A Little, Brown Review Book

BIOCHEMISTRY

A Review with Questions and Explanations

Second Edition

Paul Jay Friedman, M.D.

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Paul Jay Friedman, M.D.

Research Medical Center
Kansas City, Missouri

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Second Edition

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Biochemistry

To Ralph B. Friedman, my father and the best teacher I've ever had,
and Diane A. Friedman, my wife, who provided the encouragement and assistance I needed to complete this book.

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Preface

All the atoms of the earth bear witness, O my Lord, to the greatness of Thy power and of Thy sovereignty; and all the signs of the universe attest to the glory of Thy might.

GLEANINGS FROM THE WRITINGS OF BAHA'U'LLAH

This medical review text was written to explain the fundamentals of biochemistry to medical students and to cover thoroughly the topics in a standard freshman medical biochemistry course and in the National Boards (Part I).

When I was a medical student, I found that most existing texts were 800–1200 pages and included so many diverse facts that they were overwhelming. Those texts did not focus enough on the concepts most relevant to medical biochemistry. The emphasis on medicine was limited, and often those texts did not relate biochemical reactions to organs and diseases.

I have designed *Biochemistry* to make learning as it should be—enjoyable. People learn best when they ask questions and then seek the appropriate answers. Each chapter comes equipped with a set of problems along with their step-by-step solutions. By sharpening their problem-solving skills, students develop a comprehension of biochemistry that obviates the need for thoughtless memorization on the night before an exam.

While preparing this second edition, I catalogued all of the suggestions that readers have sent to me since 1977. At least half of them found their way into the new edition, resulting in almost 150 changes in the text as well as the addition of a new chapter of 41 review problems with answers.

In order to supplement and amplify this text, the beginning biochemistry student should refer to *Biochemistry for Medical Sciences* by Isidore Danishefsky (Little, Brown: 1980), which focuses on introductory medical biochemistry in a thoroughly instructional style.

I thank Sandra Wells Ludwig for her services as medical illustrator.

P. J. F.

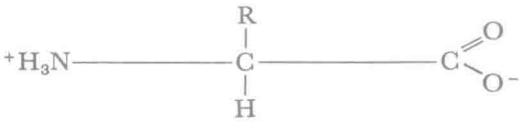
Biochemistry

Contents

1 Structure of Amino Acids

Structural biochemistry forms the foundation to the understanding of metabolic pathways. To dismiss the study of structure as unessential and dull is as senseless as refusing to learn anatomy. When we separate the chaff—in this case, the superfluous structural details—from the grain—the essential structural features—we are then left with a digestible foodstuff.

All living organisms contain α-amino acids, and all α-amino acids share the same structural backbone:



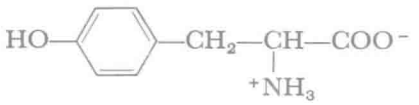
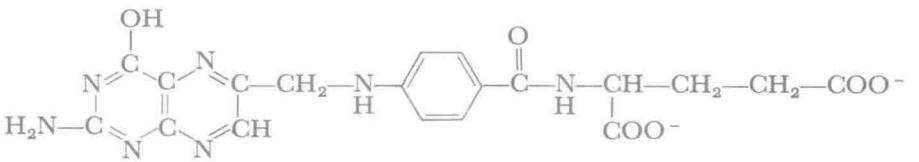
α-Amino group α-Carbon atom α-Carboxyl group

In other words, all α-amino acids possess three common features:

1. They have an α-carboxyl group. The α denotes that this group binds to the central or α-carbon atom, which is asymmetric.
2. They possess an α-amino group.
3. They contain a side chain, or R group, that is bound to the α-carbon.

Problems 1–6

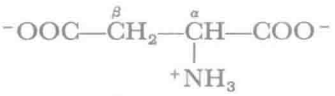
Which of these structures are α-amino acids? (Turn to the end of the chapter for the answers.)

1. $^{+}H_3N—CH_2—COO^{-}$
2. $CH_3—CH_2—COO^{-}$
3. 
4. 
5. $^{+}H_3N—CH_2—CH_2—COO^{-}$
6. $^{+}H_3N—CH_2—CH_2—CH_2—COO^{-}$

You have now proved for yourself that it is quite easy to identify a molecule as an α-amino acid. Your next task is to classify twenty of the common, naturally occurring α-amino acids. *In general, you need not memorize the exact structure of each R group;* knowing the proper classification is sufficient. You should also be familiar with the three-letter abbreviation for each amino acid.

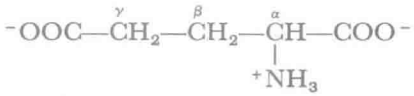
Each amino acid may be classified as *acidic*, *neutral*, or *basic*, depending on the charge on the R group at pH 7.0 (see Ch. 2). Acidic R groups bear a negative charge at pH 7.0 because they are strong proton donors. The two acidic α-amino acids—i.e., those with acidic R groups—are *aspartic acid* and *glutamic acid*:

Aspartic acid (Asp)



β-carboxyl

Glutamic acid (Glu)

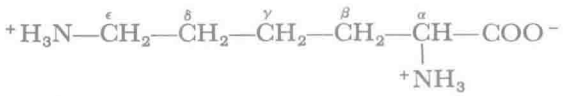


γ-carboxyl

Glutamic acid differs from aspartic acid only in the number of CH_2 groups contained in its side chain. Each acid carries a charge of minus one at neutral pH.

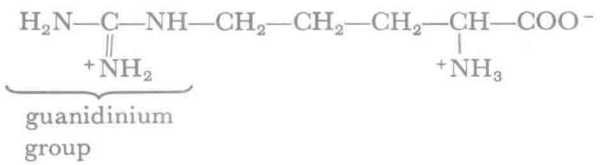
Basic R groups carry a positive charge at pH 7.0 because they avidly bind protons. The three common basic α-amino acids are *lysine*, *arginine*, and *histidine*.

Lysine (Lys)



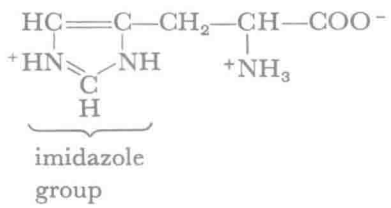
ε-amino group

Arginine (Arg)



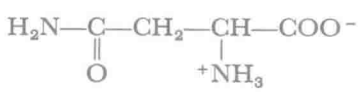
guanidinium group

Histidine (His)

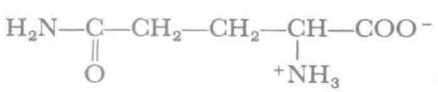


imidazole group

Two α-amino acids are commonly mislabeled as basic when in fact they are neutral: *glutamine* and *asparagine*. They are the amides of glutamic and aspartic acids, respectively. Although they are polar, the amide groups neither protonate nor dissociate.



Asparagine (Asn)

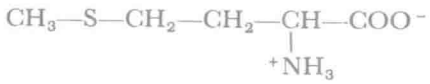


Glutamine (Gln)

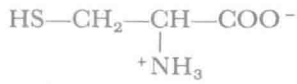
In addition to their charge at neutral pH, amino acids may be classified according to whether or not they contain sulfur atoms, hydroxyl or aromatic groups, and branched or straight-chain hydrocarbons in their side chains (R groups). Each neutral amino acid may also be designated as *polar* or *nonpolar*.

Cysteine and *methionine* contain sulfur. The free sulfhydryl group of cysteine makes it a polar molecule. The —SH groups of cysteine can bind to one another to form the disulfide bridges that stabilize the structure of proteins. *Cystine* is a dimer of cysteine, in which two molecules of cysteine are joined via their sulfur atoms. Methionine, as the name implies, has a methylated thiol group (sulfur atom), which is nonpolar.

Methionine (Met)

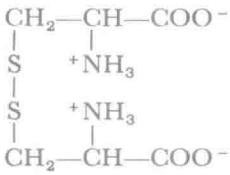


Cysteine (Cys)



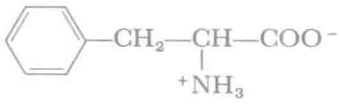
sulfhydryl group

Cystine

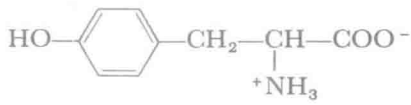


Three α-amino acids contain aromatic groups: *phenylalanine*, *tyrosine*, and *tryptophan*. Phenylalanine consists of a phenyl ring bound to the methyl group of alanine (which is shown subsequently). Tryptophan contains an indole group, which consists of a phenyl ring fused to a five-membered, nitrogen-containing ring. Both phenylalanine and tryptophan are nonpolar. The hydroxyl group of tyrosine, or *p*-hydroxyphenylalanine, renders it polar.

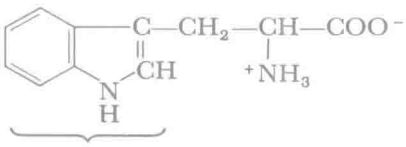
Phenylalanine (Phe)



Tyrosine (Tyr)



Tryptophan (Trp)



indole group

Three α-amino acids have branched hydrocarbon chains: *leucine*, *isoleucine*, and *valine*. Since their side chains are purely hydrocarbons, they are therefore nonpolar.

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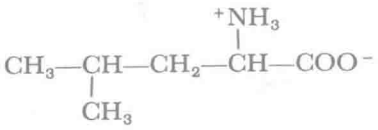
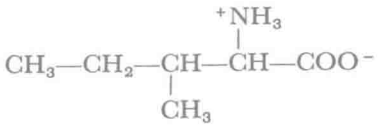
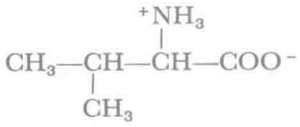


Leucine (Leu)

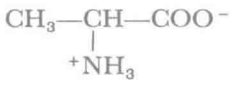


Isoleucine (Ile)

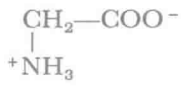


Valine (Val)

The R groups of *alanine* and *glycine* are a methyl group and a hydrogen atom, respectively. Glycine, the simplest of the amino acids, is highly polar, since it is dominated by its charged carboxyl and amino groups. The methyl side chain of alanine tends to make it nonpolar.

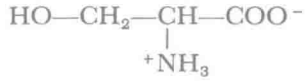


Alanine (Ala)

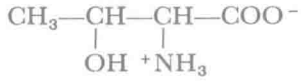


Glycine (Gly)

The R groups of *serine* and *threonine* contain hydroxyl groups, like tyrosine, which render them polar.

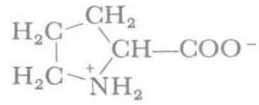


Serine (Ser)



Threonine (Thr)

One so-called amino acid is actually an "imino" acid. The imino nitrogen in proline's five-membered ring binds to two carbon atoms. Because of the rigidity of this ring, proline residues will kink a chain of amino acids. Proline is nonpolar.

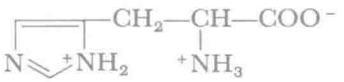


Proline (Pro)

Ninhydrin reacts with the free α-amino groups of amino acids and proteins to produce a purple color. The ninhydrin reaction can be used to estimate the quantity of amino acid present in a sample. The quantitation of individual amino acids involves their separation by chromatographic techniques.

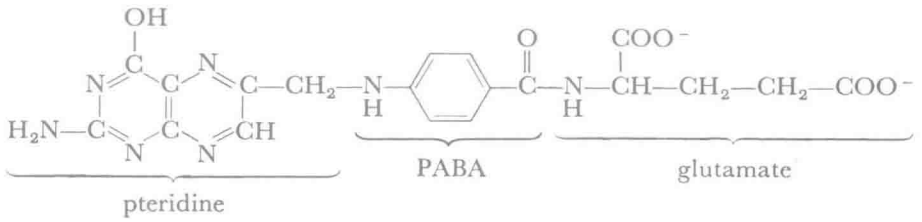
Problems 7–18

Describe each amino acid below in terms of its sulfur content, aromatic and hydroxyl groups, and branched or straight hydrocarbon chains. Designate each as acidic, neutral, or basic, and classify the neutral amino acids as polar or nonpolar.

7. $^{+}H_3N-CH_2-CH_2-CH_2-CH_2-\underset{\displaystyle {}^{+}NH_3}{\underset{|}{CH}}-COO^{-}$
8. Phenylalanine
9. $CH_3-S-CH_2-CH_2-\underset{\displaystyle {}^{+}NH_3}{\underset{|}{CH}}-COO^{-}$
10. Glycine
11. $CH_3-\underset{\displaystyle CH_3}{\underset{|}{CH}}-\underset{\displaystyle {}^{+}NH_3}{\underset{|}{CH}}-COO^{-}$
12. Glutamine
13. 
14. Proline
15. $^{-}OOC-CH_2-\underset{\displaystyle {}^{+}NH_3}{\underset{|}{CH}}-COO^{-}$
16. Asparagine
17. $CH_3-\underset{\displaystyle OH}{\underset{|}{CH}}-\underset{\displaystyle {}^{+}NH_3}{\underset{|}{CH}}-COO^{-}$
18. Arginine

ANSWERS

1. Yes. This structure represents the simplest and smallest amino acid: glycine. Its R group is a hydrogen atom.
2. No. This molecule has a carboxyl group but lacks an amino group. It is a three-carbon fatty acid, propionic acid.
3. Yes. It has both an α-carboxyl and α-amino group. Its R group is an aromatic alcohol, which identifies this as tyrosine.
4. Although this molecule as a whole is not an amino acid, it does contain an amino acid. Look again carefully and find the amino acid:



This molecule is the B vitamin, pteroylglutamic acid, one form of folic acid. The α-amino group of glutamic acid is linked through a peptide bond to *p*-aminobenzoic acid (PABA).

5. Did this β-amino acid fool you? Notice that the amino group binds to the β-carbon atom; hence, it is a β-amino acid, not an α-amino one.
6. This is a γ rather than an α-amino acid. It is γ-aminobutyric acid (GABA), an inhibitory neurotransmitter.
7. Basic (note the ϵ-amino group of lysine), straight-chain.
8. Neutral, aromatic, nonpolar.
9. Neutral, sulfur-containing (you should be able to identify this as methionine because the sulfur is methylated), nonpolar.
10. Neutral, polar.
11. Neutral, branched-chain, nonpolar.
12. Neutral (the amide does not protonate or dissociate), polar.
13. Basic (the imidazole group will have a positive charge; you should be able to identify this as histidine).
14. Neutral, nonpolar imino acid.
15. Acidic.
16. Neutral, polar.
17. The hydroxyl-containing group, like that of alcohols, is polar but does not dissociate; hence, it is a neutral amino acid.
18. Basic (the guanidine group of arginine has a positive charge at pH 7.0).

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2 Acids, Bases, and Buffers

This may be one of the most challenging chapters in this book, and you should plan to spend at least fifteen hours to master this area. Never skip the problems; they are essential to your learning.

In biochemistry the most workable definitions of acids and bases are those of Brønsted, who defined an *acid* as a proton donor and a *base* as a proton acceptor. For each acid and each base, there is its *conjugate base* and *conjugate acid*, respectively, from which it differs by the proton lost or gained.

THE HENDERSON-HASSELBALCH EQUATION

Let RH represent an acid and R^- its conjugate base. Its dissociation may be represented by:

$$RH \rightleftharpoons H^+ + R^-$$

The ionization, or dissociation, constant of this acid, K_a, is defined by the equilibrium expression:

$$K_a = \frac{[H^+][R^-]}{[RH]} \tag{2-1}$$

where the square brackets indicate the molar concentrations of the substances. By rearrangement and substitution utilizing the definitions $pH = -\log[H^+]$ and $pK_a = -\log K_a$, we get:

$$[H^+] = \frac{K_a[RH]}{[R^-]}$$

$$-\log[H^+] = -\log K_a + \log \frac{[R^-]}{[RH]}$$

$$pH = pK_a + \log \frac{[R^-]}{[RH]} \tag{2-2}$$

Equation 2-2 is the *Henderson-Hasselbalch equation*, which may also be written:

$$pH = pK_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

Problem 1

Calculate the hydrogen ion concentration, $[H^+]$, in:

A. plasma at pH 7.4
B. gastric juice at pH 2.7

Problem 2

Calculate the pH of:

A. 0.001 M HCl
B. 0.20 M NaOH
C. 0.05 M CH_3COOH ($K_a = 1.86 \times 10^{-5}$)

Problem 3

Using the Henderson-Hasselbalch equation, compute the $[R^-]/[RH]$ ratio when:

A. $pH = pK_a$
B. $pH = pK_a + 1$
C. $pH = pK_a + 2$
D. $pH = pK_a - 1$
E. $pH = pK_a - 2$

If the pH differs by two or more units from the pK_a, you will not, for most practical purposes, need to use the Henderson-Hasselbalch equation to calculate the concentrations of the components of the dissociation reaction, because 99% or more of the substance will exist as the proton acceptor (if $pH \geq pK_a + 2$) or the proton donor (if $pH \leq pK_a - 2$).

The meaning of the Henderson-Hasselbalch equation can be easily conceptualized. If the pH drops below the pK_a, the conjugate base (R^-) is protonated to the acid (RH); hence, the ratio $[R^-]/[RH]$ falls below one. On the other hand, if the pH rises above the pK_a, the acid (RH) liberates its proton and the $[R^-]/[RH]$ ratio rises above one.

TITRATION AND BUFFERS

Titration is the incremental addition of a strong acid or base to a solution while measuring its pH up to the point, say, of neutralization. After the desired pH is reached, one calculates the moles of acid or base added, and from that figure, one determines the quantity of titratable acid or base in the solution.

The results of titration demonstrate whether or not the substance in solution is acting as a *buffer*, that is, a compound that changes pH relatively slowly in response to the addition of strong acid or base. Most buffers exhibit their buffering action only within a narrow pH range.

An important application of titration to medicine is in renal physiology. The *titratable acidity* of urine is defined as the number of millimoles of NaOH required to titrate one liter of urine up to physiologic pH (7.4). The principal titratable acid found in the urine is phosphate, which exists in three different forms and has two pK_a values:

$$H_3PO_4 \overset{pK_{a_1} = 2.1}{\rightleftharpoons} H^+ + H_2PO_4^- \overset{pK_{a_2} = 7.2}{\rightleftharpoons} H^+ + HPO_4^{-2}$$

Since the pH of urine never drops below 4.5, there is virtually no H_3PO_4 in urine, because $pK_{a_1} = 2.1$ (recall the value of $[R^-]/[RH]$ when $pH \geq pK_a + 2$).

Problem 4

Your patient, a Nobel prize-winning biochemist, asks you to calculate the ratio $[HPO_4^{-2}]/[H_2PO_4^-]$ in blood at pH 7.4 and urine at pH 7.2, 6.2, and 5.5. You mutter "Bullfeathers!" under your breath as you draw your pocket calculator and get to work.

Problem 5

The next morning the biochemist verifies your calculations. Now he wants you to compute the titratable acidity of the phosphate in urine at pH 7.2, 6.2, and 5.5, assuming the phosphate concentration is 0.01 M and ignoring other urinary buffers. Hearing you stammer a series of feeble excuses, he volunteers

to help you. "For pH 7.2, you determined (see Problem 4) that $[HPO_4^{-2}]/[H_2PO_4^-] = 1.0$. Therefore, a 0.01 M (or 10 mM) phosphate solution at pH 7.2 will exist as 5.0 mM HPO_4^{-2} and 5.0 mM $H_2PO_4^-$. Now for pH 7.4, you determined (see Problem 4) that $[HPO_4^{-2}]/[H_2PO_4^-] = 1.6$. Hence, at pH 7.4, this solution will exist as 6.2 mM HPO_4^{-2}, since $(1.6/2.6) \times 10$ mM = 6.2 mM, and as 3.8 mM $H_2PO_4^-$, since $(1/2.6) \times 10$ mM = 3.8 mM. In titrating 1 liter of this solution from pH 7.2 to pH 7.4, 1.2 mmoles of $H_2PO_4^-$ are converted to HPO_4^{-2} (5.0 mmoles − 3.8 mmoles = 1.2 mmoles $H_2PO_4^-$ converted). This liberates 1.2 mmoles of hydrogen ion:

$$1.2\ H_2PO_4^- \longrightarrow 1.2\ HPO_4^{-2} + 1.2\ H^+$$

which must be neutralized by NaOH:

$$1.2\ H^+ + 1.2\ NaOH \longrightarrow 1.2\ Na^+ + 1.2\ H_2O$$

In other words, 1.2 mmoles of NaOH are required to titrate 1 liter of this urine solution from pH 7.2 to physiologic pH. Thus, the titratable acidity of one liter of urine containing 10 mM phosphate at pH 7.2 is 1.2 mmoles. Now follow this example for the cases of pH 6.2 and pH 5.5."

Amino Acids as Buffers

Proteins function as one of the most important buffer systems in blood and tissues, and their buffering ability derives from the dissociable groups on their constituent amino acids.

Glycine, the simplest amino acid, has two dissociable groups, the α-amino and α-carboxyl groups:

$$^+H_3N{-}CH_2{-}COOH \overset{pK_{a_1} = 2.3}{\rightleftharpoons} {}^+H_3N{-}CH_2{-}COO^- \overset{pK_{a_2} = 9.6}{\rightleftharpoons} H_2N{-}CH_2{-}COO^-$$

Glycine can therefore exist in three forms, depending on the pH:

1. Completely protonated (^+H_3N—, —COOH). Net charge of positive one.
2. Protonated α-amino group with unprotonated α-carboxyl group (^+H_3N—, —COO^-). This is the *isoelectric* species of glycine because it has zero net charge.
3. Completely unprotonated (H_2N—, —COO^-). Net charge of negative one.

To determine the form and net charge of glycine at pH 3.0, for example, consider each dissociable group separately. Since pK_{a_1} for the α-carboxyl group is 2.3, this group will exist mainly as COO^-. More than about 10%, however, will be in the COOH form, because $pH - pK_a < 1.0$. Applying the Henderson-Hasselbalch equation,

$$3.0 = 2.3 + \log[COO^-]/[COOH]$$

$$[COO^-]/[COOH] = 5$$

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