FUNDAMENTALS OF ENZYMOLOGY

RATE ENHANCEMENT, SPECIFICITY, CONTROL, AND APPLICATIONS

Garfield P. Royer

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To Jean Royer Kohr
in memoriam

PREFACE

On a number of occasions students, industrial scientists, and engineers interested in biotechnology have asked me to recommend an introductory book on enzymes that goes beyond the general biochemistry text. Although I know of comprehensive texts on enzyme mechanisms, enzyme kinetics, and control of enzyme action, I could not think of a general book that included some discussion of applications. I thought a balanced, intermediate treatise on the basics of enzyme catalysis and applications would be a useful reference.

The book is also appropriate as a text for a graduate or advanced undergraduate course. The table of contents resembles the prospectus of Biochemistry 821, a graduate course in enzymology at Ohio State University. The fundamentals are presented in the first seven weeks of the quarter; the remaining three weeks are devoted to topics from current literature that illustrate one or more basic points.

I would like to thank Jane Chapman and Doris Buchanon for help in preparation of the manuscript. Also, I acknowledge my wife, Alvilda, for her encouragement and help in proofreading.

G. P. ROYER

Worthington, Ohio
December 1981

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CHAPTER ONE

STRUCTURE, LOCALIZATION, AND ISOLATION

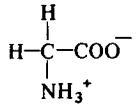
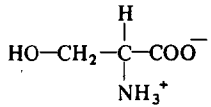
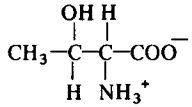
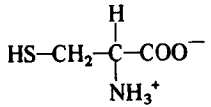
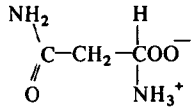
Enzymes are sophisticated catalysts found in all living cells. They are distinguished from catalysts of nonbiological origin by their efficacy, specificity, and sensitivity to control. An enzyme can be activated, transform a substrate* selectively at an impressive rate, and be deactivated until needed again. The catalytic power of enzymes permits biochemical reactions to go under mild conditions (37°C, 1 atm); precise specificity permits many reactions to go simultaneously with order; sophisticated control of enzymes allows the organism to respond rapidly to environmental changes.

For many years the size, complexity, and instability of enzyme molecules precluded an accurate understanding of their composition and structure. It is now well known that enzymes are proteins with molecular weights ranging from 10,000 to 500,000. Proteins are polyamides composed of the 20 coded L-α-amino acids and their derivatives, such as glycosyl and phosphoryl adducts (Table 1.1). Metal ions (Zn^{2+}, Mg^{2+}, K^{+}, etc.) are present in about 35% of the known enzymes. Coenzymes such as those shown in Table 1.2 are frequently present at the active centers of enzymes.

Enzymes are generally globular in shape, which means that the peptide chain

*"Substrate" is defined as the reactant in an enzyme-catalyzed reaction.

TABLE 1.1 The Amino Acids[a]

	Structure	pK_a's	Special Properties of Side Chains
Glycine (75)		2.4, 9.8	Provides flexible link, often at bends in peptide chain
Serine (105)		2.2, 9.2	Hydroxyl group is the nucleophile at the active sites of the serine proteinases Site of phosphorylation in phosphoproteins and phosphotransferases Site of glycosylation
Threonine (119)		2.1, 9.1	Site of phosphorylation in phosphoproteins Site of glycosylation
Cysteine (121)		1.9, 10.5, 8.4 (thiol group)	Active site nucleophile in a variety of enzymes. Can be oxidized to the disulfide under mild conditions
Asparagine (132)		2.0, 8.8	Site of glycosylation

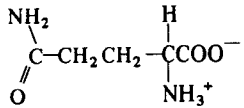
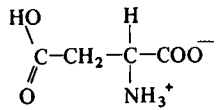
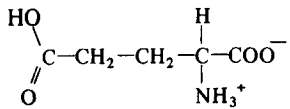
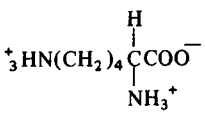
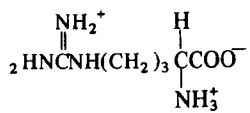
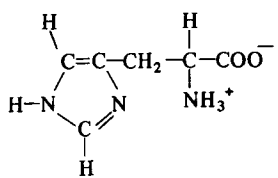
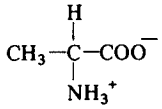
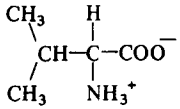
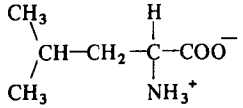
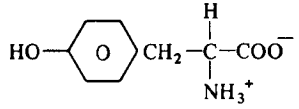
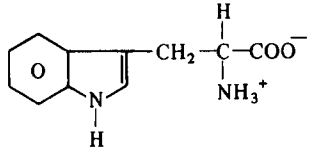
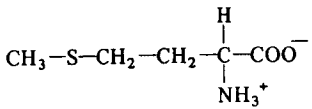
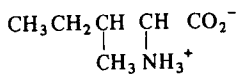
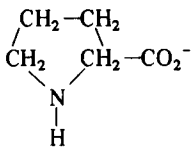
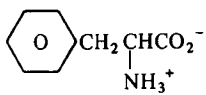
Glutamine (146)		2.2, 9.1	
Aspartic acid (133)		1.9, 9.6, 3.7 (side chain —COOH)	General acid General base Nucleophile
Glutamic acid (147)		2.2, 9.2, 4.3	General acid General base Nucleophile
Lysine (146)		2.2, 9.2, 10.8	Nucleophile Coenzyme anchor
Arginine (174)		1.8, 9.0, 12.5 (guanidino group)	Frequently occurs at binding sites
Histidine (155)		1.8, 9.2, 6.0	General acid General base Nucleophile

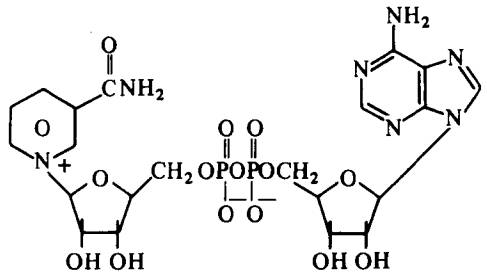
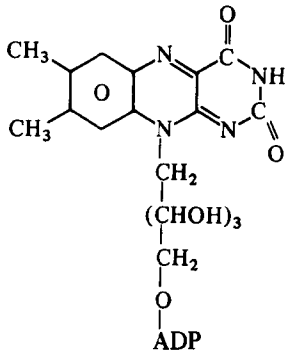
TABLE 1.1. Continued.

	Structure	pK_a's	Special Properties of Side Chains
Alanine (89)		2.3, 9.7	
Valine (117)		2.3, 9.6	
Leucine (131)		2.4, 9.6	
Tyrosine (181)		2.2, 9.1, 10.1 (phenolic–OH)	Apolar General acid $E_{274.6} = 1420$ $E_{294} = 2330$ (phenolate anion)
Tryptophan (204)		2.4, 9.4	UV fluorescence Apolar $E_{279.8} = 5600$ Electron donor in charge transfer complexes

Methionine (149)		2.3, 9.2	Apolar
Isoleucine (131)		2.4, 9.7	
Proline (115)		2.0, 10.6	Imino acid, helix breaker, frequently in sharp turns of protein chains
Phenylalanine (165)		2.2, 2.9	

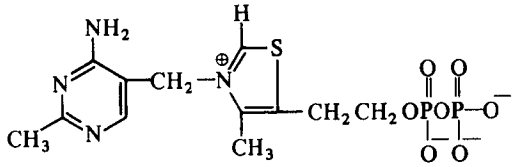
[a] pK_a's from *Handbook of Biochemistry* (Herbert A. Sober, Ed.), CRC Press, Cleveland (1970).

TABLE 1.2. Coenzymes

Structure	Function
Nicotinamide Adenine Dinucleotide (NAD)	Redox Reactions
	
Flavin Adenine Dinucleotide (FAD)	Redox Reactions
	

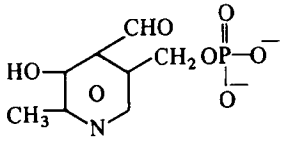
Thiamin Pyrophosphate

Aldehyde Group Transfer



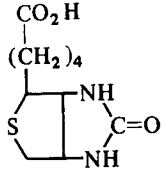
Pyridoxal Phosphate

Amino group transfer



Biotin

Carboxyl Group Transfer



Tetrahydrofolic Acid

1 Carbon Transfer

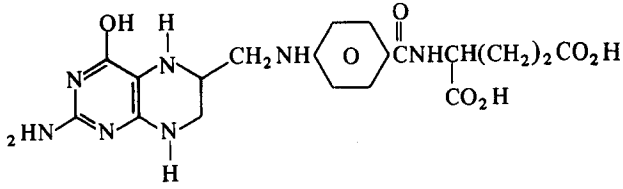
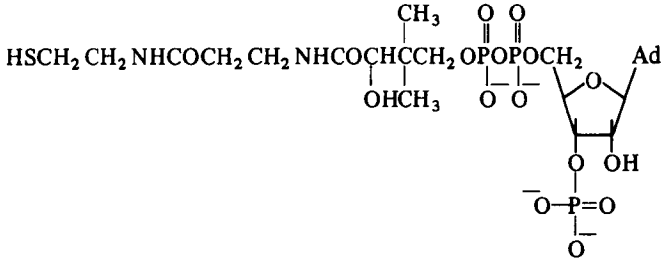
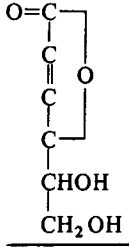


TABLE 1.2 Continued

Structure	Function
Coenzyme A	Acyl Group Transfer
	
Ascorbic Acid	Cofactor in Hydroxylation Reactions
	

folds back on itself repeatedly. The active center is comprised of amino acid side chains, which may be widely separated in the linear amino acid sequence. (It is known that, in ribonuclease, essential histidines at positions 12 and 119 in the linear sequence are close together at the active center.) The catalytic and substrate-binding sites constitute the active center, which represents only a small fraction of the total enzyme surface. Is the remainder of the molecule important in enzyme function? Disruption of the protein structure by physical or chemical agents (denaturation) leads to loss of control (desensitization) and activity. There is a large body of indirect evidence to suggest that substrates bring about conformational changes in the protein during the catalytic process. There is no doubt that the integrity of regulatory sites distinct from the active center (allosteric sites) is required. These areas will be discussed in detail later. At this point we can say, based on evidence concerning denaturation and the study of model compounds, that the portion of an enzyme outside the active center is important, and not simply excess baggage resulting from constraints on the evolutionary process.

A. FORCES IMPORTANT IN THE STRUCTURE AND FUNCTION OF PROTEINS

Before discussing the structure of enzymes and how they work, we shall look at the forces involved in the stabilization of protein structure and enzyme-substrate interactions. Proteins are complicated molecules and water structure is far from simple. However, some general guidelines for the consideration of forces in aqueous solution can be put forward.

1. *Two states must be considered.* One cannot predict, for instance, the solubility of a compound on the exclusive consideration of solute-solvent interaction. The interactions in the crystal must also be taken into account. In thermodynamic parlance, the relative chemical potentials of the molecules in the starting and final states must be known to predict the extent and direction of a given transformation or chemical reaction.

2. *In biopolymers a multiplicity of weak bonds can contribute significantly to the stabilization of a structure.* Van der Waals' attraction between one atom of the substrate and the enzyme may be of little consequence. However, the summation of many such weak interactions can lead to stabilization of an enzyme-substrate complex.