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Outlines of Enzyme Chemistry

A modern and balanced
treatment which stresses basic principles.

Outlines of
ENZYME CHEMISTRY

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Nomenclature

Physical Constants

k	rate constant
k^0	zero-order rate constant
k^1	first-order rate constant
K_{eq}	equilibrium constant
K_{app}	apparent equilibrium constant
Ka	acidic dissociation constant
pKa	log $1/Ka$
pKa'	apparent pKa
v	velocity
V	maximum velocity
K_m	Michaelis constant
T.N.	turnover number
Ki	enzyme-inhibitor dissociation constant
$\Delta F°$	standard free energy change
E_0'	normal oxidation-reduction potential
F	faraday
M	molecular weight
s	sedimentation coefficient
D	diffusion coefficient
$\bar{V}$	partial specific volume
M_{sD}	molecular weight by sedimentation and diffusion
u	electrophoretic mobility
pI	isoelectric point
O.D.	optical density
ϵ	molecular extinction coefficient

Coenzymes

DPN^+	oxidized diphosphopyridine nucleotide
DPNH	reduced diphosphopyridine nucleotide
TPN^+	oxidized triphosphopyridine nucleotide
TPNH	reduced triphosphopyridine nucleotide
NMN^+	nicotinamide mononucleotide

AMP, ADP, ATP CMP, CDP, CTP GMP, GDP, GTP UMP, UDP, UTP	mono-, di-, and triphosphates of adenosine, cytidine, guanosine, and uridine
FMN	flavin mononucleotide
FAD	flavin adenine dinucleotide
CoA	coenzyme A (acetylation)

Enzymes

En	enzyme
EnS	enzyme-substrate compound
EnI	enzyme-inhibitor compound
ADH	alcohol dehydrogenase
LDH	lactic dehydrogenase

Preface

This book is designed to serve the following twofold purpose: (*a*) to introduce senior undergraduate and beginning graduate students to the general subject of enzyme chemistry, and (*b*) to provide background material for research workers in fields other than enzymology. Although many excellent advanced treatises on enzymes have already appeared, they are useful only to specialists, and, furthermore, their cost renders them prohibitive for class use.

The book is based on lecture material presented in a one-semester course given in the Department of Biochemistry of the University of California. This course is designed for students who have had a thorough grounding in chemistry and physiology as well as in biochemistry.

Over half of the book has been devoted to the general properties of enzymes. Brief sections have been included on specific coenzymes, enzymes, and enzyme systems. The study of metabolic problems at the enzyme level has also received attention. However, because there are too many individual enzymes to permit a discussion of each of them, we have studiously avoided writing a "catalogue of enzymes." We have, instead, attempted to treat the subject matter in outline form in order to bring into focus the entire field of enzyme chemistry.

Photographs of several eminent enzyme chemists appear in three chapters. It is obvious, however, that these few are by no means the only individuals who have made outstanding contributions to the field of enzymology. For practical reasons it has been possible to include photographs of only a small number of workers from representative fields of research.

We are deeply indebted to the many reviewers and others who helped in various ways to make this book a reality. We are particularly grateful to R. A. Alberty, P. D. Boyer, B. Chance, D. E. Green, A. C. Griffin, F. M. Huennekens, B. L. Horecker, M. J. Johnson, E. P. Kennedy, H. A. Lardy, H. Neurath, and E. R. Stadtman.

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Berkeley, California
January, 1955

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Part 1

GENERAL PRINCIPLES

1

Introduction and History

1. Introduction

The subject of enzymology can rightly lay claim to scientific interest from both the theoretical and the practical points of view.

An enzyme is, in a sense, a "living" molecule. Conditions or substances that destroy life, such as heat, corrosive reagents, acids, bases, poisons, and antibiotics, also destroy or inhibit enzymes. Similarly, it can often be shown that compounds such as vitamins and hormones exert their effects through their influence on enzymes. Thus it is only through an intimate knowledge of the chemistry of the untold number of enzymes in nature that we can logically interpret the action of chemical substances on life.

Every living organism may be considered a gross catalyst. It abstracts energy from its conversion of the substrate, food, to waste products. The fundamental reaction [1]

$$6CO_2 + 6H_2O + \text{Energy} \rightleftharpoons C_6H_{12}O_6 + 6O_2$$

is carried out from left to right in green plants and from right to left in animals, many microorganisms, and plants. The reversible arrow in this equation represents a whole host of enzymes which we know to be affected by vitamins, hormones, and the like.

Practical interest in enzymes stems from their great usefulness in such diverse industries as the malting of barley, curing of meat, dairying,[2] tobacco curing, *ad infinitum*. An interesting use of enzymes is in the dried-egg industry. Dried eggs contain a certain amount of glucose;

[1] Most of the reactions written in this book will be provided with a reversible set of arrows. However, if the equilibrium lies very far to one side or the other a longer arrow will be used for the direction that is greatly favored.

[2] Phosphatase activity of milk is a time-honored check for the degree of pasteurization.

this reactive compound combines with certain constituents of egg powder to give unpleasant odors and flavors in the dried product. The enzyme, glucose oxidase, from mold mycelium, is now used to catalyze the reaction

$$\text{Glucose} + \tfrac{1}{2}O_2 \rightleftharpoons \text{Gluconic acid}$$

In fact, the dried-egg industry is very happy to increase the weight of its product by adding one oxygen atom per mole of glucose! The gentle and specific action of enzymes is further used in the dry-cleaning industry. Here, a preparation of hydrolytic enzymes is applied to the stubborn spot.

Perhaps we may even hope that eventually we shall be able to interpret the nature of disease at the enzyme level.

2. History

The reader is referred to comprehensive reviews on the origin of enzymology (1). For our purposes we will simply consider that the modern era in enzyme research began with Büchner's (2) extraction of enzymes from yeast. This experiment showed that enzymes could be removed from cells in a soluble, active form and has led to a direct study of their physical and chemical properties. The historical aspects of the role of enzymes in fermentation is beautifully developed in the classical book by Harden (4).

Although, at the moment of writing, over 70 enzymes have been obtained in the crystalline state, we still possess only the most rudimentary knowledge of their mechanism of action. To unravel the details of the catalytic transformation, to *characterize chemically* the active site on the enzyme molecule, to explain why all enzymes apparently are proteins with high molecular weights—these remain among the most challenging problems in biochemistry.

If the reader has not yet experienced an informal introduction to the subject of enzymology, then he should by all means read the fascinating little book by Szent-Györgyi (3).

3. Future

Research on enzymes may be carried out by scientists in many fields other than biochemistry and in recent years mathematicians, physicists, biologists, among others, have become interested in this subject. A prime reason for such a diversity of interest lies in the fact that an enzyme may be used as a purely physicochemical model or, on the other hand, it may be studied *in vivo* as a cog in the complicated machinery of the cell. It is a common experience that those scientists whose train-

ing and experience lie outside of "pure" biochemistry have made the most spectacular discoveries in this rapidly expanding field.

References

1. J. B. Sumner and K. Myrbäck, *The Enzymes*, Academic Press, New York, 1950.

2. E. Büchner, H. Büchner, and M. Hahn, *Die Zymasegärung*, Munich and Berlin, 1903.

3. A. v. Szent-Györgyi, *On Oxidation, Fermentation, Vitamins, Health and Disease*, Williams and Wilkins Co., Baltimore, 1939.

4. A. Harden, *Alcoholic Fermentation*, Longmans, Green & Co., London, 1932.

2

Equilibria and Ionization

1. Introduction (1–4)

Most textbooks of physical chemistry give a thorough development of equilibria and ionization. However, in biochemistry, certain equilibria are more important than others and should therefore be stressed. In textbooks of physical chemistry these are lost in a maze of detail. For these reasons it has seemed profitable to review these subjects briefly here.

Ionization is a phenomenon of the utmost importance to biochemistry in general and to enzymology in particular. The theory of ionization finds application in the preparation of buffers, isolation work, identification and analytical procedures, characterization of coenzymes, enzymes and the reactions catalyzed by them, etc. Specific examples of the close relationship between enzymology and ionization will be cited later.

2. Mass Action Law

One of the first men to enunciate the concept of mass action was C. Berthollet (see reference 1). In 1799 he theorized that certain deposits of Na_2CO_3 in Egypt had their origin in the reaction

$$CaCl_2 + Na_2CO_3 \rightleftharpoons CaCO_3 + 2NaCl$$

The equilibrium of this reaction lies so far to the right that the reverse reaction is normally never encountered. However, according to Berthollet, a high concentration of sodium chloride could act to push the reaction from right to left. This concept provided the notion both of reversibility and of the effect of concentration on extent of reaction.

3. Equilibrium Constant

In the usual enzymatic reaction where there are two substrates and two products we may write the following equilibrium constant, K_{eq}, for

their reaction:

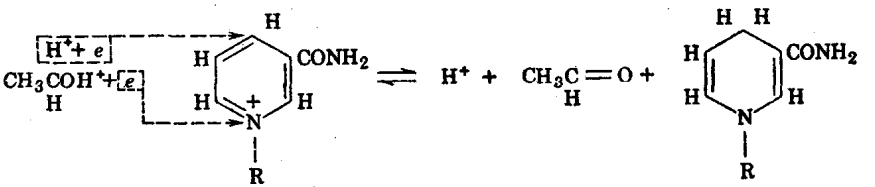
$$A + B \rightleftharpoons C + D$$

$$K_{eq} = \frac{[C][D]}{[A][B]}$$

A change in the concentration of any one of the four molecular species demands an adjustment in the ratio of the other three species present. When the equilibrium point is reached, the forward and back reactions have the same speed. All chemical reactions are striving to reach the equilibrium point, but the rate is often infinitely slow. *This is where enzymes play their key role.* By virtue of their catalytic powers, they speed up the reaction so that equilibrium is established much sooner than would otherwise be the case.

It should be clearly understood that all enzymatic reactions are reversible. "Irreversibility" is used in the same loose sense as "insoluble." It has qualitative meaning only.

To take just one example of the profound significance of ionization in enzymology the following reaction may be considered.



Diphosphopyridine nucleotide, DPN$^+$ Reduced diphosphopyridine nucleotide, DPNH

The enzyme alcohol dehydrogenase transfers a hydrogen atom from alcohol to the coenzyme. The other hydrogen atom may be thought to "separate" into a proton and an electron; the electron neutralizes the positive charge on the nitrogen atom in the pyridine ring. Therefore, the reduction of the ring at physiological *p*H leads to the generation of 1 equivalent of acid by virtue of the fact that a strongly basic quaternary nitrogen atom is converted to a weakly basic tertiary nitrogen atom.

One may write a partial or "apparent" [1] equilibrium constant for this reaction, K_{app}, which at *p*H 7.0 and room temperature happens to be (5, 6) as follows:

$$K_{app} = \frac{[CH_3CHO][DPNH]}{[C_2H_5OH][DPN^+]} = 1 \times 10^{-4}\ M$$

[1] The term "apparent" rather than "thermodynamic" will be used throughout this book where proper attention has not been paid to such factors as *p*H, ionic strength, and relative concentration of free and bound reactants.

The "thermodynamic" equilibrium constant, K_{eq}, at any *p*H value between, for example, 7 and 11, would be:

$$K_{eq} = \frac{[CH_3CHO][DPNH][H^+]}{[C_2H_5OH][DPN^+]} = 1 \times 10^{-11}\ M$$

The relationship between K_{eq} and K_{app} from *p*H 7 to 11 is summarized in the tabulation. Thus the K_{app}, which contains the reactants and

*p*H	$[H^+]$		K_{app} (M)		K_{eq} (M)
7	10^{-7}	$\times$	1×10^{-4}	=	1×10^{-11}
8	10^{-8}	$\times$	1×10^{-3}	=	1×10^{-11}
9	10^{-9}	$\times$	1×10^{-2}	=	1×10^{-11}
10	10^{-10}	$\times$	1×10^{-1}	=	1×10^{-11}
11	10^{-11}	$\times$	1	=	1×10^{-11}

products of practical interest, is strictly dependent on *p*H. The reaction is shifted to the right in basic solution. In the absence of a buffer a reaction mixture containing DPN^+, substrate, and the appropriate dehydrogenase would become strongly acidic as the reduction proceeded.

4. Equilibria and Enzyme Concentration

Many textbooks carry the statement that enzymes do not affect the equilibrium point. This assertion may not be strictly true where reactants, products, and enzyme are all present in approximately equimolar ratios. In a brilliant investigation, Theorell and Bonnichsen (5) showed that molar proportions of crystalline alcohol dehydrogenase could in fact shift the apparent equilibrium point of its reaction by combining with the product DPNH. These observations may be of physiological significance where the enzyme is present in large amounts and is concentrated in intracellular bodies such as mitochondria. In addition, it shows on which side of the equation we may expect to find a stable enzyme-substrate compound (see Chapter 13).

5. Ionization of Weak Acids

Accepting Brönsted's representation (7), an acid, HA, dissociates into protons and an anion.

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

Here, the K_{eq} may be referred to as the dissociation or ionization constant of the acid, Ka.

$$Ka = \frac{[H^+][A^-]}{[HA]} = \frac{[H^+][Salt]}{[Acid]}$$

Ka may be defined as being equal to the $[H^+]$ at half neutralization.[2] At this point, $\frac{[A^-]}{[HA]} = 1$ and Ka is equal to the $[H^+]$.

Thus a comparison of the $[H^+]$ at half neutralization gives a convenient manner of comparing, for different acids, the magnitude of Ka. The "strength" of acids is directly proportional to the value of Ka. Some of the mineral acids are dissociated so completely that a Ka cannot be written for them.

The physiological $[H^+]$ of fluids and tissues is close to 10^{-7} mole per liter. The desirability that the $[H^+]$ be expressed as a small number was one of the principal reasons why Sörensen proposed the use of $-\log [H^+]$ or $\log 1/[H^+]$ or pH. We may therefore rearrange the above equation as follows:

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

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

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

$$p\mathrm{H} = -\log Ka + \log \frac{[A^-]}{[HA]}$$

The abbreviation for $-\log Ka$ is pKa, and is the form of the ionization constant which appears in the Henderson-Hasselbach equation,

$$p\mathrm{H} = pKa + \log \frac{[A^-]}{[HA]}$$

A pKa value as measured with the glass electrode, without proper regard for "activities" (effective concentrations) of the reactants, ionic strength, etc., is designated as an "apparent pKa" or pKa'. Very exact methods for the determination of the pKa are given elsewhere (8, 14).

6. Example for the Ionization of a Weak Acid

Acetic acid

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$$

$$Ka = \frac{[H^+][CH_3COO^-]}{[CH_3COOH]} = 1.75 \times 10^{-5}$$

[2] Brackets, [ ], are used here to denote molar concentration rather than activity. As a further simplification, the effect of water has been neglected.

Since log 1.75 = 0.24, $pKa = 5 - 0.24 = 4.76$. The complete titration curve for acetic acid is shown in Fig. 2.1.[3]

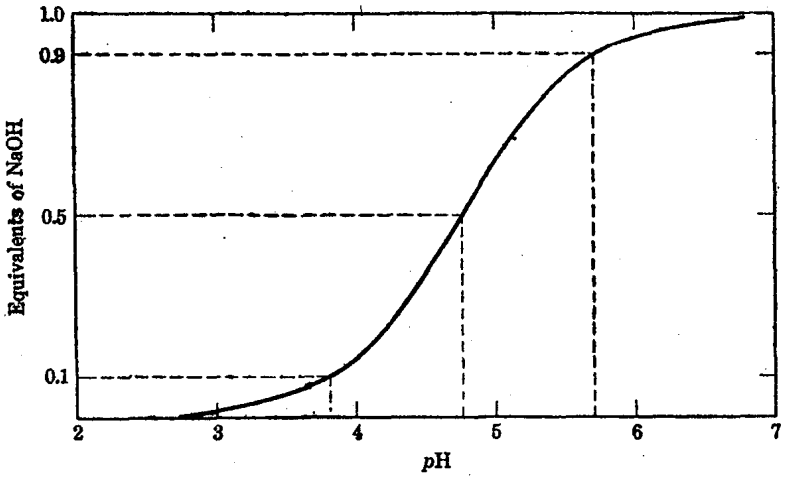


Fig. 2.1. The titration curve of acetic acid.

7. The Dissociation of Water

Water is both an acid and a base. It dissociates according to the equation

$$H_2O \rightleftharpoons H^+ + OH^-$$

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]}$$

Water, however, is so slightly dissociated that the $[H_2O]$ is very large and may itself be regarded as a constant. Hence, at 25°,

$$K_w = [H^+][OH^-] = 10^{-14}$$

The ion product of 10^{-14} is evaluated by various methods, including the conductivity of water and hydrolysis of an ester. Changing these terms to their negative logarithms,

$$pK_w = 14 = pH + pOH$$

[3] A small amount of strong acid (or base, if acid is used as the titrating fluid) may be added at the start of the titration in order that the entire curve may be obtained.