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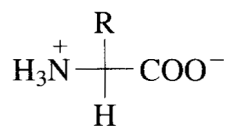
Amino Acids

This chapter introduces you to the structure and chemistry of amino acids. The chapter begins by discussing the zwitterionic character of amino acids at physiological pH, followed by a brief introduction to the amide linkage known as the peptide bond. Amino acids are categorized by the chemistry of their unique R groups and by their mode of biosynthesis. The standard amino acids are specified by the genetic code; nonstandard amino acids include the D stereoisomers and modified forms of the standard amino acids. This chapter also introduces you to the conventions used to describe stereoisomers, namely the Fischer convention and the *RS* system. Amino acids contain ionizable groups, so this chapter discusses the acid–base properties of amino acids and introduces you to the concept of the isoelectric point. A clear understanding of the acid–base properties of amino acids is critical for appreciating the structural and catalytic behavior of proteins. The chapter closes with a brief discussion of the biological significance of some amino acid derivatives.

Essential Concepts

Amino Acid Structure

1. All proteins are composed of 20 standard amino acids, which are specified by the genetic code.
2. The standard amino acids are called α -amino acids because they have a primary amino group and a carboxyl group bound to the same carbon atom (the α carbon). Only proline has a secondary amino group attached to the α carbon, but it is still commonly referred to as an α -amino acid.
3. The generic structure of an amino acid at pH 7 is shown below.



At pH 7, the amino acid is a zwitterion, or dipolar ion. A unique side chain, or R group, characterizes each amino acid.

4. Amino acids are polymerized by condensation reactions to form a chain called a polypeptide. Each polypeptide is polarized: One end has a free amino group and the other end has a free carboxyl group, referred to as the N-terminus and the C-terminus, respectively.
5. The R groups of the standard 20 amino acids are classified into three categories based on their polarities and charge at pH 7: the nonpolar amino acids, the polar uncharged amino acids, and the charged amino acids (see Table 4-1).
6. Among the nonpolar amino acids, glycine (shorthand Gly or G) has a hydrogen atom as its R group. Alanine (Ala; A), valine (Val; V), leucine (Leu; L), isoleucine (Ile; I), and methionine (Met; M) have aliphatic chains as R groups (Met has a sulfur rather than a methylene group). Tryptophan (Trp; W) and phenylalanine (Phe; F) contain bulky indole and phenyl groups, respectively.
7. The polar uncharged amino acids include asparagine (Asn; N), glutamine (Gln; Q), serine (Ser; S), threonine (Thr; T), tyrosine (Tyr; Y), and cysteine (Cys; C). Amide functional groups occur in Gln and Asn. Alcoholic functional groups occur in Ser and Thr. Tyrosine and cysteine are characterized by a phenolic group and a thiol group, respectively.
8. Among the charged amino acids, aspartate (Asp; D) and glutamate (Glu; E) contain carboxylic groups in their R groups. Lysine (Lys; K), arginine (Arg; R), and histidine (His; H) contain a butylammonium group, a guanidino group, and an imidazole group, respectively.
9. The pK values of ionizable groups depend on the electrostatic influences of nearby groups. Inside of proteins, the pK values of ionizable R groups may shift by several pH units from their values in the free amino acids.

Stereochemistry

10. Except for glycine, the standard amino acids have asymmetric structures and rotate the plane of polarized light; thus, they are optically active. These molecules cannot be superimposed on their mirror images. Such nonsuperimposable pairs of molecules are called enantiomers. The asymmetric atom of an optically active molecule is called the chiral center and the molecule is said to have the property of chirality.
11. Fischer projections are used to represent the absolute configuration of substituents around a chiral center. In the Fischer convention, horizontal lines extend above the plane of the paper, while vertical lines extend below the surface of the paper. The α -amino acid shown above is a Fischer projection of an L-amino acid. The specific arrangement of substituents around the chiral carbon is related to that of L-glyceraldehyde.

12. A molecule with n chiral centers has 2^n different possible stereoisomers. Molecules with two or more chiral centers are better described by the *RS* system, in which each substituent bound to the chiral center is prioritized according to its atomic number. Hence, the exact molecular arrangement of a molecule can be unambiguously described.
13. Biochemical reactions almost invariably produce pure stereoisomers, in large part due to the precise arrangement of chiral groups inside of enzymes, which restricts the geometry of the reactants.

Amino Acid Derivatives

14. Nonstandard amino acids in polypeptides arise from posttranslational modifications of the R groups of amino acid residues of the polypeptide. These modifications have critical roles in the structure and function of proteins. Many unpolymerized nonstandard amino acids are synthesized by chemical modifications of one of the standard amino acids. Cells use many of these amino acids as signaling molecules, particularly in the central nervous system. Among the nonstandard amino acids are also the D isomers of the standard amino acids; many of these occur in bacterial cell walls and bacterially produced antibiotics. The tripeptide glutathione is a cellular reducing agent.

Key Equation

$$pI = \frac{1}{2}(pK_i + pK_j)$$

Guide to Study Exercises (text p. 92)

1. See Table 4-1.
2. *Polarity.* The nonpolar amino acids are alanine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, and valine. Six of the polar amino acids are uncharged: these are asparagine, cysteine, glutamine, serine, threonine, and tyrosine. Five polar amino acids are charged; these are arginine, aspartate, histidine, glutamate, and lysine.

Structure. There are many ways to classify amino acids based on the structures of their side chains. Glycine has the smallest side chain, just an H atom. Amino acids with aliphatic side chains are alanine, isoleucine, leucine, valine, and proline (which is really a cyclic imino acid). The sulfur-containing amino acids are cysteine and methionine; the aromatic amino acids are phenylalanine, tryptophan, and tyrosine. Serine and threonine have simple alcoholic side chains. Similarly, aspartate and glutamate have small carboxylic acid side chains; their amide forms are asparagine and glutamine. Arginine, histidine, and lysine have basic side chains with nitrogenous groups.

Type of functional group. Eight of the amino acids have side chains without functional groups: alanine, glycine, isoleucine, leucine, phenylalanine, proline, tryptophan, and valine. Methionine, whose thiomethyl group is unreactive, also belongs to this class of amino

acids. Three amino acids are alcohols: serine, threonine, and tyrosine. Cysteine, with its thiol group, could also be classified with the alcohols. Three of the amino acids have basic functional groups: arginine, histidine, and lysine. Aspartate and glutamate are acidic amino acids. Their counterparts asparagine and glutamine are amides.

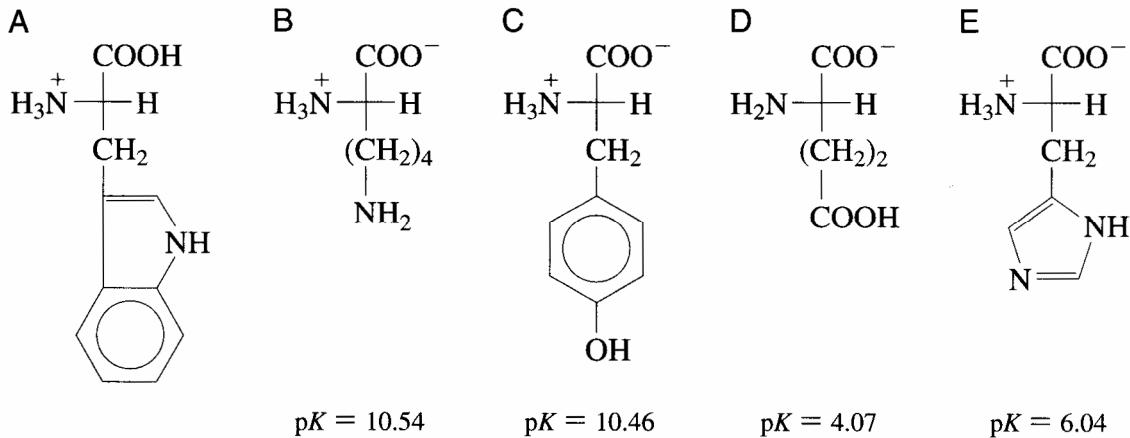
Acid–base properties. All of the amino acids have an acidic group (COOH) and a basic group (NH₂) attached to the α carbon. Two of the amino acids have very acidic side chains: aspartate and glutamate. In certain cases, the side chains of cysteine and tyrosine can also ionize. Three of the amino acids have basic side chains: arginine, histidine, and lysine. (Section 4-1)

- Five amino acids have side chains that are typically ionized at physiological pH (the side chains of cysteine and tyrosine sometimes ionize). Of these five, two are negatively charged: The β -COOH group of aspartic acid has a pK of 3.90, and the γ -COOH group of glutamic acid has a pK of 4.07. Therefore, at pH 7, these groups are entirely in the COO⁻ form. Two amino acids are positively charged at physiological pH since their pK 's are much greater than the physiological pH of 7: The guanidino group of arginine has a pK of 12.48, and the ϵ -amino group of lysine has a pK of 10.54. Only one amino acid, histidine, whose imidazole group has a pK of 6.04, exists in both the neutral and protonated forms at physiological pH. Keep in mind that the pK of an ionizable group is influenced by its microenvironment, so the pK values of amino acids may vary by several pH units when the amino acid is part of a folded polypeptide chain. (Sections 4-1C and D)
- The Fischer convention describes the absolute configuration of a chiral molecule by referring it to one of the two stereoisomers of glyceraldehyde. The comparison is made by mentally replacing the H, OH, CHO, and CH₂OH groups of D- or L-glyceraldehyde with the groups around the chiral center of the other molecule. (Section 4-2)
- Amino acids in proteins may be covalently modified by hydroxylation, methylation, acetylation, carboxylation, and phosphorylation. In addition, larger groups such as lipids and carbohydrates may be attached. (Section 4-3A)

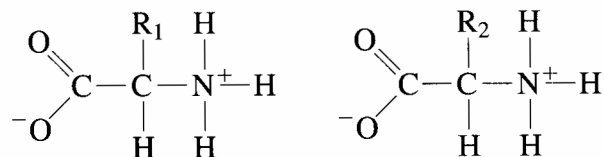
Questions

Amino Acid Structure

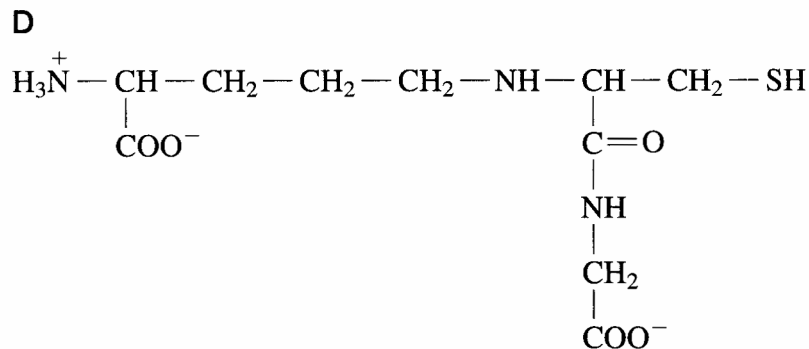
- Without consulting the text, draw a generic L- α -amino acid using the Fischer convention.
- Examine the amino acids on the following page. Assume that the pK of the carboxyl group attached to the α carbon is 2.0 and that of the primary amine attached to the α carbon is 9.5. The pK 's of ionizable R groups are shown below each structure. (a) Categorize these amino acids as nonpolar, uncharged polar, or charged polar at pH 7. (b) Which of the structures cannot exist as shown at any pH in aqueous solution? (c) Name each of the amino acids.



- Which of the amino acids in Table 4-1 can be converted to another amino acid by mild hydrolysis that liberates ammonia?
- Which of the amino acids in Table 4-1 can generate a new amino acid by the addition of a hydroxyl group?
- The ionic characters of which amino acids are likely to be sensitive to pH changes in the physiological range? Explain.
- Circle the functional groups that are eliminated in the formation of a peptide bond between the amino acids shown below. Draw the structure of the dipeptide.

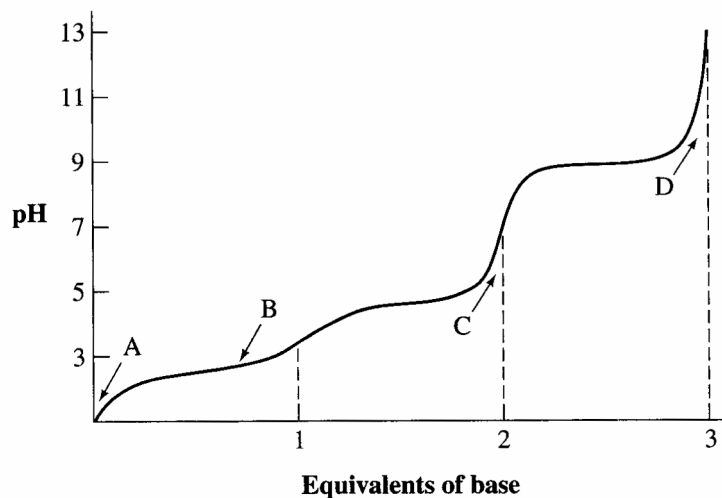


- What percentage of the histidine imidazole group is protonated at pH 7.2?
- What percentage of the cysteine sulfhydryl group is deprotonated at pH 7.6?
- In proteins, the imidazole groups of histidine play key roles in catalysis involving reversible protonation/deprotonation events. The pK of the imidazole group is influenced by surrounding amino acids and in many enzymes is near 7.
 - What is the significance of this apparent pK in catalysis?
 - Some unprotonated imidazole groups participate in hydrogen bonding a substrate to an enzyme. Do you expect the pK of these imidazole groups also to be near 7?



12. Why is the pK of the carboxyl group of glycine ($pK = 2.3$) less than that for acetic acid ($pK = 4.76$)?
13. Calculate the pI 's of aspartate, lysine and serine.
14. In proteins, the pK of the C-terminus is about 3.8, while that of the N-terminus is about 7.8. Rationalize these differences from the pK values of the α -carboxyl and the α -amino groups in free amino acids.
15. Is a protein as good a *cellular* buffer at physiological pH as its constituent amino acids would be if they were present as free amino acids in proportional concentrations in the cell? Explain.
16. 100 mg of anhydrous powder of lysine is not completely soluble in 10 mL of water but dissolves completely when base is added. Explain.
17. Examine the paper by McCoy, Meyer, and Rose (*J. Biol. Chem.* 112, 283–302, 1935) at the web site for the Journal of Biological Chemistry (<http://www.jbc.org>).
 - (a) What was the name of Unknown II? What is its name today?
 - (b) What remained to be solved about its structure?

18. Shown below is a titration curve for glutamic acid. Draw the structure of the species that predominates at each labeled point.

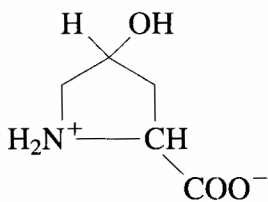


Stereochemistry

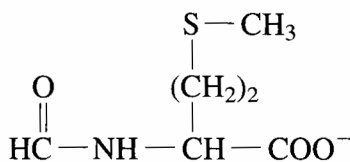
19. Which amino acid found in proteins has no optical activity?
20. Draw Fischer projections of L-aspartate and L-cysteine and show why L-Asp is (*S*)-Asp and L-Cys is (*R*)-Cys. (Refer to Box 4-2)
21. Which amino acids in Table 4-1 have two or more prochiral centers? A prochiral center can be made chiral by substituting a different group for one of the two identical groups attached to it.

Amino Acid Derivatives

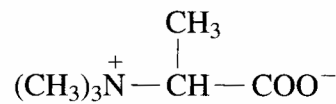
22. Which of the nonstandard amino acids below cannot occur in the interior of a polypeptide chain? Explain.



4-hydroxyproline



***N*-formylmethionine**



***N,N,N*-trimethylalanine**

23. From which of the standard amino acids are the following physiologically active amines derived? What modifications gave rise to these products? (See Figure 4-16 for structures.)

(a) GABA

(b) histamine

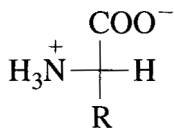
(c) thyroxine

(d) dopamine

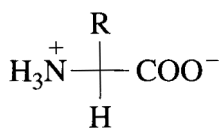
24. How many conjugated double bonds occur in the fluorescent tyrosine derivative of green fluorescent protein?

Answers to Questions

1.



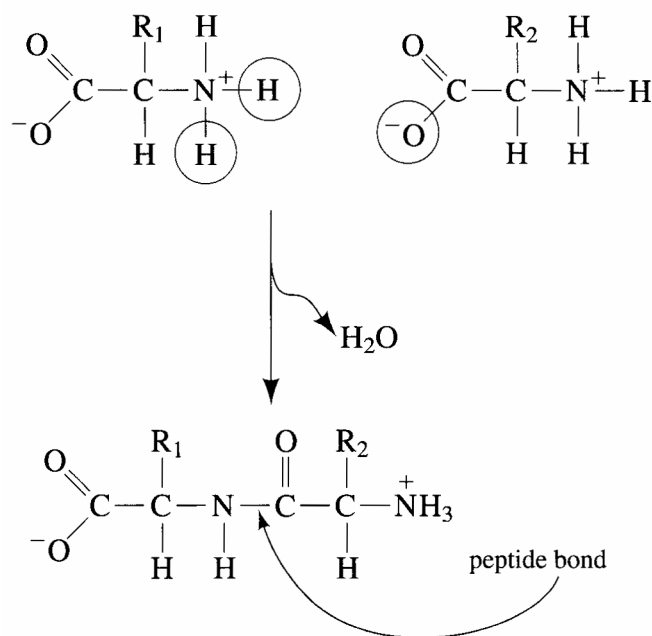
or



Remember, in Fischer projections, horizontal bonds extend out from the paper while vertical bonds extend behind the paper.

2. (a) Nonpolar: **A**; uncharged, polar: **C** and **E** (because **E** has a pK of 6.0, it is largely uncharged at pH 7); charged, polar: **B** and **D** (at pH 7, the ϵ -amino group of **B** is positively charged, and the γ -carboxyl group of **D** is negatively charged).
 (b) **B** and **D** cannot exist at any pH in aqueous solution. In **B**, the ϵ -amino group will become deprotonated at lower pH values than the α -amino group since its pK is lower. Similarly, the γ -carboxyl group of **D** is more acidic than the α -amino group.
 (c) **A** is tryptophan; **B** is lysine; **C** is tyrosine; **D** is glutamate; and **E** is histidine.
3. Glutamine and asparagine can be converted to glutamate and aspartate, respectively.
4. Alanine gives rise to serine, and phenylalanine gives rise to tyrosine.
5. Histidine and cysteine are the most likely to be sensitive in the physiological pH range since the pK values of their R groups are 6.04 and 8.37, respectively.

6.



7. Use the Henderson–Hasselbalch equation and rearrange terms to find the percentage of protonated species:

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

$$\frac{[\text{A}^-]}{[\text{HA}]} = 10^{(\text{pH} - \text{pK})}$$

$$= 10^{(7.2 - 6.04)}$$

$$= 10^{1.16} = 14.45$$

To obtain the percentage of the protonated species,

$$\% \text{ HA} = \frac{[\text{HA}]}{[\text{HA}] + [\text{A}^-]} \times 100$$

$$= \frac{1}{1 + 14.45} \times 100$$

$$= 6.47\%$$

Hence, 6.47% is in the protonated form.

8. Use the Henderson–Hasselbalch equation and rearrange terms to find the percentage of deprotonated species:

$$\text{pH} = \text{p}K + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

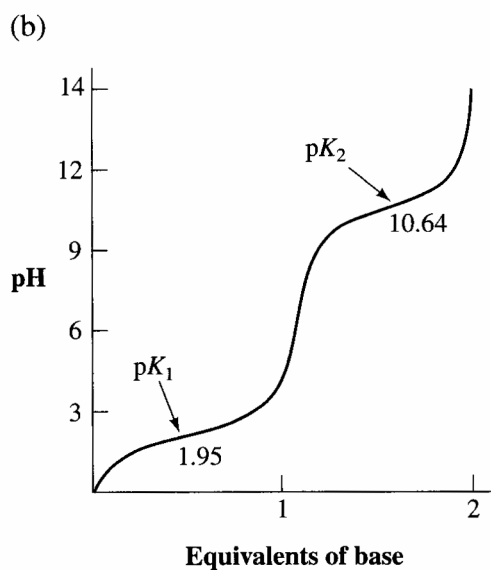
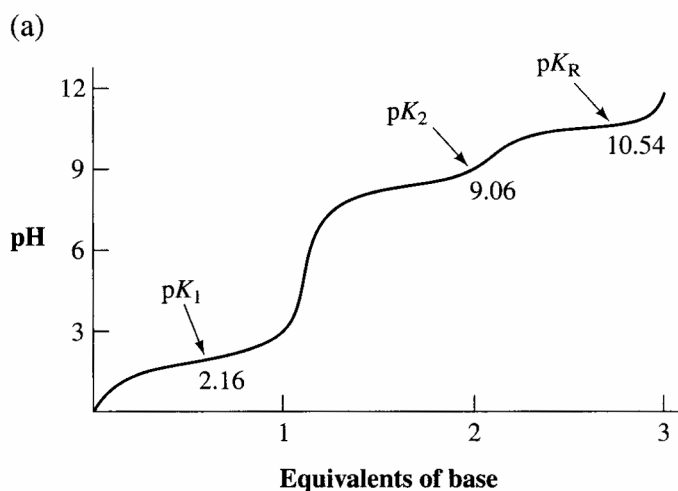
$$\begin{aligned} \frac{[\text{A}^-]}{[\text{HA}]} &= 10^{(\text{pH} - \text{p}K)} \\ &= 10^{(7.6 - 8.37)} \\ &= 10^{-0.77} = 0.17 \end{aligned}$$

$$\begin{aligned} \% \text{A}^- &= \frac{[\text{A}^-]}{[\text{HA}] + [\text{A}^-]} \times 100 \\ &= \frac{0.17}{1 + 0.17} \times 100 \\ &= 14.53\% \end{aligned}$$

Therefore, about 14.53% of the sulfhydryl groups are deprotonated.

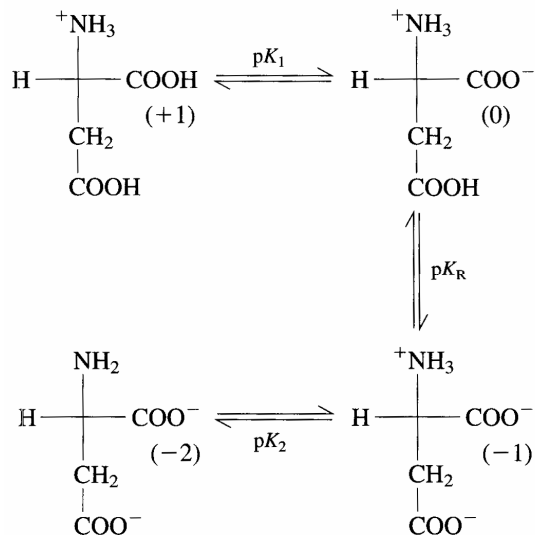
9. (a) The intracellular pH is also near 7, so about half the imidazole groups will be protonated. In other words, the imidazole groups can easily abstract a proton or give one up, as is required for catalysis, and remain unchanged at the end of the reaction.
- (b) It is more likely that the p*K* of these groups is near the p*K* of free histidine (6.04) so that they are available to form hydrogen bonds with potential substrates.

10. (a) Lysine; tyrosine is similar but its pK_2 is closer to 9.2.
 (b) Proline; it has only two pK 's, one at about 2 and the other well above 10. The only other amino acid with a pK_2 above 10 is cysteine, but it has three pK 's.



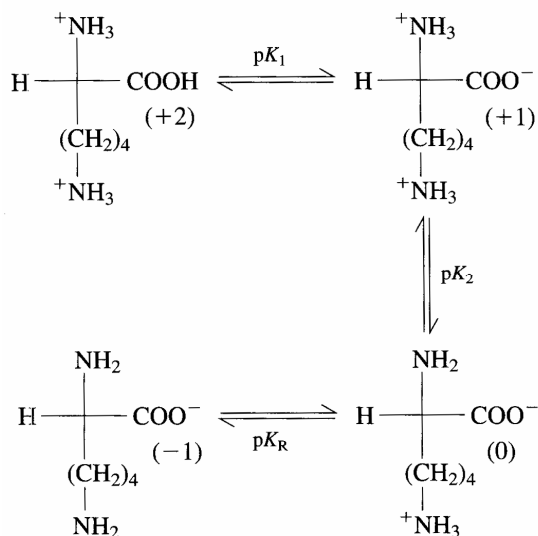
11. A, C, and D contain peptide bonds not commonly found in proteins. In A, the γ -carboxylate group of the first amino acid participates in an amide linkage (the second and third amino acids are linked by a conventional peptide bond). C contains a linkage between the carboxylate of a β -amino acid and the amino group of an α -amino acid. In D, the side chain of the first amino acid is linked to the amino group of the second. Tripeptide B can be referred to as serylvalylglycine.
12. The positively charged amino group of glycine electrostatically stabilizes the nearby carboxylate ion. Hence, the carboxylic acid group of glycine dissociates more readily to form the carboxylate ion than does the carboxylic acid group of acetic acid.

13. The pI is approximately midway between the pK 's of the two ionizations involving the neutral species. In amino acids with ionizable side chains, the relevant pK 's may be pK_1 and pK_R or pK_R and pK_2 . For aspartate, the neutral species results from ionization of the α -COOH (pK_1) and the β -COOH (pK_R):



$$pI = (pK_1 + pK_R)/2 = (1.99 + 3.90)/2 = 2.95.$$

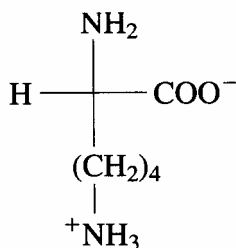
For serine, which has no ionizable R group, $pI = (pK_1 + pK_2)/2 = (2.19 + 9.21)/2 = 5.7$.



For lysine, the pI lies between pK_2 and pK_R :

$$\text{Hence, } pI = (pK_2 + pK_R)/2 = (9.06 + 10.54)/2 = 9.8.$$

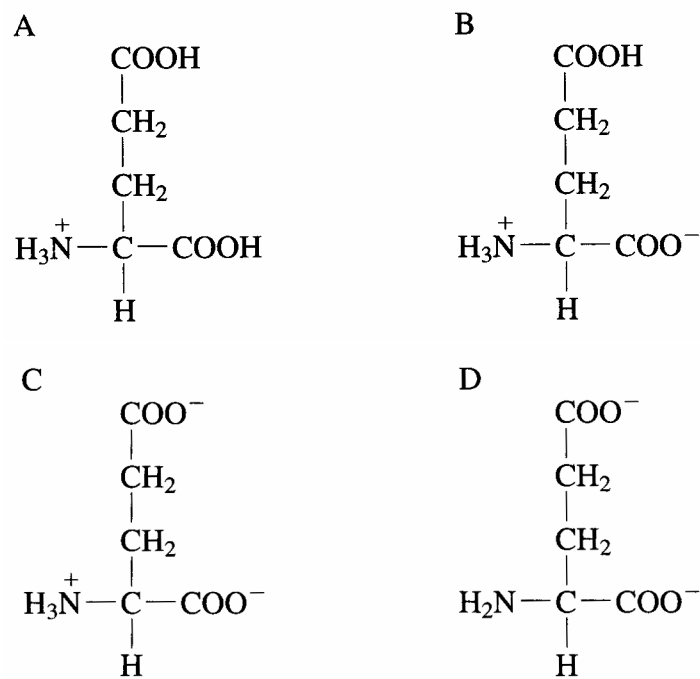
14. In free amino acids, the neighboring α -carboxyl and α -amino groups affect each other's pK values. For instance, the negatively charged carboxyl group stabilizes the protonated amino group, making it a weaker acid and thus raising its pK value. Similarly, the positively charged amino group stabilizes the anionic carboxyl group, making it a stronger acid and thereby lowering its pK value. In proteins, the free amino group and the free carboxyl group are separated by at least ~ 40 amino acid linkages, and hence have little or no effect on each other's pK values.
15. Yes. The formation of the peptide bond between each amino acid in the intact protein eliminates two ionizable groups. However, at physiological pH (pH 5–9), the α -carboxyl and the α -amino groups of amino acids are poor buffers, because their respective pK values are far from the physiological pH range. However, at physiological pH, a protein containing a number of histidines and cysteines, for instance, may have a buffering capacity similar to that of an equimolar solution of its constituent amino acids; the side groups of these amino acids are free to ionize regardless of whether they are free or in a protein. Other ionizable R groups have pK 's too far from physiological pH to be effective buffers.
16. Lysine solid is of necessity neutral and therefore has the structure



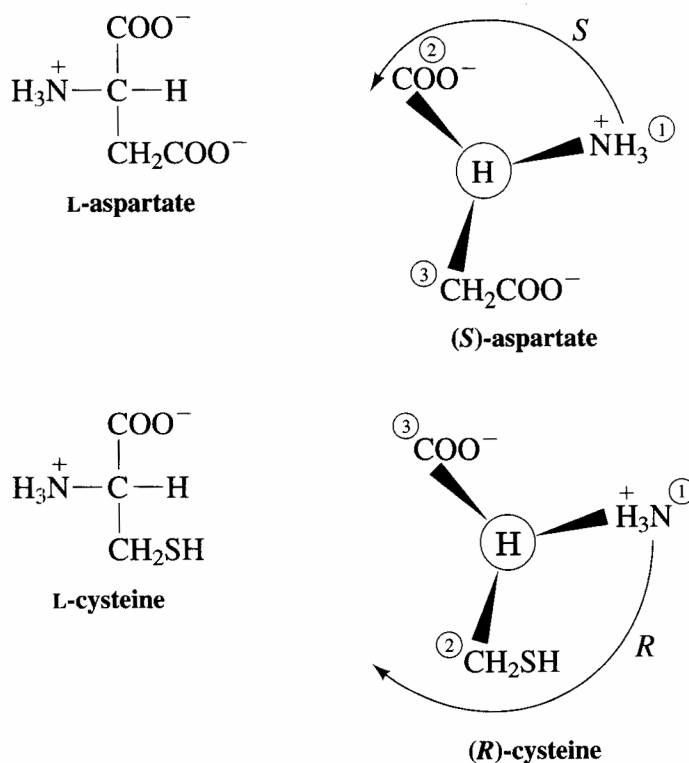
Neutral species are not as soluble as charged species. Adding base causes lysine's ϵ - NH_3^+ group to be converted to the neutral ϵ - NH_2 , giving the molecule a net negative charge and higher solubility.

17. (a) α -Amino- β -hydroxybutyric acid (later named threonine)
 (b) The chirality of the β -carbon.

18.

19. Glycine; its C_α has two identical substituents, H.

20. In a Fischer projection, the vertical bonds by convention point into the paper while the horizontal bonds point out from the paper. When the carbon groups are aligned vertically as drawn below, the amino group is on the left in an L-amino acid; it is on the right in a D-amino acid. To identify *R* or *S* configurations, swing the α -carbon hydrogen behind the α carbon. Then assign a priority number based on the atomic mass of the atom or group bound to the α carbon. The configuration is *R* or *S* according to whether the groups with decreasing priority have a clockwise or counterclockwise orientation, respectively. If two substituent atoms are the same (C, for example), the other atoms attached to them are used to assign priority. Note that for cysteine, sulfur has a higher atomic mass than oxygen, which reverses the priority of the groups. (See figure below.)



21. The amino acids with two or more prochiral centers are Arg, Gln, Glu, Leu, Lys, Met, and Pro.
22. *N*-formylmethionine and *N,N,N*-trimethylalanine cannot occur in the interior of a polypeptide, because they lack free amino groups to form peptide bonds.
23. (a) GABA is derived from glutamate via decarboxylation of the α -carboxyl group.
 (b) Histamine is derived from histidine also by decarboxylation of the α -carboxyl group.
 (c) Thyroxine is derived from tyrosine by addition of a phenyl group and iodination.
 (d) Dopamine is derived from tyrosine via hydroxylation of the phenyl group and decarboxylation of the α -carboxyl group.
24. Six (see Box 4-3)