INTRODUCTION TO ENZYMOLOGY

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INTRODUCTION TO ENZYMOLOGY

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PREFACE

Enzymology is a term that covers a broad area of biology and chemistry. The information accumulating in this area is of interest and importance to workers and students with diverse backgrounds and objectives. This book is designed to develop in the reader sufficient familiarity with enzymes that he is encouraged to continue his contact with the rapidly expanding body of knowledge about biological catalysis and is prepared to include information about enzymes in his scientific thinking and to apply this information to his work. The organization of this book has evolved from a course in enzyme chemistry taught by the author at the National Institutes of Health, where the classes consist of investigators primarily interested in clinical medicine, organic chemistry, bacteriology, biophysics, plant physiology, and other subjects, in addition to students in biochemistry.

Enzyme chemistry is not a neat package that can be presented without loose ends. It is possible to develop a theoretical generalized representation of enzymes and illustrate the theory with examples. I believe it is more descriptive of the status of the field and more meaningful for the student to describe individual enzymes and enzyme systems in some detail and to use the information at hand to develop concepts. Accordingly, topics have been selected to indicate the large variety of chemical reactions known to be catalyzed by enzymes.

For the most part these topics have been arranged as components of metabolic sequences in order to emphasize the interrelations of enzyme activities, the chemical mechanisms employed by biological systems, and the multiple factors to be considered in interpreting biological phenomena. A large part of biochemistry has been included, but practical considerations force the elimination of many topics that have intrinsic interest and illustrative value equal to those included.

In each section certain aspects have been stressed to illustrate points of general significance. Since many such points can be established with several different systems, the treatment given to some topics may appear exaggerated, while that of others seems unduly perfunctory. I regret particularly that this uneven treatment eliminates many references to work of great value in the advancement of the field. The references included, however, are not intended to serve as complete documentation for the statements in the text; they are selected only to assist the reader

in making his acquaintance with the original literature and to help him gain some historical perspective.

The rapid growth of modern enzymology has not yet permitted complete descriptions of biochemical processes at the enzyme level. Some of the gaps and limits of our knowledge are indicated in this text. The reader will undoubtedly find that some of the voids have been filled during the time when this book was being prepared. The chemistry of the enzymes themselves may also be expected to be revealed in elaborate detail in the near future. It is my hope that the interpretations I have included in the following pages will serve as a logically sound basis for supplementation, rather than contradiction, by the research of the future.

In the preparation of this text I have been greatly aided by many of my colleagues at the National Institutes of Health and other institutions. I am happy to acknowledge the contributions of Drs. Bruce N. Ames, G. Gilbert Ashwell, Simon Black, Jules A. Gladner, Joseph L. Glenn, Ronald C. Greene, Osamu Hayaishi, Leon A. Heppel, William B. Jakoby, Leonard Laster, Bruce Levenberg, Elizabeth S. Maxwell, R. Carl Millican, Jesse C. Rabinowitz, Earl R. Stadtman, DeWitt Stetten, Jr., Celia W. Tabor, Herbert Tabor, Gordon M. Tomkins, Edith C. Wolff, Barbara E. Wright and Charles I. Wright, who have improved the text through scientific and literary suggestions. I am particularly grateful to Dr. Sidney P. Colowick for his criticisms on the entire manuscript, and to my wife, Anne, for her assistance in the technical aspects of producing this book and for her careful nurturing of manuscript and author.

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CHAPTER I

INTRODUCTION

In recent years the study of enzymes has expanded to such proportions that not only has it become a specialized field, but it has become an important element in studies in many other disciplines. The present body of knowledge about enzymes has been derived from workers who entered the field from many directions, and consequently is composed of fragments which at first glance appear to have little relation to each other. Indeed, a systematic organization of sciences has no need for a category "Enzyme Chemistry." Nevertheless, certain elements of physical chemistry, organic chemistry, physiology, microbiology, and other well-defined areas have been applied to an area of special interest, and from an expanding study of biochemistry, enzymology has grown until its special problems and interests warrant special texts. The true justification for this arbitrary selection of information is the interest of the investigators who have focused their attention on enzymes as the active, and therefore interesting, element of biological systems.

Reactions in which biological material participates, now known to be enzyme catalyzed, were studied throughout the development of modern chemistry. The term catalysis was coined by Berzelius (1836) to describe the effect of substances that cause reactions to occur in their presence. In addition to inorganic reactions, the fermentation of sugar was included among the original examples of catalysis. The existence of substances that digest meat had been known since the experiments of Spallanzani (1783) and de Reaumur (1752). The conversion of starch to sugars was shown by Kirchhoff (1814) to be caused by an extract of wheat. In 1830 Robiquet and Boutron demonstrated hydrolysis of the glycoside amygdalin by a factor in bitter almonds.

The term catalysis was applied to the biological phenomena listed above and to several other reactions discovered in the next several years. The active component of bitter almonds was named *emulsin* in 1837. Other carbohydrate-splitting activities (ptyalin in saliva, amylase in malt) had already been described. Pepsin and trypsin, protein-digesting agents from the stomach and pancreas, were also discovered during this period. The activities of these materials were contrasted with the materials responsible for fermentation. Early theories of Willis and of Stahl to explain fermentation as a disruption caused by violent motion of

particles of the fermenting substance were adapted by Liebig to form a chemical theory of fermentation. Several investigators, notably Schwann, Cagniard-Latour, and Kützing, were meanwhile determining the dependence of fermentation on living cells. Their publications led to a controversy that raged for over twenty years, until Pasteur (1850) provided convincing evidence that fermentation was associated with living, growing organisms. The catalytic agents active only as components of living cells were designated *organized ferments*, while the cell-free activities were called *unorganized ferments*. The latter were named *enzymes* by Kühne.

Modern enzyme chemistry may be dated from the successful studies of Buchner, who in 1897 obtained cell-free preparations of yeast that were capable of fermenting sugar. Before Buchner's work there had been numerous intensive attempts that had failed either because of loss of activity or because of the difficulty in removing all viable cells. Indeed it is possible that Pasteur himself, who argued uncompromisingly for the association of fermentation with living cells, failed to anticipate Buchner only because he did not happen to use an appropriate strain of yeast. Buchner successfully defended his conclusions against all attacks, and as his work was confirmed, the enzyme theory became accepted. Today the word *enzyme* is used to designate all biological catalysts; the infrequently used *ferment* has the same connotation.

Some Generalizations about Enzymes as Catalysts

Catalysts accelerate the rates of chemical reactions. In the case of many enzyme-catalyzed reactions there is no detectable reaction in the absence of the catalyst. It should be noted, however, that enzymes do not add energy to a reacting system; their function is only to influence the rates, not the extents of reactions. Enzymes are remarkable among catalysts for their specificities and for their efficiencies of catalysis at low temperatures. A given enzyme generally affects only one type of chemical bond, usually in a restricted group of compounds. Absolute specificity, the ability to react with only one compound of all similar compounds tested, is frequently observed. All catalysis is positive, in that the catalyst increases a rate of reaction. So-called negative catalysis does not exist except as an indirect result of a positive reaction. If one reaction modifies a system by changing one or more of the reagents of a second reaction, the second reaction may appear to be inhibited by the catalyst of the first. There are, however, no mysterious forces by which a negative catalyst can prevent the interaction of other molecules.

Enzymes are the organic catalysts elaborated by all organisms. As catalysts, they are defined by their activities; primarily, all enzymes must be defined in terms of rates of chemical reactions. The rate of an enzyme-

catalyzed reaction is proportional to the concentration of enzyme when all other conditions are constant (Fig. 1). Ultimately enzymes may be described as particular molecules, and measured in terms of some constituent, weight, etc., but the measurement of any property other than activity may fail to give a true determination of the amount of enzyme in a preparation because subtle alterations in the enzyme molecule may either increase or decrease the activity. At the present time there is no complete chemical description of even the simplest enzyme. One of the objectives of current research is to learn the relation between structure and function of enzymes. Until this goal is reached, the kinetic definition of each enzyme is the only one that describes the enzyme.

The kinetic description of an enzyme requires the measurement of a rate of a reaction. Although examples are known of enzyme reactions at

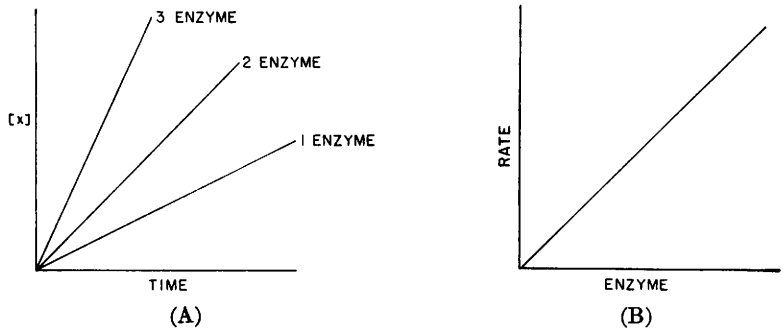

FIG. 1. (A) represents the increase in the concentration of x, the product of a reaction, with time in the presence of 1, 2, and 3 increments of enzyme. The slopes of the lines of (A) may be used as rates to give the plot of (B), which shows proportionality of rate to enzyme concentration.

interfaces, in general enzymes act in aqueous solutions. The compound or compounds initially present in solution which are modified through the agency of an enzyme are called *substrates*. The substrates are converted to *products*. To measure the rate of a reaction, it is necessary to measure the concentration of either a substrate or a product. The rate of change in concentration with time is a measure of the enzyme.

Reaction Rates. There are many embarrassing examples in the enzyme literature of errors in the assay method. These are generally avoided if it is ascertained that a rate of reaction is measured, and *that this is proportional to* enzyme concentration. The rate of reaction may be linear with time, independent of the concentrations of substrates or products. Such reactions are termed *zero order*. If the reaction rate is proportional to the concentration of one substrate it is called *first order* (Fig. 2). These are

the simple types of reaction that are convenient to use in kinetic studies. The rate of a zero-order reaction is given by the equation $x = kt$, and is evaluated as the slope of the line, x/t. Any points on the line thus suffice for the rate measurement. A first-order reaction rate changes continually with time, as the rate-limiting substrate is consumed. A simple relation between the concentration of substrate $[S]$ and the rate v is $v = k[S]$ when k is a constant. From this equation another may be derived that relates k to the amount of substrate present at given times. In this equation

$$k = \frac{1}{t} \ln \frac{a}{a - x}$$

a is the concentration of substrate at the beginning of a time period, t, and x is the concentration at the end of the period. The constant k may be evaluated by substituting experimental values for a, x, and t.

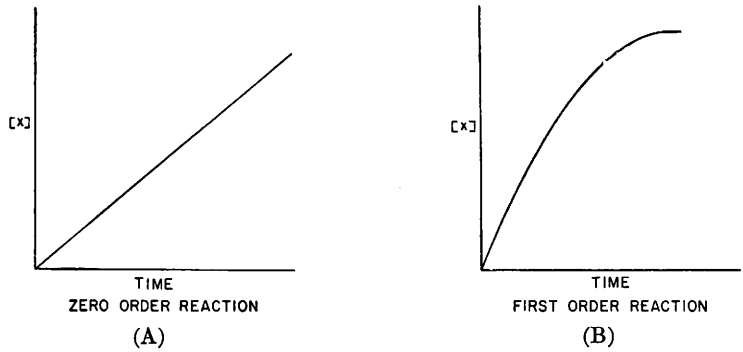

FIG. 2. These curves illustrate the rate of formation of reaction product, x, when the reaction rate is (A) zero order or (B) first order.

It may be evaluated from any portion of the curve, and is proportional to the amount of enzyme. Graphically, a plot of log substrate concentration versus time gives a straight line with first-order reactions, and the negative slope of the line is proportional to the reaction constant. Occasionally it is not possible to use conditions that produce zero- or first-order rates. The rate may be a function of more than one substrate (second or higher order) or it may be influenced by the accumulation of products. The products may act as inhibitors or they may react back to yield the original substrate or other products. An obvious complication is the existence of additional reactions which may involve either substrates or products. When higher order reactions or other complications obscure rate measurements, various empirical devices are employed to establish an assay. The most familiar of these are measurement of initial rates that may tend to approach zero-order kinetics and therefore be

proportional to enzyme, and the establishment of a reference curve of apparent activity versus enzyme concentration. The latter must be recalibrated whenever circumstances, such as state of enzyme purity, are changed. When amounts of enzyme are selected to give rates within the range used for calibration, empirical assay methods are often valid and extremely useful.

Valid Assays. A valid assay of an enzyme must measure the rate at which particular substrates are converted to specified products. Therefore, analysis of a single component of a system may give misleading results. Most importantly, a valid assay must measure a rate. Two values do not establish a rate because they do not describe the course of the

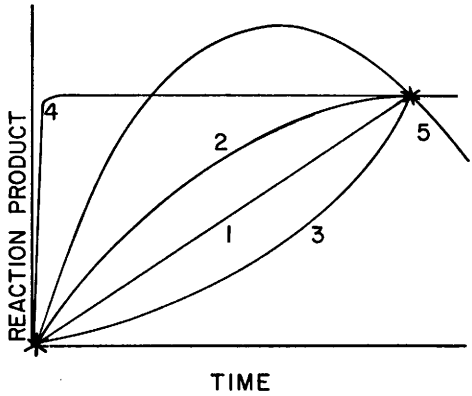

FIG. 3. Some representative routes to the same point. * = Measured values. Curve 1, zero-order reaction. Curve 2, first-order reaction. Curve 3, autocatalytic reaction. Curve 4, reaction gone to completion. Curve 5, secondary reaction.

reaction between the times of the analyses. One of the most common analytical errors is the measurement of a single value after a period of incubation of enzyme with substrate. It cannot be over-emphasized that single-point assays are not valid until the reaction has been studied thoroughly, and it is known that the points measured fall on a meaningful part of a curve (Fig. 3).

Finding that the amount of product formed or substrate removed is proportional to the amount of enzyme added is essential for a meaningful assay, but it is not sufficient to establish that the assay is valid, since a contribution of the enzyme preparation may be a substrate that determines the extent of a reaction.

Enzyme Units. Amounts of enzyme are conventionally expressed in *units.* A unit is an arbitrary designation that depends on the assay used. Under specified conditions a rate constant or any measure of substrate

utilized or product formed in a given time can define a unit. Some conventional units are: micromoles O_2 consumed per hour, Δ optical density at a specified wavelength per minute, micrograms inorganic phosphorus (in phosphate) formed in 10 minutes, etc.

It is often desirable to express the degree of purification of an enzyme. When the chemical nature of enzymes was not known it was conventional to express the *specific activity* as units of enzyme per milligram dry weight. With the identification of enzymes with proteins, specific activity has been expressed as units per milligram nitrogen or per milligram protein as determined for example by the biuret reaction, by ultraviolet light absorption, by tyrosine (phenol) content, or by turbidity produced by protein precipitants. None of these methods is free from objections. Any of them may be of value for specific purposes and none may claim to give absolute values.

As mentioned above, units are defined for particular assay conditions. There are many conditions which must be controlled, including temperature, pH, ionic strength, specific ion concentrations, substrate concentrations, presence of activators, stabilizers, and inhibitors. The role of these factors will be illustrated for various enzymes in later sections. At this point it will merely be mentioned that there are no generalities that describe the effect of varying any of these conditions. Each enzyme must be studied as an individual case; some are indifferent to conditions that effect others profoundly, and some environmental changes, as temperature, influence competing phenomena, as rate of catalyzed reaction and rate of enzyme destruction.

Enzymes and Energy. The mechanisms by which enzymes act are only beginning to be discerned. Evidence to be discussed later for specific enzymes allows the generalization that enzymes participate directly in chemical reactions. In mediating a difficult over-all reaction, one with a large energy of activation, the enzyme does not supply energy. Instead, it circumvents the difficult reaction by substituting a sequence of reactions in which the enzyme participates, and which includes steps that proceed easily at ordinary temperatures. If the reaction $A + B \rightarrow C + D$ has a sufficiently high energy of activation, thermal agitation of the molecules of A and B at ordinary body temperatures may not be sufficient for the reaction to proceed measurably. If, however, the reaction $A + X \rightarrow AX$ proceeds easily, and $AX + B \rightarrow C + D + X$, a mechanism is provided for obtaining a net reaction in an indirect manner. The rate of reaction is increased according to the difference in activation energy of the uncatalyzed reaction and the rate-limiting step of the enzymatic pathway. In ways that are as yet not completely understood for any reaction, the activation energies for the partial reactions in an

enzyme-catalyzed sequence are lower than the activation energy of the uncatalyzed reaction. The participation of an enzyme in chemical reactions that restore the enzyme to its original state has been described as a "cyclic" or "shuttle" process. Such processes may theoretically be repeated indefinitely, but in practice enzymes "wear out" through poorly defined reactions that may or may not be related to the reaction catalyzed.

Substrate Concentration and Rate of Reaction. In ordinary chemical reactions the rate of reaction increases with increase in concentration of reactants. The kinetics of enzyme-catalyzed reactions differ from those of uncatalyzed reactions because the enzyme must "adsorb" or "bind" substrates prior to conversion to products. The nature of the interaction between enzyme and substrate will be considered in some individual cases; in many systems the enzyme reacts efficiently with low concentrations of substrate (10^{-3} M or less), while in others the rate of reaction increases with substrate concentration up to very high concentrations. In general a maximum rate can be measured or calculated from the curve of rate versus substrate concentration. This maximum rate occurs when the concentration of substrate is sufficient to *saturate* the enzyme.

Michaelis Constant. A measure of the affinity of an enzyme for its substrate is the Michaelis constant, K_m. This constant is an equilibrium constant of the dissociation of an enzyme–substrate combination. It might be considered desirable to express enzyme–substrate affinity in terms of the amount of substrate needed to saturate the enzyme, but it is often difficult or technically impossible to measure the maximum rate directly, and it is obviously difficult to select the substrate concentration that just permits the maximum rate. These difficulties are avoided by determining instead the concentration of substrate required for permitting half the maximum rate. As described below, this value can be obtained by measuring reaction velocities in the presence of various rate-limiting amounts of substrate, without direct experimental determination of the maximum rate. The original formulation[1] for the determination of the constant now known as K_m assumes that the rate of equilibration between enzyme and substrate is fast compared with the subsequent reaction leading to products; that is, in the conversion of a compound A to B, the velocity constants k_1 and k_2 are large compared

$$A + \text{Enzyme} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A \cdot \text{Enzyme} \xrightarrow{k_3} \text{Enzyme} + B$$

with k_3. In this case the equilibrium constant,

$$K_{eq} = [A] \cdot [\text{Enzyme}]/[A \cdot \text{Enzyme}]$$

[1] L. Michaelis and M. L. Menten, *Biochem. Z.* **49**, 339 (1913).

determines the concentration of $A \cdot$ Enzyme at a given substrate concentration. The over-all rate of reaction is proportional to $A \cdot$ Enzyme concentration, and therefore is used to measure the amount of enzyme–substrate combination present at any time. Ordinarily the concentration of enzyme is very small compared with substrate, so it is assumed that A is not diminished through formation of $A \cdot$ Enzyme, and the constant is evaluated by determining the substrate concentration that permits a half-maximal rate. At this substrate concentration the concentrations of free and complexed enzyme are equal, and $K_{eq} = [A]$. This K_{eq} is K_m.

The derivation above contains assumptions that may not be justified. A more complete derivation shows that what is actually measured is $k_2 + k_3/k_1$, not k_2/k_1. If k_3 is *not* small compared with k_2, the measured K_m will not be a true dissociation constant.

In the first derivation of the Michaelis constant given, Enzyme was used to represent free enzyme, not that part bound to substrate. An alternate expression of the same concepts is obtained by letting E = total enzyme, ES = the concentration of complex, and S = substrate whose concentration is essentially constant. Then

$$K_m = [E - ES]S/ES$$

and

$$ES = \frac{[E][S]}{K_m + S}$$

The velocity of the over-all reaction, v, is equal to a constant, k_3, times the concentration of ES. Therefore,

$$v = k_3[ES] = \frac{k_3[E][S]}{K_m + S}$$

When all of the enzyme is present as enzyme–substrate complex, the velocity is maximum, so for k_3E the term V_{max} may be substituted. Then

$$v = \frac{V_{max}[S]}{K_m + S} \tag{1}$$

or, rearranging,

$$K_m = \frac{V_{max}}{v} - 1 \tag{2}$$

Several methods have been used to evaluate the constants K_m and V_{max}. The one most often used is that of Lineweaver and Burk,[2] who rearranged equation (1) to obtain the form

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{S} + \frac{1}{V_{max}}$$

[2] H. Lineweaver and D. Burk, *J. Am. Chem. Soc.* **56**, 658 (1934).

When $1/v$ is plotted against $1/S$, a straight line is obtained with a slope of K_m/V_{max} and an intercept of $1/V_{max}$ (Fig. 4). Other useful formulations are

$$\frac{S}{v} = \frac{1}{V_{max}} \times S + \frac{K_m}{V_{max}} \qquad \text{(Lineweaver and Burk)}$$

$$\frac{v}{S} \times K_m = V_{max} - v \qquad \text{(Augustinsson)}^3$$

Each substrate for a given enzyme for both forward and reverse reactions has a characteristic K_m under specified reaction conditions. It is possible to determine K_m values for all of the substrates involved in reversible reactions and to determine V_{max} in each direction. The reaction rates in the two directions may be so different that the amount of enzyme

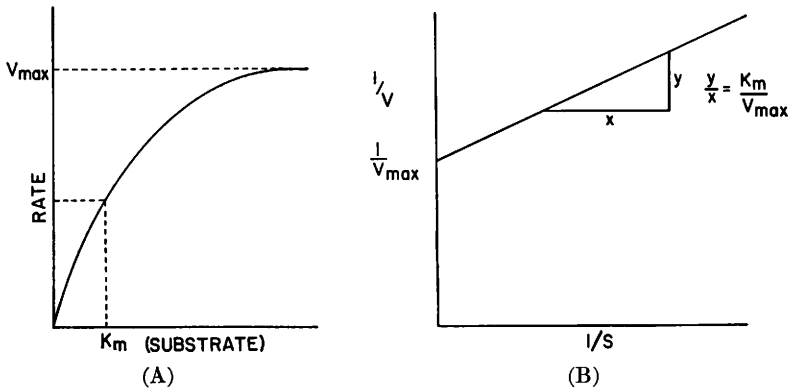

FIG. 4. (A) indicates the increase in reaction rate with increase in substrate concentration and constant amounts of enzyme. The maximum rate is obtained graphically, and K_m is determined from the curve as the value on the abscissa corresponding to ½ the maximum rate. (B) shows a typical Lineweaver-Burk plot.

required for measuring a rate in one direction may be many times the amount needed for the reverse reaction, but as long as the enzyme concentration is small compared with the substrate concentration, K_m values are not influenced by enzyme concentration. V_{max} values are proportional to the amount of enzyme used.

Inhibition. Graphic analysis of kinetic data according to the method of Lineweaver and Burk has a useful application in the study of inhibitors. Many substances limit the activity of enzymes by reacting with the protein or some other component in such a way as to destroy or decrease the catalytic ability. Other materials inhibit by forming the same sort of complex that a substrate does. In this latter case, the two materials

[3] K. B. Augustinsson, *Acta Physiol. Scand.* **15**, *Suppl.* 52 (1948).

compete for the enzyme, and the net rate of reaction is a function of the concentrations of substrate and inhibitor. A dissociation constant for the inhibitor, K_I, corresponds to the Michaelis constant of the substrate, K_m or K_s.

Lineweaver-Burk plots can be used to show that an inhibitor is a competitor with respect to substrate and to evaluate the affinity of the enzyme for the inhibitor. When plots are made for experiments carried out with and without inhibitor, two lines are obtained that extrapolate to the same intercept, V_{max}. The slopes of the two lines will be different; the presence of inhibitor causes the slope to be greater (Fig. 5).

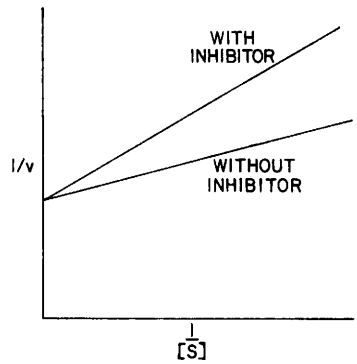

FIG. 5. Lineweaver-Burk plot of a reaction, with the rate measured at various substrated concentrations in the presence and absence of a competitive inhibitor.

The difference is in the apparent K_m; the apparent K_m is equal to

$$K_m + \frac{K_m\ (\text{Inhibitor})}{K_I}$$

It is often convenient to evaluate K_I by varying the inhibitor concentration and maintaining a constant substrate concentration. A formulation recently devised by Adams[4] shows that

$$\frac{1}{v} - \frac{1}{V_{max}} = \frac{K_s}{V_{max}S} + \frac{K_sI}{V_{max}SK_I}$$

When $1/v - 1/V_{max}$ is plotted against I, the intercept is $K_s/V_{max}S$ and the slope is $K_s/V_{max}SK_I$, so the ratio of intercept to slope is K_I.

Enzymes may be inhibited by materials that react with sites other than those that interact directly with a substrate and by reagents that bind sites irreversibly. When these types of inhibition occur, Lineweaver-Burk plots of the inhibited reaction do not have the same intercept as

[4] E. Adams, *J. Biol. Chem.* **217**, 325 (1955).

found for the uninhibited system; V_{max} is decreased by the inhibitor. K_m may or may not be affected by a noncompetitive inhibitor, so the slope may or may not be altered. The difference in intercept is the usual criterion for distinguishing between competitive and noncompetitive inhibitions. A noncompetitive inhibitor may also compete with substrate so a qualitative observation of partial reversal of an inhibition by excess substrate is not adequate to describe the action of an inhibitor.[5]

Determinations of K_m and K_I have become routine in enzyme chemistry because of the theoretical and practical information derived from these values. From the theoretical point of view, the affinity of an enzyme for a substrate is important in determining the nature of the bonds between them, especially when the relative affinities for a variety of related structures (both substrates and inhibitors) can be determined. Variations in K_m with changes in the physical environment give additional information about the nature of the binding forces. Practically, the K_m permits decisions to be made about the concentrations required to obtain desired rates of reaction, the relative success of different enzymes that use the same substrate, the feasibility of using an enzyme assay for analysis of substrate at given concentrations, the ability of an enzyme to "pull" another reaction by removing a product, the relative rates of reaction when two substrates are present, and the relative effects of competitive inhibitors.

The Haldane Relationship. Another of the properties of enzyme systems frequently measured is the equilibrium constant of the over-all reaction. This is the means for determining a fundamental thermodynamic property, the free energy (F) of a reaction. Free energy will be discussed later. At this point a relation between enzyme kinetics and equilibrium is of interest. The equilibrium constant for a reaction

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$$

is $[B]/[A]$ and is also equal to k_1/k_2. With nonenzymatic reactions the velocities in the two directions are simply the products of reagent concentration and rate constant. In enzyme-catalyzed reactions, the velocities do not increase without limit as substrate concentration increases. The equilibrium constant can be evaluated as a function of reaction velocities, however, in the case of enzyme reactions also. The relationship between enzymatic reaction rates and chemical equilibrium was derived first by Haldane, and bears his name. Simple derivations were devised by Horecker and Kornberg and by Colowick, whose reasoning is

[5] A theoretical analysis of enzyme–inhibitor interaction and experimental applications to an enzyme system are described by A. Goldstein in *J. Gen. Physiol.* **27**, 529 (1944).

given. For the over-all reaction $A \rightleftharpoons B$, a Michaelis constant exists for each substrate:

$$E + A \rightleftharpoons EA \xrightarrow{k_{3_A}} E + B \qquad E + B \leftrightharpoons EB \xrightarrow{k_{3_B}} E + A$$

$$K_A = \frac{[E][A]}{[EA]} \qquad K_B = \frac{[E][B]}{[EB]} \tag{1}$$

$$\text{dividing: } \frac{K_A}{K_B} = \frac{[A][EB]}{[B][EA]} \tag{2}$$

$$\text{Rearranging } \frac{[B]}{[A]} = \frac{[EB]K_B}{[EA]K_A} \tag{3}$$

At equilibrium the rates of reaction in the two directions are equal. The maximum rates in each direction, V_A and V_B, are obtained when all of the enzyme is bound to one substrate. The rate of reaction in each direction at equilibrium is that fraction of V_{max} given by the actual enzyme–substrate complex divided by total enzyme.

$$V_A = E_{tot}k_{3_A} \qquad V_B = E_{tot}k_{3_B}$$

$$v = \frac{V_A[EA]}{[E_{tot}]} = \frac{V_B[EB]}{[E_{tot}]} \tag{4}$$

$$\frac{V_A}{V_B} = \frac{[EB]}{[EA]} \tag{5}$$

Substituting the terms of (5) into (3)

$$\frac{[B]}{[A]} = K_{eq} = \frac{V_A}{V_B} \times \frac{K_B}{K_A}$$

(The Haldane relationship between the equilibrium constant, maximum velocities, and Michaelis constants.)

For more complex reactions, the Michaelis constants for all components of the system must be included. When the substrates and products have similar affinities for the enzyme, the equilibrium constant approaches the ratio of the maximum rates, which can often be measured as initial rates. Examples will be shown later of reactions in which the correction of the ratio of rates by the ratio of Michaelis constants is more than an order of magnitude.

General References

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A very thorough, analytical historical review of the embryonic and neonatal development of enzyme chemistry has been recorded elegantly by A. Harden in his monograph "Alcoholic Fermentation," Longmans, Green & Co., London, 1923.

CHAPTER II

HYDROLYSIS OF PEPTIDES AND PROTEINS

Enzymes that hydrolyze proteins and other compounds composed of amino acids were among the first biological catalysts to be discovered, and they have continued to be prominent in studies of enzyme structure, kinetics, activation, and mechanism of action. The crystallization of the enzyme urease by Sumner was followed by the crystallization of various proteolytic enzymes in the laboratory of Northrop. These studies established that catalytic activity is associated with what appear to be pure proteins; all well-defined enzymes isolated subsequently have also proved to be proteins, although many contain additional components. The study of enzymatic reactions involving proteins as substrate, therefore, gives insight into the chemical nature of enzymes as well as the mechanisms by which they act.

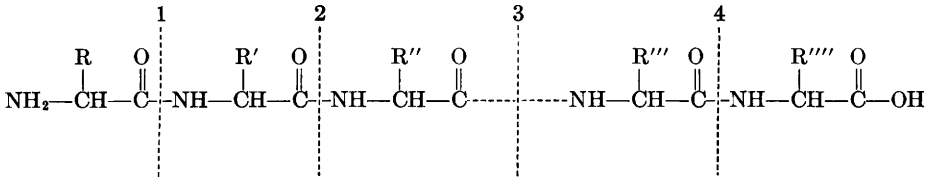

FIG. 6. Peptide chain showing N-terminal linkage (1) and C-terminal linkage (4), which are hydrolyzed by appropriate exopeptidases, and additional peptide bonds (2) and (3), which are hydrolyzed only by endopeptidases.

Originally proteolytic enzymes (proteases) were studied by their ability to digest crude substrates; later highly purified proteins were used. Proteins are polymers of 20 different α-amino acids in which the amino acid residues form substituted amides. Amides of amino acids are called peptides. In addition to the proteases, peptidases, enzymes capable of hydrolyzing small synthetic peptides, were found by Fisher.[1] Considerable progress was made when Bergmann and his collaborators discovered that small synthetic substrates could also serve as substrates for proteolytic enzymes.[2]

The distinction between enzymes capable of attacking large protein molecules and those that attack only small peptides is not as complete as once thought. Two large groups of peptide-splitting enzymes have

[1] E. Fisher and P. Bergell, *Ber.* **36,** 2592 (1903).

[2] M. Bergmann, *Advances in Enzymol.* **2,** 49 (1942).