

Chap. 5 "Properties of Enzymes"

Reading Assignment: pp. 130-155. (Skip Sections 5.6, 5.7 B & C, 5.9, and 5.10 A & C)

Problem Assignment: 2, 4, 5, 7 a and b, 8, 13, & 16.

I. Introduction

Nearly all reactions of metabolism are catalyzed by enzymes. Enzymes increase the rates of reactions allowing them to occur within a biologically useful time scale. Most enzymes are proteins, although a number of enzymes are RNA molecules. For both types of enzymes, the reaction occurs within a specialized site known as the active site. Active sites are complementary in shape to the reactant (the substrate), and are even more closely complementary to the transition state structure for the reaction (**Chap. 6**). Enzymes are like simple catalysts in that they are not consumed during the chemical reaction that takes place.

Enzymes are able to increase rates of reactions by 10^3 to 10^{17} fold over the rate of the uncatalyzed reaction. It is important to note that enzymes do not change the positions of chemical equilibrium, they just increase the rate at which equilibrium is attained. By having a shape complementary to the transition state structure, enzymes favor formation of this structure, and thereby, increases the rate of the reaction (**Chap. 6**).

Enzyme-catalyzed reactions are highly specific. Unlike most organic chemistry reactions, side products rarely are produced from an enzyme-catalyzed reaction. Specificity results from the structural complementarity between the enzyme and its substrate. Only substrates with the correct shapes can be bound by the enzyme, minimizing the frequency of side reactions. Most enzymes are stereospecific and act only on one stereoisomeric form of the substrate.

Metabolism is regulated largely by modulating the activity of enzymes that catalyze key steps in metabolic pathways. Enzyme activity can be regulated by changing enzyme concentration in response to a hormonal signal, changing the activity of existing enzyme molecules by binding of modulators, and pharmacologically by drugs that act as enzyme inhibitors, e.g., aspirin.

II. The six classes of enzymes.

1. Oxidoreductases. Catalyze oxidation-reduction reactions. Most often called dehydrogenases.
2. Transferases. Catalyze group transfer reactions. This group includes kinases which transfer a phosphoryl group from ATP.
3. Hydrolases. Catalyze bond cleavage by hydrolysis.
4. Lyases. Catalyze substrate lysis to produce a double bond. If the reverse reaction is catalyzed, the enzyme may be called a synthase.
5. Isomerases. Catalyze structural changes within a single molecule.
6. Ligases. Catalyze the ligation or joining together of 2 substrates. If ATP is required in the reaction the enzyme usually is referred to as a synthetase.

III. Kinetic experiments reveal enzyme properties.

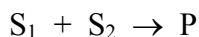
Enzyme kinetics is the study of the rates of enzyme catalyzed reactions. Basic studies shed light on the mechanisms of reactions and the specificity of enzymes for substrates. In the clinic, the determination of enzyme activity level can be used to diagnose diseases such as myocardial infarction (heart attack).

A. Chemical kinetics.

Consider a reaction $S \rightarrow P$, where S is the substrate and P is the product. The rate (or velocity, v) of the reaction is the change in $[P]$ per unit time (t). This can be expressed in a rate equation for the reaction, where k is the rate constant:

$$\Delta[P] / \Delta t = v = k[S].$$

This equation indicates that the reaction velocity is a linear function of $[S]$, where the rate constant k is the slope of a plot of v vs $[S]$. Fig. 5.1 Because the rate depends on the concentration of just one substrate, the reaction is said to have a kinetic order of one, i.e., is a first-order reaction. In this case, the units of k are s^{-1} . If there are 2 substrates



then the rate equation is

$$\Delta[P] / \Delta t = v = k[S_1][S_2]$$

and the kinetic order is 2, i.e., is a second-order reaction. In this case the units of k are $M^{-1}s^{-1}$. Reactions involving 3 or more substrates can occur, but won't be dealt with here.

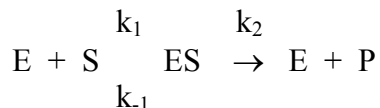
B. Enzyme kinetics, and basic rate determinations.

During an enzyme catalyzed reaction, the enzyme (E) first combines with the substrate to generate an enzyme-substrate complex, and then the product is formed and dissociates from the enzyme. The kinetic scheme for an enzyme catalyzed reaction can be written as



If the rate limiting step is the breakdown of $ES \rightarrow E + P$, then the initial velocity (v_0) of the reaction will be linearly proportional to the amount of E present, provided S is in great excess and all of the enzyme is combined with S, i.e., the enzyme is "saturated." This basic behavior can be exploited to determine the amount of enzyme in a sample of blood, for example, using a simple calibration curve (**Fig. 5.2**).

In actuality the 2 steps of this reaction are reversible. However, if one measures initial velocity (v_0) before P builds up then the equation can be written as



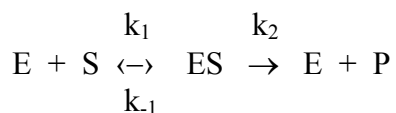
In this equation k_1 is the rate constant for the association of E and S, and k_{-1} is the rate constant for the dissociation of the ES complex. k_2 is the rate constant for formation of E and P. At prolonged times after the onset of the reaction when P has accumulated, the rate $\Delta P/\Delta t$ falls off due to the reverse reaction $E + P \rightarrow EP$ resulting in progress curves such as shown in **Fig. 5.3**. This illustrates the importance of measuring initial velocities before the reaction begins to slow down due to product accumulation, so that one can calculate the amount of enzyme present.

IV. Michaelis-Menten equation.

A. Basic assumptions.

For many enzymes, a plot of initial reaction velocity (v_0 , measured in units of moles of product per liter per second) vs. substrate concentration $[S]$ gives a hyperbolic curve like the one in **Fig. 5.4**. At very low substrate concentration, v_0 increases almost linearly with increasing $[S]$, while at high $[S]$, v_0 is independent of $[S]$ and approaches a maximum velocity, V_{\max} .

The French mathematicians Leonor Michaelis and Maud Menten derived a mathematical equation that describes the kinetic behavior of most enzymes. In their derivation, they proposed that 1) the enzymatic reaction occurs in two stages, and 2) the rate of product formation is determined by the amount of enzyme substrate (ES) complex present, as shown in the equation:



They also assumed that the rate of the second stage of the reaction ($ES \rightarrow E + P$) is slower than the first. For this reason, the second stage of the reaction sets the rate of the overall reaction, i.e., is "rate limiting". Because 1) the rate of the overall reaction is dependent on $[ES]$, and 2) the second stage is rate-limiting, the velocity of the reaction (v_0) is given by the equation:

$$v_0 = k_2[ES].$$

A maximal rate is obtained when all of the enzyme (denoted by $[E]_{\text{total}}$) is in the form of the ES complex, and under these conditions:

$$V_{\max} = k_2[E]_{\text{total}}.$$

B. Derivation of the Michaelis-Menten equation.

Starting with these assumptions it is possible to derive the MM equation. The derivation takes the standpoint that a "steady state", in which the concentration of ES remains relatively constant over time, soon develops after the substrate and enzyme are mixed. The amount of ES formed depends on the concentration of the substrate and its affinity for the enzyme. When the steady state is achieved, the rate of ES formation equals the rate of ES breakdown, i.e.,

$$\text{Rate of ES formation} = k_1([E]_{\text{total}} - [ES])[S]$$

$$\text{Rate of ES breakdown} = k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)[ES]$$

and therefore,

$$k_1([E]_{\text{total}} - [ES])[S] = (k_{-1} + k_2)[ES].$$

(Note that the concentration of the free enzyme $[E]$ in upper of these 2 kinetic equations, has been written as $[E]_{\text{total}} - [ES]$.) The steady state equation is then rearranged to collect all of the rate constants on one side in the so-called Michaelis constant, K_m :

$$\frac{k_{-1} + k_2}{k_1} = K_m = \frac{([E]_{\text{total}} - [ES])[S]}{[ES]}$$

This equation is solved (in several steps) for $[ES]$:

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

Next, the initial velocity (v_0) term is introduced into the equation after multiplying both sides by the k_2 rate constant and substituting $v_0 = k_2[ES]$ from above:

$$v_0 = k_2[ES] = \frac{k_2[E]_{\text{total}}[S]}{K_m + [S]}$$

Finally, the term V_{max} is substituted into the equation for $k_2[E]_{\text{total}}$ resulting in the final form of the MM equation:

$$v_0 = \frac{V_{\text{max}} [S]}{K_m + [S]}$$

Does the equation actually describe for the shape of the hyperbolic curve shown in **Fig. 5.3**? This can be addressed using some simple mathematical proofs. At high substrate concentrations (the extreme right side of the curve), $[S] \gg K_m$, we can neglect the term K_m in the MM equation. Under these conditions,

$$v_0 = \frac{V_{\text{max}} [S]}{[S]} = V_{\text{max}}.$$

Thus, at very high substrate concentrations v_0 becomes independent of $[S]$, and the curve becomes flat at the limiting value V_{max} .

Now, at very low substrate concentrations (the extreme left side of the curve) where $[S] \ll K_m$, we can neglect the term $[S]$ in the denominator of the MM equation. Under these conditions,

$$v_0 = \frac{V_{\max} [S]}{K_m} = (V_{\max}/K_m) [S]$$

This indicates that at very low substrate concentrations the velocity is linearly proportional to [S], i.e., the shape of the curve is approximately linear. These points about curve shape at extremes of substrate concentration also are made in **Fig. 5.5**.

V. Meaning of k_{cat} , K_m , and V_{\max} .

A.

In a large percentage of cases, the rate-limiting step is indeed $\text{ES} \rightarrow \text{E} + \text{P}$, and the equations $v_0 = k_2[\text{ES}]$ and $V_{\max} = k_2[\text{E}]_{\text{total}}$ are directly applicable to the enzyme catalyzed reaction. However, in a significant number of cases, the enzymatic rate may be dependent not only on k_2 , but on other rate constants (e.g., k_{-1}) as well. In these cases, it is better to use a generic constant k_{cat} for the rate limiting step. k_{cat} lumps together all of the constants that actually influence the rate of the reaction. Then, the v_0 and V_{\max} equations become

$$v_0 = k_{\text{cat}}[\text{ES}]$$

and

$$V_{\max} = k_{\text{cat}}[\text{E}]_{\text{total}}.$$

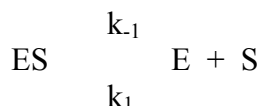
k_{cat} (like k_2) is a first order rate constant and has the units s^{-1} . When the enzyme is operating at maximal velocity, it is a direct measure of the number of moles of product formed per second in the active site. This is the so-called turnover number for the enzyme, and the higher the turnover number the more efficient the enzyme is in carrying out the reaction (**Table 5.1**). The enzyme carbonic anhydrase which converts dissolved CO_2 into carbonic acid has one of the fastest turnover numbers known and thus is well designed for efficient CO_2 transport to the lungs.

B. K_m .

The K_m kinetic constant actually has units of moles/liter (M). It can be mathematically proven that the reaction velocity is half-maximal when $[\text{S}] = K_m$. To prove this, $[\text{S}]$ is substituted for K_m in the MM equation:

$$v_0 = \frac{V_{\max} [\text{S}]}{[\text{S}] + [\text{S}]} = \frac{V_{\max} [\text{S}]}{2[\text{S}]} = \frac{V_{\max}}{2}$$

The K_m constant also provides information about the affinity of an enzyme for its substrate. If the breakdown of $\text{ES} \rightarrow \text{E} + \text{P}$ truly is rate limiting, then k_2 can be neglected from the K_m constant and $K_m \approx k_{-1}/k_1$. Under these conditions, K_m is equivalent to the dissociation constant (K_{ES}) for the ES complex:



for which

$$K_m = K_{ES} = ([E][S])/[ES] = k_{-1}/k_1.$$

This demonstrates that "the lower the K_m , the higher the affinity of the enzyme for its substrate."

The K_m is an intrinsic property of an enzyme and is independent of the enzyme concentration used in a reaction. The K_m of an enzyme often is just slightly higher than the typical intracellular concentration of its substrate (**Fig. 5.4**). Because the hyperbolic curve is roughly linear in this range of substrate concentration, a slight change in substrate concentration produces a significant change in reaction rate, which is advantageous to metabolism. In contrast, if the K_m were much lower than the typical intracellular substrate concentration, then there would be little change in reaction velocity even for substantial changes in S concentration.

C. V_{max} .

V_{max} is calculated from the equations $V_{max} = k_2[E]_{total}$ or $V_{max} = k_{cat}[E]_{total}$. These equations show that V_{max} is dependent on the concentration of enzyme used to set up a reaction. In other words, the V_{max} measured for a reaction will depend on the amount of enzyme present. This allows one to determine the concentration of a clinically important enzyme in a blood specimen, for example, by determining the V_{max} for the sample and using a calibration curve such as shown in **Fig. 5.1** to calculate the amount of enzyme present. The value of V_{max} also can be used to calculate the turnover number (k_{cat}) for an enzyme, as was explained above.

VI. Determination of K_m and V_{max} by Lineweaver-Burk plots.

It is difficult to accurately determine K_m and V_{max} values from hyperbolic kinetic curves due to uncertainty as to where the limit V_{max} occurs. To better determine these values, data often are graphed in the form of the Lineweaver-Burk plot, which plots $1/v_0$ vs $1/[S]$ (**Fig. 5.6**). If one takes the reciprocal of the MM equation, then the LB equation is obtained:

$$1/v_0 = (K_m/V_{max})(1/[S]) + 1/V_{max}$$

This equation is in the form $y = mx + b$ which is the general equation for a straight line. The y-intercept of a LB plot is $1/V_{max}$ and the x-intercept is $-1/K_m$. Thus, the values of V_{max} and K_m are obtained by determining the intercepts of the line with the axes.

VII. Reversible enzyme inhibition.

The study of enzyme inhibition provides information about the structure of active sites, mechanisms of metabolic control, and provides useful information pertinent to the development of pharmaceuticals. Enzyme inhibitors are classified into two broad categories--irreversible and reversible. Irreversible inhibitors usually combine covalently with the enzyme and cannot easily be removed. Reversible inhibitors bind noncovalently and thus reversibly to their target enzyme. When reversible inhibitors are bound to the enzyme, the enzyme is inactive. One common type of reversible enzyme inhibition (competitive inhibition) will be discussed. For reversible inhibitors, one can calculate an inhibition constant K_i , which is the same as the EI dissociation constant. The lower the value of K_i , the stronger the inhibitor binds to the enzyme.



$$K_i = [E][I]/[EI]$$

A. Competitive inhibitors and competitive inhibition.

A competitive inhibitor has a structure that is similar to that of the substrate for the enzyme. For example, the structure of benzamidine, which is a competitive inhibitor of the protease trypsin, is reminiscent of arginine, which is one of the amino acids recognized by this enzyme (**Fig. 5.10**). Due to structural resemblance to the substrate, a competitive inhibitor competes with the substrate for binding to the active site. Then when bound to the enzyme, the competitive inhibitor prevents binding of S (**Fig. 5.8**). Because the EI complex cannot bind S, v_0 values are less when the competitive inhibitor is present than when it is absent. The kinetic scheme for competitive inhibition and the appearance of Lineweaver-Burk plots as a function of [I] are shown in **Fig. 5.9**. Both Lineweaver-Burk and hyperbolic plots (discussed in class) reveal that V_{\max} is unaffected by the presence of the competitive inhibitor. This is so because at very high substrate concentrations S can effectively compete with I for binding to the enzyme. However, the value of the apparent K_m (K_m^{app}), which is the S concentration needed to achieve half-maximal velocity, is increased in the presence of the competitive inhibitor. In other words, the apparent affinity of the enzyme for S decreases in the presence of I. The affinity appears to decrease because I competes with S for binding to the active site.

VIII. Irreversible enzyme inhibition.

Irreversible inhibitors typically react covalently with their target enzymes causing permanent inactivation. Often a key amino acid side-chain in the active site is alkylated or acylated by the inhibitor. A classic example of an irreversible inhibitor is the nerve gas diisopropyl fluorophosphate (DFP) (**Fig. 5.15**). DFP inactivates the enzyme acetylcholinesterase by combining with a serine located within the active site. Acetylcholinesterase is secreted into the synapses of cholinergic nerves, where it serves to degrade acetylcholine molecules once a nerve signal has passed through the synapse. Acetylcholine must be removed from the synapse to allow the neurons to return to their resting state before conduction of another impulse. Other enzymes that contain serines in their active site, such as trypsin and chymotrypsin, also are inactivated by DFP. Another example of an irreversible enzyme inhibitor is aspirin, whose mode of action will be discussed in class.

IX. Regulation of enzyme activity.

In order for metabolism to proceed in an economical fashion, flux through metabolic pathways must be regulated. This is achieved through both long- and short-term regulation of the activity of regulatory enzymes that catalyze key steps in pathways. Long-term regulation is achieved through altering the level of synthesis of regulatory enzymes in response to a hormone or other signal. This type of regulation typically takes hours or days to achieve. Short-term, second-by-second regulation is accomplished by noncovalent binding of modulators (effectors) to regulatory enzymes, or by covalent modification of regulatory enzymes. In both cases, the shape of the enzyme is altered, and with it, its ability to carry out the reaction. Because enzyme shape is affected, this type of regulation is known as allosteric (other shape) regulation. To reduce wasteful synthesis and degradation of metabolites, the activity of an enzyme that catalyzes the first step of a metabolic pathway or a step that requires the input of energy usually is regulated. Allosteric regulation can be

used to positively or negatively regulate activity, depending on the modulator-enzyme combination. The general aspects of allosteric regulation are discussed here.

A. Regulation of allosteric enzymes by noncovalent binding of effectors.

The activity of allosteric enzymes can be controlled by reversible binding of negative effectors (inhibitors) and positive effectors (activators), which usually don't resemble the structure of the substrate, to separate regulatory sites. Allosteric effectors bind noncovalently to regulatory enzymes, changing either the K_m or V_{max} of the reaction. Allosteric enzymes almost always are multi-subunit enzymes and regulation is achieved through changing the packing of subunits within the quaternary structure. Individual chains can be the same or different. Regulatory sites may be on the same subunit that contains the active site or on a purely regulatory subunit that itself lacks an active site. Allosteric enzymes do not obey Michaelis-Menten kinetics. Instead, they show sigmoidal v_0 vs. $[S]$ plots instead of hyperbolic plots for at least one of the substrates of the reaction. The sigmoidal plot indicates that cooperative binding of the substrate is occurring due to the binding of an effector. The general kinetic behavior of allosteric enzymes in response to activators and inhibitors is shown in **Fig. 5.21**. Activators often shift the velocity curve to the left, lowering the apparent K_m , and making the curve more hyperbolic (indicating high affinity). Inhibitors often shift the curve to the right, raising the apparent K_m , and exaggerating the sigmoidal shape.

B. Regulation of allosteric enzymes by covalent modification.

The activity of allosteric enzymes often is controlled by covalent modification. In this case, the regulatory enzyme, known as an interconvertible enzyme, is modified by the action of converter enzymes (**Fig. 5.24**). Depending on the modification, the regulatory enzyme is shifted between its inactive and active forms, and activity is slowed down or turned on, respectively. Note that converter enzymes themselves also are regulatory enzymes whose activity can be altered by the binding of some small molecule (ligand) or also by covalent modification.

Phosphorylation is a common type of covalent modification used to regulate enzyme activity. Phosphate groups typically are transferred from ATP to the hydroxyl group of a serine, threonine, or tyrosine residue of the enzyme, forming phosphate esters. Enzymes that transfer a phosphate group from ATP to the target enzyme are called kinases. Enzymes that remove the phosphate groups (by hydrolysis) are called phosphatases.

X. Multienzyme complexes and multifunctional enzymes.

Often, different enzymes that catalyze successive steps in a metabolic pathway may be held together in a multienzyme complex. Furthermore, through gene evolution, the individual catalytic domains may have become fused together within a single larger multifunctional polypeptide. The advantage is that a metabolite formed in the first step of a pathway can be directly handed off to the enzyme that carries out the next step through a process known as metabolic channeling. Channeling increases the rate at which metabolic pathways can run by reducing the time required for enzymes and their substrate to diffuse together in the bulk solution of the cytoplasm.