Second Edition

Essential Biochemistry and Molecular Biology

A Comprehensive Review

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Elsevier
New York • Amsterdam • London • Tokyo

Elsevier Science Publishing Co., Inc.
655 Avenue of the Americas, New York, New York 10010

Sole distributors outside the United States of America and Canada:

Elsevier Science Publishers B.V.
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

This book is printed on acid-free paper.

Library of Congress Cataloging-in-Publication Data

Werner, Rudolf, 1934-
Essential biochemistry and molecular biology: a comprehensive review/
Rudolf Werner. – 2nd ed.
p. cm.
rev. ed. of: Essentials of modern biochemistry. c1983.
Includes index.
ISBN 0-444-01631-7 (pbk. : alk. paper)
1. Biochemistry. 2. Molecular biology. I. Werner, Rudolf, 1934-
Essentials of modern biochemistry. II. Title.
[DNLM: 1. Biochemistry. 2. Molecular biology. QU 4 W494ea]
QP514.2.W47 1992
612'.015 — dc20
DNLM/DLC 91-34474
for Library of Congress CIP

Current printing
10 9 8 7 6 5 4 3 2 1

Manufactured in the United States of America

PREFACE

Because of the continuing explosion of scientific knowledge, many of today's textbooks are becoming too voluminous and thus tend to lose their usefulness as study guides. Students, therefore, look for smaller review books that cover all course material in a concise form. This allows the student to review the material covered during a lecture in a more efficient way. Full-size textbooks are still required as information sources and for in-depth study of certain topics.

This book provides the student with a comprehensive yet concise description of all the biochemistry and molecular biology that is medically relevant. It allows the student to find important facts without having to read through more than 1000 pages of text. In order to achieve this comprehensive coverage, I have sought to condense the entire field of medical biochemistry into essentials and thereby offer students a comprehensive coverage of all topics of medical biochemistry. Despite its limited format, the book contains all the information a medical student must have to pass the National Board Examination Part I, including topics often not found in a single textbook.

Despite its emphasis on medical biochemistry, the book should appeal to all undergraduate students in the life sciences. Since the first edition of this book, there have been numerous scientific advances in the area of molecular biology. In this second edition, I have tried to include molecular biological approaches in most chapters, and have added an additional chapter (Chapter 7) to describe the latest developments in molecular biology as they apply to modern medicine.

The first seven chapters deal with the basic biochemical and molecular biological mechanisms; the last six chapters cover more medically oriented topics. Medical correlations are mentioned whenever appropriate. (Undergraduate students, especially those who are not premedical or predental students, may want to skip over these sections.) Each chapter is preceded by a set of objectives that help students to recognize the relevant points and, at the same time, to test their understanding of the subject. References to specific chapters of the major biochemistry textbooks as well as to review articles and original research papers enable the student to consult the more extensive coverage when desired and thus deepen his or her understanding of a particular topic.

While writing this book I received valuable advice and criticism from my colleagues at the Department of Biochemistry and Molecular Biology at the University of Miami and from my students. I am especially indebted to David Anderson, Bonnie Blomberg, Keith Brew, Gerhard Dahl, Ian Dickerson, Frans Huijing, David Puett, and Fred Woessner, who critically read sections of the manuscript.

Rudolf Werner, PhD

CONTENTS

CHAPTER

1 Amino Acids, Proteins, and Enzymes

OBJECTIVES

After studying this chapter, the student should be able to

1. Draw the structures of the 20 amino acids found in natural proteins and understand their properties.
2. Remember the approximate pK values of conjugate acid-base pairs found in amino acids.
3. Be able to calculate the pI of an amino acid from the pKs of its ionizable groups.
4. Describe the nature of the peptide bond.
5. Explain the types of intramolecular forces that determine protein conformation.
6. Define the terms "primary," "secondary," "tertiary," and "quaternary" structures of proteins and name examples for each.
7. Remember the general rules that govern the structure of globular proteins.
8. Describe some of the techniques used for the purification and characterization of proteins and understand their rationale.
9. Understand how a mixture of amino acids can be analyzed.
10. Explain some of the techniques used for the determination of amino acid sequences in proteins.
11. Name the site of cleavage in a polypeptide chain by cyanogen bromide and by trypsin.
12. Understand the basis of polyacrylamide gel electrophoresis in the presence of SDS and why it can be used to determine molecular weights of protein subunits.
13. Explain the terms "activation energy" and "transition state intermediate" and relate these to enzyme action.
14. Understand the basis of Michaelis-Menten enzyme kinetics and distinguish the different types of enzyme inhibition from Lineweaver-Burk plots.
15. State the evidence for the existence of enzyme-substrate complexes.
16. Understand the mechanism of allosteric regulation of enzyme activity.
17. Name examples of the labeling and identification of active sites in enzymes.
18. Give an example of an isoenzyme.
19. Define the terms "prochiral" and "chiral centers" and explain their significance in enzyme-substrate interactions.
20. Give examples of a multi-subunit enzyme and a multienzyme complex.

Proteins are polymers of amino acids. They constitute by far the largest class of polymers found in living cells and play a central role in the cell's structure and function. In this chapter we will discuss the structures and properties of proteins. Their biosynthesis will be covered extensively in the chapter on biochemical genetics (Chapter 6).

Examples of protein function are

1. Catalysis of chemical reactions by enzymes
2. Provide structure (hair, bone, connective tissue)
3. Transport ions and metabolites (permeases), gases (hemoglobin), electrons (cytochromes), and lipids (lipoproteins) across membranes and between organs and tissues
4. Regulate metabolism (cell membrane receptors, hormones)
5. Provide contractibility (actin and myosin in muscle)
6. Transmit nerve impulses (neurotransmitters, excitable assemblies)
7. Body defense mechanisms (antibodies)
8. Maintain functions (blood clotting factors)
9. Perception (visual pigments, chemoreceptors)
10. Storage (ferritin for iron storage and ovalbumin in egg white and casein in milk for amino acid storage)

A protein or functional entity consists of one or more polypeptide chains folded into a specific three-dimensional structure (conformation) that is determined by the sequence of the amino acids in the polypeptide chain(s). Before we can discuss protein structure, we must acquaint ourselves with the structures of the amino acids found in proteins.

1.1 STRUCTURES AND PROPERTIES OF AMINO ACIDS

There are 20 amino acids that can be polymerized into linear polypeptide chains, which make up native proteins. Nineteen of these amino acids are α amino acids of the general structure shown in Figure 1.1. (The letter "α" indicates that the amino group is attached to the first carbon atom after the carboxyl group.) The twentieth amino acid, proline, is an α imino acid.

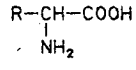
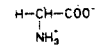
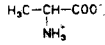
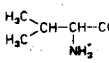
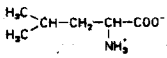
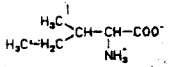
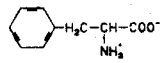
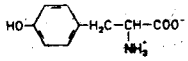
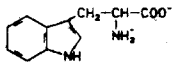
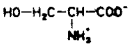
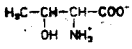
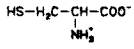
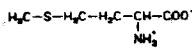
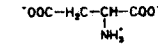
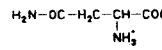
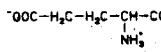
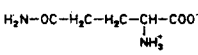
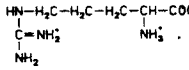
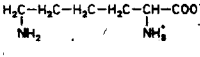
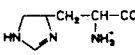


Figure 1.1.
General structure of an α amino acid.

Table 1.1 shows the structures of the natural amino acids. They are classified according to the nature of their side chains, which in Figure

Amino Acid	Symbols	Structural Formula	pK_1	pK_2	pK_3	pI
Aliphatic side chains						
Glycine	Gly (G)		2.34	9.60		5.97
Alanine	Ala (A)		2.34	9.69		6.00
Valine	Val (V)		2.32	9.62		5.96
Leucine	Leu (L)		2.36	9.60		5.98
Isoleucine	Ile (I)		2.36	9.60		6.02
Proline	Pro (P)		1.99	10.60		6.30
Aromatic side chains						
Phenylalanine	Phe (F)		1.83	9.13		5.48
Tyrosine	Tyr (Y)		2.20	9.11		5.66
Tryptophan	Trp (W)		2.83	9.39		5.89
Side chains with other groups						
Serine	Ser (S)		2.21	9.15		5.68
Threonine	Thr (T)		2.09	9.10		5.60
Side chains containing sulfur						
Cysteine	Cys (C)		1.96	10.28	8.33 (SH)	5.07
Methionine	Met (M)		2.28	9.21		5.74
Acidic amino acids and their amides						
Aspartate	Asp (D)		1.88	3.65 (β-*COOH*)	9.60 (α-NH_2)	2.77
Asparagine	Asn (N)		2.02	8.80		5.41
Glutamate	Glu (E)		2.19	4.25 (γ-*COOH*)	9.67 (α-NH_2)	3.22
Glutamine	Gln (Q)		2.17	9.13		5.65
Basic amino acids						
Arginine	Arg (R)		2.17	9.04 (α-NH_2)	12.48 (*guanidinium*)	10.76
Lysine	Lys (K)		2.18	8.95 (α-NH_2)	10.53 (ε-NH_2)	9.74
Histidine	His (H)		1.82	6.00 (*imidazole*)	9.17 (α-NH_2)	7.59

1.1. are denoted R. For reasons of brevity, their commonly used three-letter abbreviations will be used throughout this book. Amino acids with hydrophobic or nonpolar side chains are Phe, Trp, Pro, Met, Val, Leu, and Ile. All other amino acids have hydrophilic or polar side chains, with the exception of Gly and Ala, which have intermediate character.

1.1.1 Stereochemistry of Amino Acids

The α **carbon atom** of all amino acids with the exception of glycine is **asymmetric** because it is linked to four different groups. Therefore, two possible stereoisomers exist for each amino acid (except glycine) that are mirror images of each other (Figure 1.2). The two stereoisomers have very similar physicochemical properties but rotate the axis of polarized light in opposite directions. The two configurations are designated L and D. These letters refer to the absolute configuration of the isomers, not the direction of optical rotation.

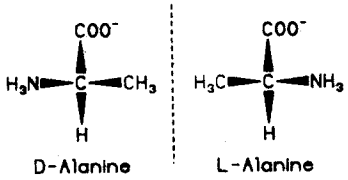


Figure 1.2.
Stereoisomers of an α amino acid.

All amino acids found in natural proteins have the L configuration. Two amino acids, Thr and Ile, possess two asymmetric centers, thus giving rise to four possible **diastereoisomers.** However, in each case only one form, the L form, is found in proteins.

Some D amino acids are found in substances other than proteins. Bacterial cell walls, for example, contain a network of polysaccharide chains that are cross-linked by short oligopeptides containing D-Ala and D-Glu. The antibiotic **penicillin** inhibits the biosynthesis of these cross-links, thus causing the formation of a defective cell wall in growing bacteria, which results in cell lysis.

1.1.2 Acid-Base Properties

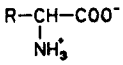


Figure 1.3.
Zwitterion form of an α amino acid.

Amino acids are **amphoteric** in nature, containing both acidic and basic groups. Under physiologic conditions (pH 7.4), amino acids exist as dipolar ions, called **zwitterions** (Figure 1.3).

Depending on their state of ionization, carboxyl and amino groups may be regarded as weak bases or weak acids, and their dissociation can be described by the single equation

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

HA denotes the proton donor or **conjugate acid** (—COOH or $—NH_3^+$) and A^- the proton acceptor or **conjugate base** ($—COO^-$ or $—NH_2$).

The extent of this ionization reaction depends on its equilibrium constant or dissociation constant

$$K = \frac{[H^+][A^-]}{[HA]}$$

in which [ ] denotes the molar concentration.

By rearrangement, we obtain

$$[H^+] = \frac{K[HA]}{[A^-]}$$