A. Derivation of the equation

Consider the release of a proton by a weak acid represented by HA:

$$\underset{\text{weak acid}}{HA} \rightleftarrows \underset{\text{proton}}{H^+} + \underset{\text{salt form or conjugate base}}{A^-}$$

The "salt" or conjugate base, A^-, is the ionized form of a weak acid. By definition, the dissociation constant of the acid, K_a, is

$$K_a \qquad [HA]$$

[Note: The larger the K_a, the stronger the acid, because most of the HA has been converted into H^+ and A". Conversely, the smaller the K_a, the less acid has dissociated and, therefore, the weaker the acid.] By solving for the $[H^+]$ in the above equation, taking the logarithm of both sides of the equation, multiplying both sides of the equation by - 1 , and substituting pH = -log $[H^+]$ and pK_a = -log K_a, we obtain the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

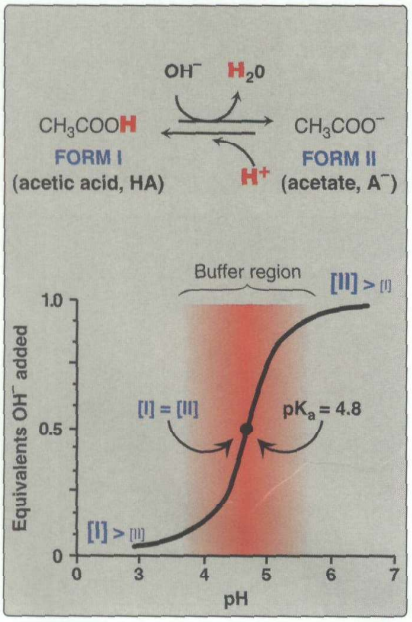


Figure 1.9
Titration curve of acetic acid.

B. Buffers

A buffer is a solution that resists change in pH following the addition of an acid or base. A buffer can be created by mixing a weak acid (HA) with its conjugate base (A^-). If an acid such as HCl is added to such a solution, A^- can neutralize it, in the process being converted to HA. If a base is added, HA can neutralize it, in the process being converted to A^-. Maximum buffering capacity occurs at a pH equal to the pK_a, but a conjugate acid/base pair can still serve as an effec tive buffer when the pH of a solution is within approximately + 1 pH unit of the pK_a. [Note: If the amounts of HA and A^- are equal, the pH is equal to the pK_a.] As shown in Figure 1.9, a solution containing acetic acid (HA = CH_3–COOH) and acetate (A^- = CH_3–COO^-) with a pK_a of 4.8 resists a change in pH from pH 3.8 to 5.8, with maxi mum buffering at pH = 4.8. [Note: At pH values less than the pK_a, the protonated acid form (CH_3–COOH) is the predominant species. At pH values greater than the pK_a, the deprotonated base form (CH_3–COO^-) is the predominant species in solution.]

C. Titration of an amino acid

1. **Dissociation of the carboxyl group:** The titration curve of an amino acid can be analyzed in the same way as described for acetic acid. Consider alanine, for example, which contains both an α-carboxyl and an α-amino group. At a low (acidic) pH, both of these groups

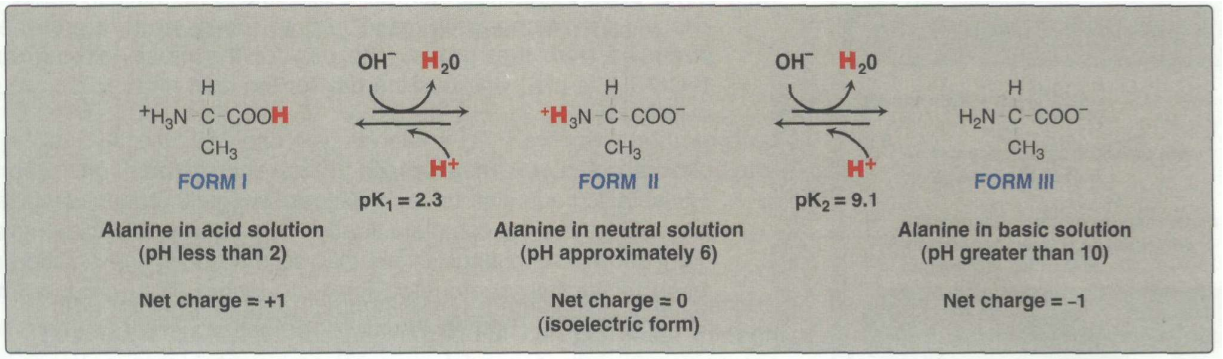


Figure 1.10
Ionic forms of alanine in acidic, neutral, and basic solutions.

are protonated (shown in Figure 1.10). As the pH of the solution is raised, the -COOH group of form I can dissociate by donating a proton to the medium. The release of a proton results in the formation of the carboxylate group, $-COO^-$. This structure is shown as form II, which is the **dipolar form** of the molecule (see Figure 1.10). [Note: This form, also called a zwitterion, is the **isoelectric form** of alanine—that is, it has an overall charge of zero.]

2. **Application of the** Henderson-Hasselbalch **equation:** The dissociation constant of the carboxyl group of an amino acid is called K_1, rather than K_a, because the molecule contains a second titratable group. The Henderson-Hasselbalch equation can be used to analyze the dissociation of the carboxyl group of alanine in the same way as described for acetic acid.

$$K_1 = \frac{[H^+][II]}{[I]}$$

where I is the fully protonated form of alanine, and II is the isoelectric form of alanine (see Figure 1.10). This equation can be rearranged and converted to its logarithmic form to yield:

$$pH = pK_1 + \log \frac{[II]}{[I]}$$

3. **Dissociation of the amino group:** The second titratable group of alanine is the amino ($-NH_3^+$) group shown in Figure 1.10. This is a much weaker acid than the -COOH group and, therefore, has a much smaller dissociation constant, K_2. [Note: Its pK_a is therefore larger.] Release of a proton from the protonated amino group of form II results in the fully deprotonated form of alanine, form III (see Figure 1.10).

4. **pKs of alanine:** The sequential dissociation of protons from the carboxyl and amino groups of alanine is summarized in Figure

1.10. Each titratable group has a pK_a that is numerically equal to the pH at which exactly one half of the protons have been removed from that group. The pK_a for the most acidic group ($-COOH$) is pK_1, whereas the pK_a for the next most acidic group ($-NH_3^+$) is pK_2.

5. Titration curve of alanine: By applying the Henderson-Hasselbalch equation to each dissociable acidic group, it is possi ble to calculate the complete titration curve of a weak acid. Figure 1.11 shows the change in pH that occurs during the addition of base to the fully protonated form of alanine (I) to produce the completely deprotonated form (III). Note the following:

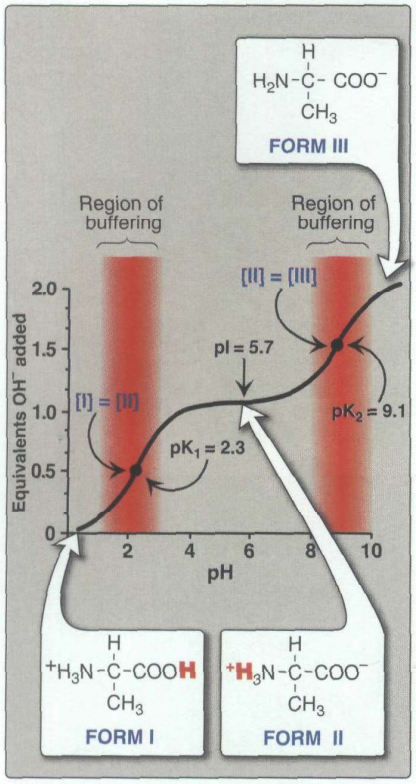


Figure 1.11
The titration curve of alanine.

a. Buffer pairs: The $-COOH/-COO^-$ pair can serve as a buffer in the pH region around pK_1, and the -NH3V-NH2 pair can buffer in the region around pK_2.

b. When pH = pK: When the pH is equal to pK_1 (2.3), equal amounts of forms I and II of alanine exist in solution. When the pH is equal to pK_2 (9.1), equal amounts of forms II and III are present in solution.

c. Isoelectric point: At neutral pH, alanine exists predominantly as the dipolar form II in which the amino and carboxyl groups are ionized, but the net charge is zero. The isoelectric point (pI) is the pH at which an amino acid is electrically neutral—that is, in which the sum of the positive charges equals the sum of the negative charges. [Note: For an amino acid, such as alanine, that has only two dissociable hydrogens (one from the α-carboxyl and one from the α-amino group), the pI is the average of pK_1 and pK_2 (pI = [2.3 + 9.1]/2 = 5.7, see Figure 1.10). The pI is thus midway between pK_1 (2.3) and pK_2 (9.1). It corresponds to the pH at which structure II (with a net charge of zero) predominates, and at which there are also equal amounts of form I (net charge of +1) and III (net charge of -1).]

6. Net charge of amino acids at neutral pH: At physiologic pH, all amino acids have a negatively charged group ($-COO^-$) and a positively charged group ($-NH_3^+$), both attached to the α-carbon. [Note: Glutamate, aspartate, histidine, arginine, and lysine have additional potentially charged groups in their side chains.] Substances, such as amino acids, that can act either as an acid or a base are defined as **amphoteric,** and are referred to as **ampholytes (amphoteric electrolytes).**

D. Other applications of the Henderson-Hasselbalch equation

The Henderson-Hasselbalch equation can be used to calculate how the pH of a physiologic solution responds to changes in the concen tration of weak acid and/or its corresponding "salt" form. For example, in the **bicarbonate buffer system,** the Henderson-Hasselbalch equa tion predicts how shifts in $[HCO_3^-]$ and pCO_2 influence pH (Figure 1.12A). The equation is also useful for calculating the abundance of ionic forms of acidic and basic drugs. For example, most drugs are

either weak acids or weak bases (Figure 1.12B). Acidic drugs (HA) release a proton (H^+), causing a charged anion (A") to form.

$$HA \rightleftarrows H^+ + A^-$$

Weak bases (BH^+) can also release a H^+. However, the protonated form of basic drugs is usually charged, and the loss of a proton produces the uncharged base (B).

$$BH^+ \rightleftarrows B + H^+$$

A drug passes through membranes more readily if it is uncharged. Thus, for a weak acid, the uncharged HA can permeate through membranes and A^- cannot. For a weak base, such as morphine, the uncharged form, B, penetrates through the cell membrane and BH^+ does not. Therefore, the effective concentration of the permeable form of each drug at its absorption site is determined by the relative concentrations of the charged and uncharged forms. The ratio between the two forms is, in turn, determined by the pH at the site of absorption, and by the strength of the weak acid or base, which is represented by the pK_a of the ionizable group. The Henderson-Hasselbalch equation is useful in determining how much drug is found on either side of a membrane that separates two compartments that differ in pH, for example, the stomach (pH 1.0-1.5) and blood plasma (pH 7.4).

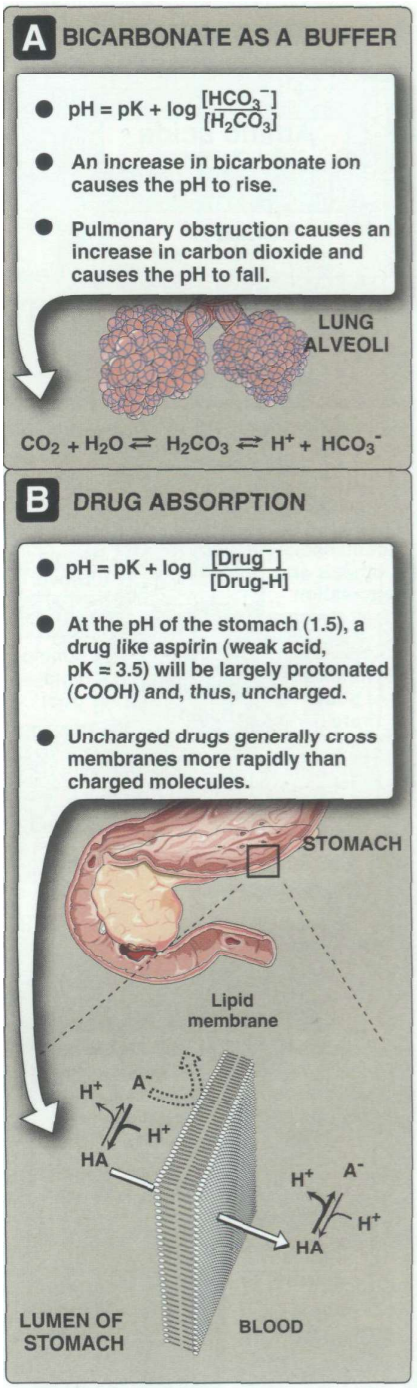


Figure 1.12
The Henderson-Hasselbalch equation is used to predict: A, changes in pH as the concentrations of HCO_3^- or CO_2 are altered; or B, the ionic forms of drugs.

IV. CONCEPT MAPS

Students sometimes view biochemistry as a blur of facts or equations to be memorized, rather than a body of concepts to be understood. Details provided to enrich understanding of these concepts inadvertently turn into distractions. What seems to be missing is a road map—a guide that provides the student with an intuitive understanding of how various topics fit together to make sense. The authors have, therefore, created a series of **biochemical concept maps** to graphically illustrate relationships between ideas presented in a chapter, and to show how the information can be grouped or organized. A concept map is, thus, a tool for visualizing the connections between concepts. Material is represented in a hierarchical fashion, with the most inclusive, most general concepts at the top of the map, and the more specific, less general concepts arranged beneath.

A. How is a concept map constructed?

1. **Concept boxes and links:** Educators define concepts as "perceived regularities in events or objects." In our biochemical maps, concepts include abstractions (for example, free energy), processes (for example, oxidative phosphorylation), and compounds (for example, glucose 6-phosphate). These broadly defined concepts are prioritized with the central idea positioned at the top of the page. The concepts that follow from this central idea are then drawn in boxes (Figure 1.13A). The size of the box and type indicate the relative importance of each idea. Lines are drawn between concept boxes to show which are related. The label on

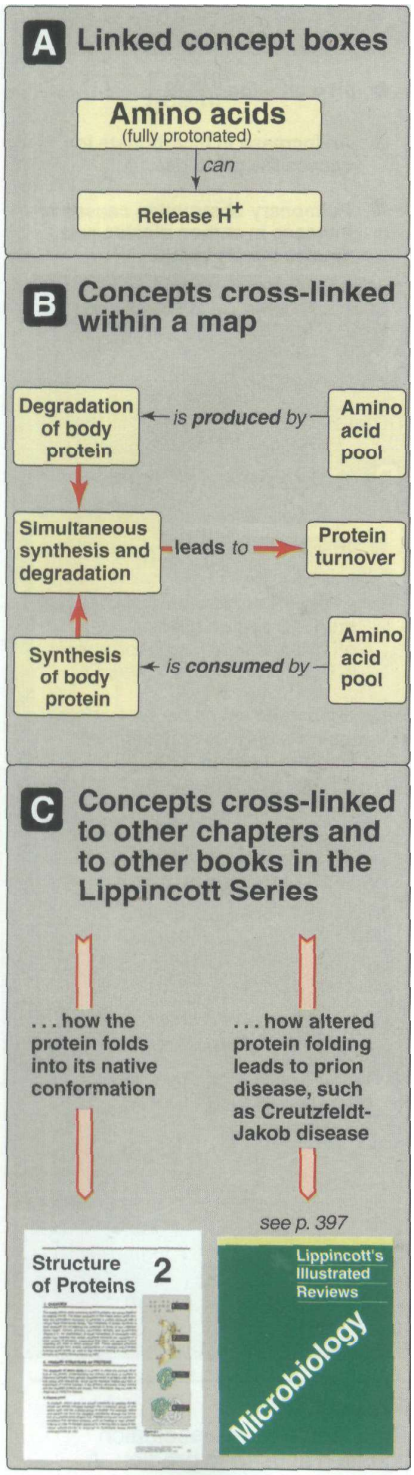


Figure 1.13
Symbols used in concept maps.

the line defines the relationship between two concepts, so that it reads as a valid statement, that is, the connection creates meaning. The lines with arrowheads indicate which direction the connection should be read.

2 **Cross-links:** Unlike linear flow charts or outlines, concept maps may contain cross-links that allow the reader to visualize complex relationships between ideas represented in different parts of the map (Figure 1.13B), or between the map and other chapters in this book, or companion books in the series (Figure 1.13C). Cross-links can thus identify concepts that are central to more than one discipline, empowering students to be effective in clinical situations, and on the United States Medical Licensure Examination (USMLE) or other examinations, that bridge disciplinary boundaries. Students learn to visually perceive non-linear relationships between facts, in contrast to cross referencing within linear text.

B. Concept maps and meaningful learning

"Meaningful learning" refers to a process in which students link new information to relevant concepts that they already possess. To learn meaningfully, individuals must consciously choose to relate new information to knowledge that they already know, rather than simply memorizing isolated facts or concept definitions. Rote is undesirable because such learning is easily forgotten, and is not readily applied in new problem-solving situations. Thus, the concept maps prepared by the authors should not be memorized. This would merely promote rote learning and defeat the purpose of the maps. Rather, the concept maps ideally function as templates or guides for organizing information, so the student can readily find the best ways to integrate new information into knowledge they already possess.

V. CHAPTER SUMMARY

Each amino acid has an **α-carboxyl group** and an **α-amino group** (except for proline, which has an **imino group).** At physiologic pH, the α-carboxyl group is dissociated, forming the negatively charged carboxylate ion (-C00~), and the α-amino group is protonated ($-NH_3^+$). Each amino acid also contains one of twenty distinctive **side chains** attached to the α-carbon atom. The chemical nature of this side chain determines the function of an amino acid in a protein, and provides the basis for classification of the amino acids as **nonpolar, uncharged polar, acidic,** or **basic.** All free amino acids, plus charged amino acids in peptide chains, can serve as **buffers.** The quantitative relationship between the concentration of a weak acid (HA) and its conjugate base (A^-) is described by the **Henderson-Hasselbalch equation.** Buffering occurs within ±1 pH unit of the pK_a, and is maximal when pH = pK_a, at which $[A^-]$ = [HA]. The α-carbon of each amino acid (except glycine) is attached to four different chemical groups and is, therefore, a **chiral** or **optically active** carbon atom. Only the L-form of amino acids is found in proteins synthesized by the human body.

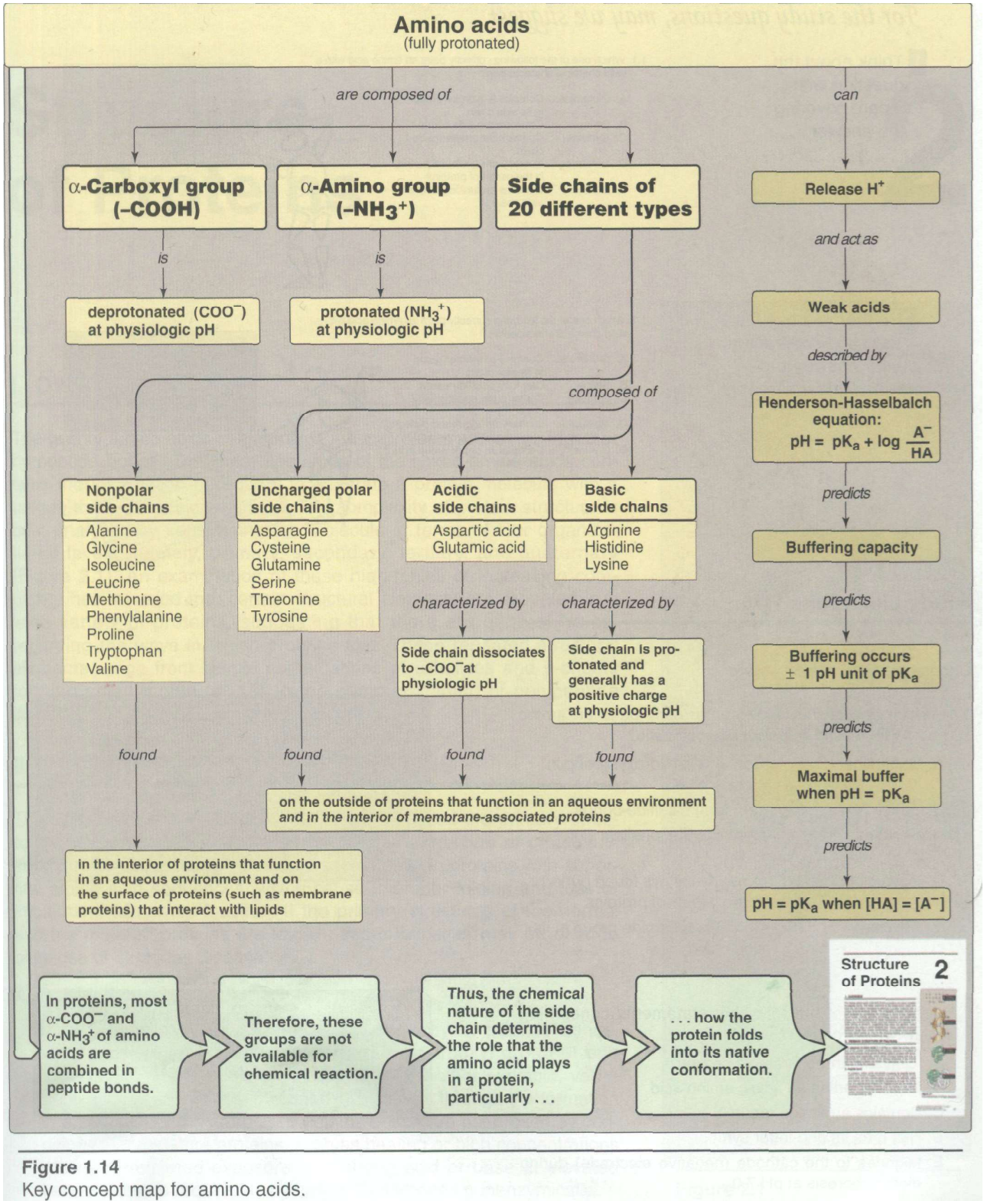


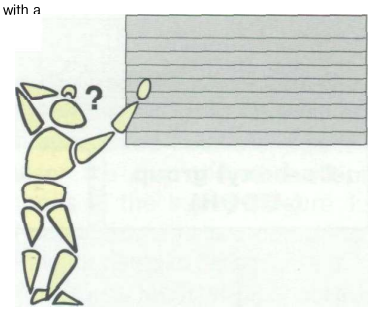
Figure 1.14
Key concept map for amino acids.

for the study questions, may we suggest...

1 Think about the question with a card covering the answer . . .

1.1 Which one of the following correctly pairs an amino acid with a valid chemical characteristic?

A. Glutamine: Contains a hydroxyl group in its side chain
B. Serine: Can form disulfide bonds
C. Cysteine: Contains the smallest side chain
D. Isoleucine: Is nearly always found buried in the center of proteins
E. Glycine: Contains an amide group in its side chain



2 . .then remove the card and confirm that your answer and reasoning are correct.

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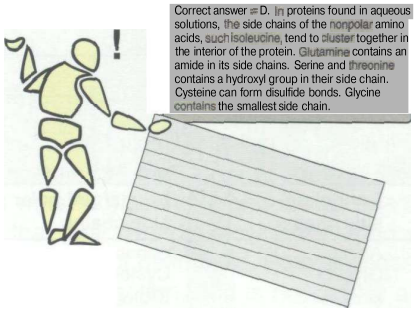

Study Questions

Choose the ONE correct answer

1.1 Which one of the following correctly pairs an amino acid with a valid chemical characteristic?

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C. Cysteine: Contains the smallest side chain
D. Isoleucine: Is nearly always found buried in the center of proteins
E. Glycine: Contains an amide group in its side chain

Correct answer = D. In proteins found in aqueous solutions, the side chains of the nonpolar amino acids, such isoleucine, tend to cluster together in the interior of the protein. Glutamine contains an amide in its side chain. Serine and threonine contain a hydroxyl group in their side chain. Cysteine can form disulfide bonds. Glycine contains the smallest side chain.

1.2 Which one of the following statements concerning glutamine is correct?

A. Contains three titratable groups
B. Is classified as an acidic amino acid
C. Contains an amide group
D. Has E as its one-letter symbol
E. Migrates to the cathode (negative electrode) during electrophoresis at pH 7.0

Correct answer = C. Glutamine contains two titratable groups, α-carboxyl and α-amino. Glutamine is a polar, neutral amino acid that shows little electrophoretic migration at pH 7.0. The symbol for glutamine is "Q."