From Lehninger (1979) Biochemistry

CHAPTER 8 ENZYMES: KINETICS AND INHIBITION

Enzymes are proteins specialized to catalyze biological reactions. They are among the most remarkable biomolecules known because of their extraordinary specificity and catalytic power, which are far greater than those of man-made catalysts.

Much of the history of biochemistry is the history of enzyme research. The name enzyme ("in yeast") was not used until 1877, but much earlier it was suspected that biological catalysts are involved in the fermentation of sugar to form alcohol (hence the earlier name "ferments"). The first general theory of chemical catalysis, published in 1835 by J. J. Berzelius, included an example of what is now known as an enzyme, diastase of malt, and pointed out that hydrolysis of starch is more efficiently catalyzed by diastase than by sulfuric acid.

Although Louis Pasteur recognized that fermentation is catalyzed by enzymes, he postulated in 1860 that they are inextricably linked with the structure and life of the yeast cell. It was therefore a major landmark in the history of enzyme research when, in 1897, Eduard Buchner succeeded in extracting from yeast cells the enzymes catalyzing alcoholic fermentation. This achievement clearly demonstrated that these important enzymes, which catalyze a major energyyielding metabolic pathway, can function independently of cell structure. However, it was not until many years later that an enzyme was first isolated in pure crystalline form. This was accomplished by J. B. Sumner in 1926 for the enzyme urease, isolated from extracts of the jack bean. Sumner presented evidence that the crystals consist of protein, and he concluded, contrary to prevailing opinion, that enzymes are proteins. His views were not immediately accepted, however, and it was not until the period 1930 to 1936, during which J. Northrop crystallized the enzymes pepsin, trypsin, and chymotrypsin (Figure 8-1), that the protein nature of enzymes was firmly established. Today nearly 2,000 different enzymes are known. Many have been isolated in pure homogeneous form, and at least 200 have been crystallized.

Figure 8-1 Crystals of bovine chyrnotrypsin.



0.1 mm

Although most of the enzymes concerned with the basic metabolic housekeeping of the cell have been identified, many important problems remain to be solved, including the genetic control of enzyme synthesis, the molecular mechanisms by which enzyme activity is regulated, and the role of multiple forms of certain enzymes in development and differentiation. Above all, we still do not know in molecular terms how enzymes catalyze chemical reactions with such efficiency, precision, and specificity.

In this and the following chapter no attempt is made to catalog and describe the large number of different enzymes known today. Instead the properties and characteristics common to most enzymes will be examined. Specific enzymes participating in various metabolic cycles will be discussed in more detail in succeeding chapters.

Naming and Classification of Enzymes

Many enzymes have been named by adding the suffix -ase to the name of the substrate, i.e., the molecule on which the enzyme exerts catalytic action. For example, urease catalyzes hydrolysis of urea to ammonia and CO,, arginase catalyzes the hydrolysis of arginine to ornithine and urea, and phosphatase the hydrolysis of phosphate esters. However, this nomenclature has not always been practical, with the result that many enzymes have been given chemically uninformative names, e.g., pepsin, trypsin, and catalase. For this reason and because the number of newly discovered enzymes is increasing rapidly, a systematic classification of enzymes has been adopted on the recommendation of an international enzyme commission. The new system divides enzymes into six major classes and sets of subclasses, according to the type of reaction catalyzed (Table 8-1). Each enzyme is assigned a recommended name, usually short and appropriate for everyday use, a systematic name, which identifies the reaction it catalyzes, and a classification number, which is used where accurate and unambiguous identification of an enzyme is required, as in international research journals, abstracts, and indexes. An example is given by the enzyme catalyzing the reaction

 $ATP + creatine \implies ADP + phosphocreatine$

The recommended name of this enzyme, that normally used, is <u>creatine kinase</u>, and the systematic name, based on the reaction catalyzed, is <u>ATP:creatine phosphotransferase</u>. Its classification number is EC 2.7.3.2, where EC stands for Enzyme Commission, the first digit (2) for the class name (transferases), the second digit (7) for the subclass (phosphotransferases), the third digit (3) for the sub-subclass (phosphotransferases with a nitrogenous group as acceptor), and the fourth digit (2) designates creatine kinase (see Table 8-1). In this book we shall use the recommended names of enzymes, as listed in the 1973 edition of Enzyme Nomenclature (see References), with a few exceptions. Table 8-1 International classification of enzymes (class names, code numbers, and types of reactions catalyzed)

- 1. Oxido-reductases (oxidationreduction reactions)
 - 1.1 Acting on CH—OH
 - 1.2 Acting on C=C

1.3 Actingon C=CH-

- 1.4 Acting on CH-NH₂
- 1.5 Acting on CH-NH-

1.6 Acting on NADH; NADPH

- 2. Transferases (transfer of functional groups)
 - 2.1 One-carbon groups
 - 2.2 Aldehydic or ketonic groups
 - 2.3 Acyl groups
 - 2.4 Glycosyl groups
 - 2.7 Phosphate groups
 - 2.8 S-containing groups
- 3. Hydrolases (hydrolysis reactions)
 - 3.1 Esters
 - 3.2 Glycosidic bonds
 - 3.4 Peptide bonds
 - 3.5 Other C-N bonds
 - 3.6 Acid anhydrides
- 4. Lyases (addition to double bonds)



4.3 C=N-

- Isomerases (isomerization reactions)
 1 Racemases
- 6. Ligases (formation of bonds with ATP cleavage)
 - 6.1 C 0
 - 6.2 C-S
 - 6.3 C-N
 - 6.4 C-C

Table 8-2 Some enzymes containing or requiring metal ions as cofactors

$Zn^{2}+$

Alcohol dehydrogenase Carbonic anhydrase Carboxypeptidase Mg²+ Phosphohydrolases

Phosphotransferases

Mn²+

Arginase Phosphotransferases

Fe^{2+} or Fe^{3+}

Cytochromes Peroxidase Catalase Ferredoxin

Cu^{2+} (Cu^{+})

Tyrosinase Cytochrome oxidase

K+

Pyruvate kinase (also requires Mg²⁺)

Na⁺

Plasma membrane ATPase (also requires K^+ and Mg^{2+})

Enzyme Cofactors

Some enzymes depend for activity only on their structure as proteins, while others also require one or more nonprotein components, called <u>cofactors</u>. The cofactor may be a <u>metal</u> <u>ion</u> or an organic molecule called a <u>coenzyme</u>; some enzymes require both. Cofactors are generally stable to heat, whereas most enzyme proteins lose activity on heating. The catalytically active enzyme-cofactor complex is called the <u>holoenzyme</u>. When the cofactor is removed, the remaining protein, which is catalytically inactive by itself, is called an apoenzyme.

Table 8-2 lists some enzymes requiring metal ions as cofactors. In such enzymes the metal ion may serve as (1) the primary catalytic center; (2) a bridging group, to bind substrate and enzyme together through formation of a coordination complex; or (3) an agent stabilizing the conformation of the enzyme protein in its catalytically active form. Enzymes requiring metal ions are sometimes called <u>metalloenzymes</u>. In some metalloenzymes the metal compodent alone already possesses primitive catalytic activity, which is greatly enhanced by the enzyme protein; e.g., the iron-porphyrin enzyme catalase, which catalyzes very rapid decomposition of hydrogen peroxide to water and oxygen. Simple iron salts also catalyze this reaction but at a much lower rate.

Table 8-3 summarizes the principal coenzymes and the types of enzymatic reactions in which they participate. Each of the coenzymes listed contains as part of its structure a molecule of one or another of the <u>vitamins</u>, trace organic substances that are vital to the function of all cells and required in the diet of certain species. The vitamins and their coenzyme forms are treated in Chapter 13 (page 335). Coenzymes usually function as intermediate carriers of functional groups, of specific atoms, or of electrons that are transferred in the overall enzymatic reaction. When the coenzyme is very tightly bound to the enzyme molecule, it is usually called a prosthetic group, e.g., the biocytin group of acetyl-CoA carboxylase, which is covalently incorporated in the

Table 8-3 Coenzymes in group-transferring reactions

Coenzyme	Entity transferred
Nicotinamide adenine dinucleotide	Hydrogen atoms (electrons)
Nicotinamide adenine dinucleotide phosphate	Hydrogen atoms (electrons)
Flavin mononucleotide	Hydrogen atoms (electrons)
Flavin adenine dinucleotide	Hydrogen atoms (electrons)
Coenzyme Q	Hydrogen atoms (electrons)
Thiamin pyrophosphate	Aldehydes
Coenzyme A	Acyl groups
Lipoamide	Acyl groups
Cobamide coenzymes	Alkyl groups
Biocytin	Carbon dioxide
Pyridoxal phosphate	Amino groups
Tetrahpdrofolate coenzymes	Methyl, methylene, formyl or formimino groups

polypeptide chain (page 345). In some cases, however, the coenzyme is only loosely bound and essentially functions as one of the specific substrates of that enzyme.

Chemical Kinetics

Before we examine the catalysis of reactions by enzymes, some relationships and terms used in measuring and expressing the rates of chemical reactions must be outlined. Chemical reactions may be classified on the basis of the number of molecules that must ultimately react to form the reaction products. Thus, we have <u>monomolecular</u>, <u>bimolec</u>ular, and <u>termolecular</u> reactions, in which one, two, or three molecules, respectively, undergo reaction.

Chemical reactions are also classified on a kinetic basis, by <u>reaction order</u>, and we have zero-order, first-order, second-order, and third-order reactions, depending on how the reaction rate is influenced by the concentration of the reactants under a given set of conditions.

<u>First-order reactions</u> are those which proceed at a rate exactly proportional to the concentration of one reactant (Figure 8-2). The simplest example is when the rate of the reaction

A — P

is exactly proportional to the concentration of A. Then the reaction rate at any time t is given by the <u>first-order rate</u> equation

$$\frac{-d[A]}{dt} = k[A]$$

where [A] is the molar concentration of A and -d[A]/dt is the rate at which the concentration of A decreases. The proportionality constant k is called the <u>rate constant</u> or <u>specific</u> reaction rate. First-order rate constants have the dimensions of reciprocal time, usually s^{-1} .

The integrated form of this equation, which is more useful for carrying out kinetic calculations, is

$$\log \frac{[A_0]}{[A]} = \frac{kt}{2.303}$$

in which [A,] is the concentration of A at zero time and [A] is the concentration at time t.

In first-order reactions, the half-time $t_{1/2}$ of the reaction is given by

$$t_{1/2} = \frac{0.693}{k}$$

a relationship that is simply derived. In first-order reactions the half-time is independent of the initial concentration of substrate.

<u>Second-order reactions</u> are those in which the rate is proportional to the product of the concentrations of two reac-

Figure 8-2 Plot of the course of a first-order reaction. The half-time t,,, is the time required for one-half of the initial reactant to be consumed.



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tants or to the second power of a single reactant. The simplest example is the reaction

$$A + B \longrightarrow P$$

The rate of this reaction, which may be designated as -d[A]/dt, -d[B]/dt, or +d[P]/dt, is proportional to the product of the concentrations of A and B, as given by the <u>second</u>-order rate equation

$$\frac{-d[A]}{dt} = k[A][B]$$

where k is the second-order rate constant. If the reaction has the form

$$2A \longrightarrow P$$

and its rate is proportional to the product of the concentration of the two reacting molecules, the second-order rate equation is

$$\frac{-d[A]}{dt} = k[A][A] = k[A]^2$$

The rate constants of second-order reactions have the dimensions $1/(\text{concentration} \times \text{time})$, or $M^{-1} s^{-1}$. The integrated form of the second-order rate equation is

$$t = \frac{2.303}{k([A_0] - [B_0])} \log \frac{[B_0][A]}{[A_0][B]}$$

where $[A_n]$ and $[B_0]$ are initial concentrations and [A] and [B] the concentrations at time t.

For second-order reactions in which the initial concentrations of the reactants are equal, the half-time is equal to $1/C_0k$, where C_0 is the initial concentration of reactants and k the second-order rate constant.

It is important to note that a second-order reaction such as

$$A + B \longrightarrow P$$

may under some conditions appear to be a first-order reaction. For example, if the concentration of B is very high and that of A very low, this reaction might appear to be firstorder because its rate will be nearly proportional to the concentration of only one reactant, namely, **A.** Under these special conditions the reaction is an <u>apparent-</u> or <u>pseudo-</u> first-order reaction.

<u>Third-order</u> reactions, which are relatively rare, are those whose velocity is proportional to the product of three concentration terms. Some chemical reactions are independent of the concentration of any reactant; these are called <u>zeroorder</u> reactions. Many catalyzed reactions are zero order with respect to the reactants. When this is true, the rate of reaction depends on the concentration of the catalyst or on some

factor other than the concentration of the molecular species undergoing reaction. Reaction rates need not necessarily be pure first order or pure second order; often reactions are of mixed order.

The Free Energy of Activation and the Effects of Catalysts

A chemical reaction such as $A \longrightarrow P$ takes place because a certain fraction of the population of A molecules at any given instant possesses enough energy to attain an activated condition, called the <u>transition state</u>, in which the probability is very high that a chemical bond will be made or broken to form the product P. This transition state is at the top of the energy barrier separating the reactants and products (Figure 8-3). The rate of a given chemical reaction is proportional to the concentration of this transition-state species. The free energy of activation AG+ (the symbol * designates the activation process) is the amount of energy required to bring all the molecules in 1 mol of a substance at a given temperature to the transition state at the top of the activation barrier.

There are two general ways in which the rate of a chemical reaction may be accelerated. A rise in temperature, because it increases thermal motion and energy, increases the number of molecules capable of entering the transition state and thus accelerates the rate of chemical reactions. In many reactions, the reaction rate is approximately doubled by a 10°C rise in temperature. The rate of a chemical reaction

Figure 8-3

Energy diagram for a chemical reaction, uncatalyzed and catalyzed.





Table 8-4 Estimated free energy of acti- vation AG' for the decomposition of hydro- gen peroxide at 20°C; catalase accelerates the rate of the reaction by more than 10 ⁸ -fold		
Conditions	AG* kcal mol ⁻¹	
Uncatalyzed catalyzed by colloidal platinum catalyzed by catalase	18 13 7	
catalyzed by catalase	/	

can also be accelerated by addition of a catalyst. Catalysts combine transiently with the reactants to produce a transition state having a lower energy of activation than the transition state of the uncatalyzed reaction. Thus they accelerate chemical reactions by lowering the energy of activation (Figure 8-3). When the reaction products are formed, the free catalyst is regenerated. Table 8-4 shows the energy of activation for the decomposition of hydrogen peroxide under noncatalyzed and catalyzed conditions.

Kinetics of Enzyme-Catalyzed Reactions: The **Michaelis-Menten** Equation

The general principles of chemical-reaction kinetics apply to enzyme-catalyzed reactions, but they also show a distinctive feature not usually observed in nonenzymatic reactions, saturation with substrate. In Figure 8-4 we see the effect of the substrate concentration on the rate of the enzyme-catalyzed reaction $A \longrightarrow P$. At a low substrate concentration, the initial reaction velocity v_0 is nearly proportional to the substrate concentration, and the reaction is thus approximately first order with respect to the substrate. However, as the substrate concentration is increased, the initial rate increases less, so that it is no longer nearly proportional to the substrate concentration; in this zone, the reaction is mixed order. With a further increase in the substrate concentration, the reaction rate becomes essentially independent of substrate concentration and asymptotically approaches a constant rate. In this range of substrate concentrations the reaction is essentially zero order with respect to the substrate and the enzyme is spoken of as being saturated with its substrate. All enzymes show the saturation effect, but they vary widely with respect to the substrate concentration required to produce it. This saturation effect led some early investigators, particularly A. J. Brown and also V. Henri, to the hypothesis that the enzyme and substrate react reversibly to form a complex, as an essential step in the catalyzed reaction.

In 1913 a general theory of enzyme action and kinetics was developed by L. Michaelis and M. L. Menten, which was later extended by G. E. Briggs and J. B. S. Haldane. This theory, which is basic to the quantitative analysis of all as-

Figure 8-4 Effect of substrate concentration on the rate of an enzyme-catalyzed reaction.



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pects of enzyme kinetics and inhibition, is best developed for the simple case of a reaction in which there is only one substrate. The Michaelis-Menten theory assumes that the enzyme E first combines with the substrate S to form the enzyme-substrate complex ES; the latter then breaks down in a second step to form free enzyme and the product P:

$$E + S \xrightarrow[k_{-1}]{k_{-1}} ES$$
 (1)

$$\mathrm{ES} \xrightarrow[k_{-2}]{} \mathrm{E} + \mathrm{P} \tag{2}$$

These reactions are assumed to be reversible; the rate constants for the forward and reverse directions respectively have a positive and a negative subscript.

We now derive the Michaelis-Menten equation, which expresses the mathematical relationship between the initial rate of an enzyme-catalyzed reaction, the concentration of the substrate, and certain characteristics of the enzyme. The Michaelis-Menten equation is the <u>rate equation</u> for reactions catalyzed by enzymes having a single substrate. In this derivation, that of Briggs and Haldane, [E] represents the concentration of the free or uncombined enzyme, [ES] the concentration of the enzyme-substrate complex, and $[E_T]$ the total enzyme concentration (the sum of the free and combined forms). [S] represents the substrate concentration, which is assumed to be far greater than [E], so that the amount of S bound by E at any given time is negligible compared with the total concentration of S.

It is the purpose of this derivation to define a general expression for v_0 , the initial velocity of an enzyme-catalyzed reaction, assuming that enzyme-catalyzed reactions take place in two steps, as shown in reactions (1) and (2). The initial velocity is of course equal to the rate of breakdown of the enzyme-substrate complex ES, according to equation (2), for which we can write the first-order rate equation

$$v_0 = k_{+2}[\text{ES}] \tag{3}$$

However, since neither k_{+2} nor [ES] can be determined directly, we must find an alternative expression for v_0 in terms of other variables that can be measured more readily. To do this we first write the second-order rate equation for the formation of ES from E and S [see reaction (1)]:

$$\frac{d[\text{ES}]}{dt} = k_{+1}([\text{E}_T] - [\text{ES}]) [S]$$
(4)

in which k_{+1} is the second-order rate constant. Although ES can also be formed from E and P by reversal of reaction (2), the rate of this back reaction may be neglected, since we are considering the beginning of the reaction in the forward direction, when [S] is very high and [P] is zero or close to zero.

Next we may write the rate equation for the breakdown of ES by the sum of two reactions; first, the reaction yielding

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the product (forward direction) and, second, the reaction yielding E + S [the reverse direction of equation (1)]. We then have

$$\frac{-d[\text{ES}]}{dt} = k_{-1}[\text{ES}] + k_{+2}[\text{ES}]$$
(5)

When the rate of formation of ES is equal to its rate of breakdown, i.e., when the reaction system has entered the steady state, defined as the condition in which the concentration of ES remains constant, then

$$k_{+1}([E_T] - [ES])[S] = k_{-1}[ES] + k_{+2}[ES]$$
(6)

Figure 8-5 illustrates the course with time of the various participants.





Figure 8-5

Time-course of the formation of an enzymesubstrate complex and initiation of the steady state, as derived from computer solutions of data obtained in an actual experiment on a typical enzyme. The portion shaded in the top graph is shown in magnified form on the lower graph.



Rearranging equation (6), we obtain

$$\frac{[S]([E_T] - [ES])}{[ES]} = \frac{k_{-1} + k_{+2}}{k_{+1}} = K_M$$
(7)

The lumped constant K_M , which replaces the term $(k_{-1} + k_{+2})/k_{+1}$, is called the Michaelis-Menten constant.

From this equation the steady-state concentration of the ES complex can be obtained by solving for [ES]:

$$[ES] = \frac{[E_T][S]}{K_M + [S]}$$
(8)

We have seen (above) that the initial rate v_0 of an enzymatic reaction is

$$v_0 = k_{+2} [ES]$$
 (3)

We can now substitute for the term [ES] in equation (3) its value from equation (8):

$$v_0 = k_{\pm 2} \frac{[E_T][S]}{K_M + [S]}$$
(9)

When the substrate concentration is so high that essentially all the enzyme in the system is present as the ES complex, i.e., when the enzyme is <u>saturated</u>, we reach the maximum initial velocity V_{max} , given by

in which $[E_T]$ is the total enzyme concentration. Now, substituting for k_{+2} $[E_T]$ its value from equation (10) we obtain

$$v_0 = \frac{V_{\max} [S]}{K_M + [S]}$$
(11)

This is the Michaelis-Menten equation, the rate equation for a one-substrate enzyme-catalyzed reaction. It relates the initial velocity, the maximum velocity, and the initial substrate concentration through the Michaelis-Menten constant. It is important to note that although the Michaelis-Menten equation appears to have no term for enzyme concentration, it is actually contained in the term V_{max} , which we have seen is equal to k_{+2} [E_T].

An important numerical relationship emerges from the Michaelis-Menten equation in the special case when the initial reaction rate is exactly one-half the maximum velocity, i.e., when $v_0 = \frac{1}{2}V_{max}$ (Figure 8-4).

$$\frac{V_{\max}}{2} = \frac{V_{\max} [S]}{K_M + [S]}$$

If we divide by V_{max} , we obtain

$$\frac{1}{2} = \frac{[S]}{K_M + [S]}$$

K _M , mM
25
0.15
1.5
2.5
12.0
32
122
9.0
0.12
2.0
57
0.025
0.018
0.9
0.1
0.04
4.0

Table 8-5 K_M for some enzymes

Table 8-6	Effect of substrate struc-
ture on V _m	ax for n-amino acid oxi-
dase (relat	ive to p-alanine = 100)

Substrate	Relative V_{max}
D-Tvrosine	297
D-Proline	231
D-Methionine	125
D-Alanine	100
D-Valine	55
D-Histidine	9.7
Glycine	0.0

On rearranging, this becomes

$$K_{M} + [S] = 2[S]$$
$$K_{M} = [S] \qquad \bigvee_{o} \left(\frac{\bigvee_{o}}{2} \right)$$

Thus we see that K_M , the Michaelis-Menten constant, is equal to the substrate concentration at which the initial reaction velocity is half maximal. K_M for a one-substrate reaction usually has the dimensions moles per liter and is independent of the enzyme concentration.

The value of K_M for any given enzyme can easily be approximated from a series of simple experiments in which the initial reaction velocity is measured at different initial concentrations of the substrate with a fixed concentration of enzyme. The approximate value of K_M is obtained graphically from a plot of initial velocity vs. initial substrate concentration (Figure 8-4), which has the form of a rectangular hyperbola. At very low substrate concentrations, the initial velocity v_0 is nearly proportional to [S]; that is, the reaction shows essentially first-order behavior. At very high substrate concentrations the reaction rate approaches V_{max} asymptotically and is essentially zero order, i.e., nearly independent of substrate concentration. A few enzymes, such as catalase, appear not to show saturation with substrate; this is because the rate of decomposition of the ES complex to form products is so fast that it cannot easily be made rate-limiting.

Table 8-5 gives the K_M values for a number of enzymes. Note that K_M is not a fixed value but may vary with the structure of the substrate, with pH, and with temperature. For enzymes having more than one substrate, each substrate has a characteristic K_M . Under intracellular conditions, enzymes are not necessarily saturated with their substrates. The maximum velocity V_{max} , which, we recall, is equal to k_{+2} [E_T], also varies widely from one enzyme to another for a given enzyme concentration. V_{max} also varies with the structure of the substrate (Table 8-6), with pH, and with temperature.

The Michaelis constant of an enzyme is an important and useful characteristic, fundamental not only to the mathematical description of enzyme kinetics but also to the quantitative assay of enzyme activity in tissues and enzyme purification. Moreover, the substrate concentration yielding halfmaximal velocity provides a useful index for the analysis of some enzyme regulatory mechanisms (page **236**). A striking example from recent medical research shows the usefulness of K_M in another way. Some types of animal and human leukemia (a form of cancer in which white blood cells proliferate abnormally) can be suppressed by intravenous administration of the enzyme <u>asparaginase</u>, which catalyzes the reaction

Asparagine + H₂O \implies aspartate + NH₄⁺

This finding led to the conclusion that asparagine present in the blood is an essential nutrient for the growth of the malignant white cells; intravenous asparaginase causes hydrolysis of asparagine to aspartate, which cannot satisfy the require-

ment for asparagine. During a search for sources of asparaginase suitable for treatment of leukemia, the puzzling discovery was made that not all asparaginases are effective in suppressing experimental leukemia. The reason was finally found: asparaginases from different animal, plant, and bacterial sources differ widely in their K_M for asparagine. Since the concentration of asparagine in the blood is very low, the administration of an asparaginase from another species can be therapeutically effective only if its K_M value is low enough to hydrolyze asparagine rapidly at the low concentration at which it is present in the blood.

The kinetic behavior of most enzymes is more complex than that of the simple, idealized one-substrate reaction we have just discussed. For one thing, our formulation has assumed that in a one-substrate reaction there is only one enzyme-substrate complex, but it now appears likely that many one-substrate reactions may involve two or three complexes, as indicated in the sequence

$$E + S \Longrightarrow ES \Longrightarrow EZ \Longrightarrow EP \Longrightarrow E + P$$

where EZ is an intermediate complex and EP an enzymeproduct complex. Moreover, only a minority of enzymatic reactions have a single substrate; most enzymes have two or more substrates and may have two or more products. Kinetic analysis of enzymatic reactions involving multiple reactants and multiple products is complex; nevertheless, the Michaelis-Menten relationship remains the starting point for analysis of the kinetics of all enzymatic reactions.

The Michaelis Constant K_M and the Substrate Constant K_S

The Michaelis constant, as we noted above, is an experimentally determined, operationally defined quantity: the substrate concentration at which the reaction velocity is half maximal. In the idealized case used in the derivation above it is represented by

$$K_M = \frac{k_{-1} + k_{+2}}{k_{+1}} \tag{7}$$

but in some enzymatic reactions k_{-1} is very large compared with k_{+2} , in which case the rate constant k_{+2} becomes negligibly small and equation (7) simplifies to the expression

$$K_M \approx \frac{k_{-1}}{k_{+1}}$$

where K_M is approximately equal to the dissociation constant of the enzyme-substrate complex K_s , also called the substrate constant:

$$K_{\rm S} = \frac{[\rm E][\rm S]}{[\rm ES]}$$

Unfortunately, K_M and K_S are frequently but wrongly regarded as synonymous. K_M should not be regarded as the dissociation constant of the ES complex unless specific information is available that k_{+2} is very small compared with k_{-1} .

Transformations of the Michaelis-Menten Equation

The Michaelis-Menten relationship [equation (11)] can be algebraically transformed into other forms that are more useful in plotting experimental data. One common transformation is derived simply by taking the reciprocal of both sides' of the Michaelis-Menten equation (11):

$$\frac{1}{v_0} = \frac{K_M + [S]}{V_{\max} [S]}$$

Rearranging, we have

$$\frac{1}{v_0} - \frac{K_M}{V_{\max}[S]} + \frac{[S]}{\tilde{V}_{\max}[\tilde{S}]}$$

which reduces to

$$\frac{1}{v_0} = \frac{K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}}$$
(12)

Equation (12) is the Lineweaver-Burk equation. When $1/v_0$ is plotted against 1/[S], a straight line is obtained. This line will have a slope of K_M/V_{max} , an intercept of $1/V_{max}$ on the $1/v_0$ axis, and an intercept of $-1/K_M$ on the 1/[S] axis (Figure 8-6). Such a <u>double-reciprocal plot</u> has the advantage of allowing a much more accurate determination of V_{max} , which can only be approximated as a limiting value at infinite substrate concentration from a simple plot of v_0 vs. [S], as seen in Figure 8-4. The double-reciprocal plot can also give valuable information on enzyme inhibition, as we shall see later (page 199).

Another useful transformation of the Michaelis-Menten equation is obtained by multiplying both sides of equation (12) by V_{max} and rearranging to yield

$$v_0 = -K_M \frac{v_0}{[S]} + V_{\max}$$

A plot of v_0 against $v_0/[S]$, called the <u>Eadie-Hofstee plot</u> (Figure 8-7), not only yields V_{max} and K_M in a very simple way but also magnifies departures from linearity which might not be apparent in a double-reciprocal plot.

Effect of pH on Enzymatic Activity

Most enzymes have a characteristic pH at which their activity is maximal; above or below this pH the activity declines. Although the pH-activity profiles of many enzymes are bell-shaped, they may vary considerably in form (Figure 8-8). The pH-activity relationship of any given enzyme depends on the acid-base behavior of enzyme and substrate, as well as many other factors that are usually difficult to ana-



Figure 8-6





