH = 11.0,

$$11.0 = 10.5 + \log \frac{[-\text{NH}_2]}{[-\text{NH}_3^+]}$$

$$0.5 = \log \frac{[-\text{NH}_2]}{[-\text{NH}_3^+]}$$

$$3.2 = \frac{[-\text{NH}_2]}{[-\text{NH}_3^+]}$$

thus,

$$\frac{[-\text{NH}_2]}{[-\text{NH}_3^+]} = \frac{3.2}{1}$$

and the fraction of protonated molecules is

$$\frac{1}{4.2} = 24\% \text{ at pH} = 11.0$$

- c. The more stable an ionic species, the more the equilibrium will shift to favor it. Delocalization of charge on an ion stabilizes it, whereas concentration of charge destabilizes it. Both $-\text{NH}_3^+$ and $-\text{COO}^-$ groups tend to withdraw electrons from nearby atoms and become relatively more negatively charged. Since the $\alpha\text{-NH}_3^+$ group must compete with the $\alpha\text{-COO}^-$ group for electrons, it carries a greater positive charge than the $\epsilon\text{-NH}_3^+$ group. Thus the $\alpha\text{-NH}_3^+$ group is less stable than the $\epsilon\text{-NH}_3^+$ group and the equilibrium for its dissociation is shifted away from $-\text{NH}_3^+$ toward $-\text{NH}_2$ and H^+ . Consequently, the K_a is higher and the $\text{p}K_a$ is lower for the $\alpha\text{-NH}_3^+$ group.

- 2.15 a. Using the Henderson-Hasselbalch equation,

$$5.0 = 4.3 + \log \frac{[-\text{COO}^-]}{[-\text{COOH}]}$$

$$0.7 = \log \frac{[-\text{COO}^-]}{[-\text{COOH}]}$$

$$5.0 = \frac{[-\text{COO}^-]}{[-\text{COOH}]}$$

$$\frac{5.0}{6.0} = 83\% \text{ at pH} = 5.0$$

b.

$$3.8 = 4.3 + \log \frac{[-\text{COO}^-]}{[-\text{COOH}]}$$

$$-0.5 = \log \frac{[-\text{COO}^-]}{[-\text{COOH}]}$$

$$0.5 = \log \frac{[-\text{COOH}]}{[-\text{COO}^-]}$$

$$3.2 = \frac{[-\text{COOH}]}{[-\text{COO}^-]}$$

$$\frac{1}{4.2} = 24\% \text{ at pH} = 3.8$$

- c. The electron-withdrawing $\alpha\text{-NH}_3^+$ group exerts an inductive effect, withdrawing electrons from the $\alpha\text{-COO}^-$ group, which therefore carries less negative charge than does the $\gamma\text{-COO}^-$ group. Thus the $\alpha\text{-COO}^-$ group is more stable than the $\gamma\text{-COO}^-$ group and the equilibrium for $\alpha\text{-COOH}$ dissociation is shifted toward $\alpha\text{-COO}^-$ and H^+ . Consequently, the K_a is higher and the $\text{p}K_a$ is lower for the $\alpha\text{-COOH}$ group.

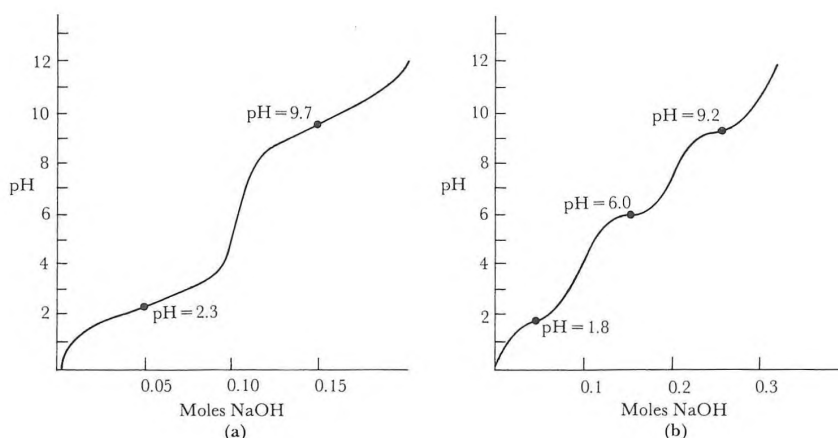


Figure 2.11
Titration curves of (a) Ala and
(b) His (Answer 2.16).

- 2.16 Titration problems can be approached using the Henderson-Hasselbalch equation (equation 2.2). For a given species of a titratable group at $\text{pH} = \text{p}K$, $[\text{A}^-]/[\text{HA}] = 1.0$; that is, 50% of the groups are in the A^- state. When $\text{pH} - \text{p}K = 1.0$, $[\text{A}^-]/[\text{HA}] = 10$, and $\frac{10}{11}$ or $\approx 91\%$ of the groups are in the A^- state. When $\text{pH} - \text{p}K = -0.5$, 24% are in the A^- state, and so on.

To titrate 0.1 mol of an amino acid will require 0.1 mol of NaOH per titratable group. (Assume that the amount of HCl present is negligible.)

- a. Ala: Inflection points will occur at pH values equal to the $\text{p}K_{\text{a}_1}$ and $\text{p}K_{\text{a}_2}$ values of 2.3 and 9.7. These points correspond to 0.05 and 0.15 mol of added NaOH. $\text{pH} = 1.3$ corresponds to $0.09 \times 0.10 \text{ mol} = 0.0090 \text{ mol}$ of NaOH added. $\text{pH} = 3.3$ corresponds to $0.91 \times 0.10 \text{ mol}$ or 0.091 mol added. $\text{pH} = 4.3$ corresponds to $0.99 \times 0.10 \text{ mol}$ or 0.099 mol of NaOH added. Thus the titration curve for the carboxyl group rises sharply until it meets the titration curve for the amino group (Figure 2.11a). As a general rule, if the $\text{p}K_{\text{a}}$ values of titratable groups are separated by more than 2.5 pH units their titration curves can be drawn independently.
- b. His: Inflection points occur at pH values of 1.8, 6.0, and 9.2, corresponding to the three $\text{p}K_{\text{a}}$ values. The titration curve is shown in Figure 2.11b.
- 2.17 a. Arg: The three inflection points are at pH values of 2.2, 9.0, and 12.5. The three titratable groups can be calculated independently.
- b. Asp: The three inflection points are at pH values of 2.1, 3.9, and 9.8. Note that the two lower $\text{p}K_{\text{a}}$ values are too close together to be completely independent, as you assumed in Problem 2.16.
- c. Each of the preceding titration curves has similar features resulting from the α -carboxyl and α -amino groups. The curves differ as a result of the absence or presence of a titratable side chain.
- 2.18 a. At $\text{pH} = \text{pI}$ the sum of the negative charges must equal the sum of the positive charges. Thus for carboxyproline the sum of the negative charges on the two carboxyl groups must equal 1 to balance the one full positive charge contributed by the α -amino group. The carboxyl groups will carry one negative charge at a pH midway between their $\text{p}K_{\text{a}}$ values. Verify this answer for yourself.

$$\text{pI} = \frac{\text{p}K_{\text{a}}(\alpha\text{-carboxyl}) + \text{p}K_{\text{a}}(\gamma\text{-carboxyl})}{2}$$

$$= \frac{2.0 + 3.8}{2}$$

$$\text{pI} = 2.9 \text{ for carboxyproline}$$

b. Similarly,

$$\begin{aligned} pI &= \frac{pK_a(\alpha\text{-amino}) + pK_a(\gamma\text{-amino})}{2} \\ &= \frac{9.8 + 10.0}{2} \end{aligned}$$

$$pI = 9.9 \text{ for aminoproline}$$

c. The ionization of the sulfhydryl group is $\text{SH} \rightleftharpoons \text{S}^- + \text{H}^+$, and $pK_a = 9.9$. At all pH values well below $\text{pH} = 9.9$ the sulfhydryl group will be uncharged. Therefore, as a first approximation, its ionization may be neglected.

$$pI = \frac{pK_{a_1} + pK_{a_2}}{2} = \frac{1.9 + 9.9}{2} = 5.9 \text{ for sulfhydrylproline}$$

At $\text{pH} = 5.9$ the sulfhydryl group is $> 99\%$ uncharged; therefore, our approximation is valid.