

13

Enzyme Kinetics: A Review

INTRODUCTION

In the preceding chapters we considered various aspects of the structure of a protein. In this and many of the succeeding chapters, we turn our attention to the function of the group of proteins known as enzymes. Enzymes are the largest class of proteins, and due to their ease of study, the most well characterized (as discussed in Chap. 1, there is often a simple way to follow their biological activity). All proteins share a common function: They interact with other molecules. In some cases the other molecule is unchanged by the interaction, while in others it is chemically altered by the interaction. Into the first category fall binding and structural proteins; into the second fall the enzymes. Proteins in both groups have specific binding sites recognized by the appropriate ligand. In some instances the ligand is a small molecule and in others it is another protein or some other macromolecule. In many cases proteins interact with either type of ligand at different binding sites.

Already we have considered some of the ways in which the chemical composition (i.e., the amino acid side chains) of a binding site can be determined. The major emphasis of the remainder of this book is to examine the area of protein–ligand interactions. We use the various characteristics of enzymes to discuss the information a protein chemist or enzymologist wishes to acquire and the experimental approaches used to acquire it. The methodology developed to study enzymes is often readily applicable to the questions that protein chemists explore concerning binding proteins or structural proteins.

In its simplest formulation, the action of an enzyme can be represented by the scheme in Fig. 13-1. In the first stage of the process the substrate ligand must recognize

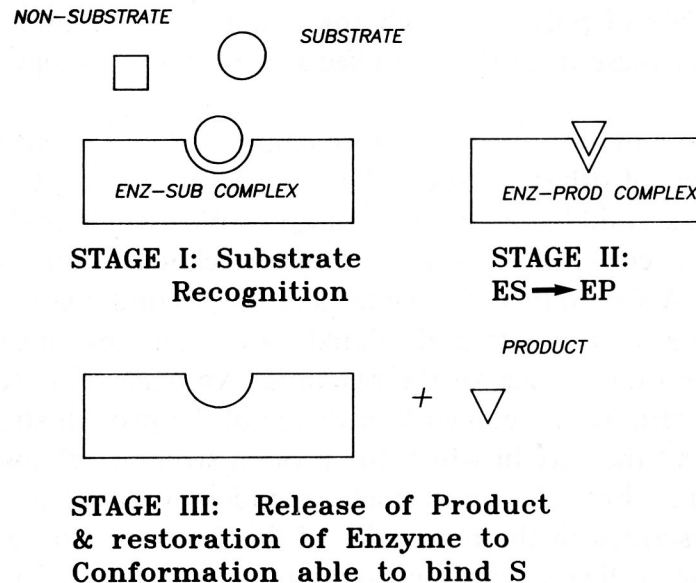


Figure 13-1 Schematic representation of the steps involved in a simple enzyme-catalyzed reaction.

and bind to its appropriate binding site. In addition to the chemical nature of the binding site, this interaction can be characterized by various kinetic and thermodynamic parameters.

The existence of a binding site on the enzyme for the substrate is the basis of one of the tenets of enzyme-catalyzed reactions; they are saturable. In its simplest terms, when all the catalytic sites of the enzyme are saturated with substrate the catalyzed reaction is proceeding at its maximum rate for that particular concentration of enzyme. A binding site, which makes specific interactions with various regions of the substrate, also confers another general property on enzymes; they are specific in their ability to recognize defined substrates. Recognition, by the binding site of the enzyme, of multiple points on the substrate confers stereo selectivity. When an enzyme binding site makes three distinct interactions with a substrate, then stereo-selective reaction is possible even in a symmetrical molecule such as citrate. This is the basis of the three-point attachment theory of enzyme specificity.

In the second stage an interconversion of substrate to product takes place, sometimes involving the formation of covalent intermediates between the substrate (or product) and some amino acid side chain or cofactor on the enzyme, and almost always involving the interaction of a variety of amino acid side chains with various stages of the substrate to product conversion.

Finally, in the third stage the product must be released from the binding site of the enzyme so that the cycle can begin again. As with the first stage, various kinetic and thermodynamic parameters describe this product release phase of the reaction.

In this simplified scheme we consider the interaction of a single ligand with a protein. In reality the majority of enzymes require two or more substrates to function and frequently a regulatory ligand as well. In some enzymes the protein molecule

consists of a number of polypeptide chains: sometimes homopolymers, sometimes heteropolymers. In these instances the effects of protein-protein interactions must also be considered.

As soon as the situation becomes more complex than the simple case shown in Fig. 13-1, a number of other questions have to be answered. Consider briefly the possible effects of a second ligand on our example. We assume that the second ligand is *not* a substrate: it could affect some parameter related to any or all of the three stages mentioned. A *description* of the effects of this second ligand requires a knowledge not only of its binding site and related parameters, but also of the effects its binding has on the other stages of the reaction. An *understanding* of the effects of this second ligand requires a detailed knowledge of the protein structure connecting the various sites and the way in which the protein structure changes in response to ligand binding. In all but a few cases such an understanding has not been attained; the approaches described in the remainder of this book are those used to obtain a detailed description of ligand-protein interactions. Such descriptions are essential before the appropriate questions can be asked, questions whose answers lead to the understanding of enzyme structure and function relationships.

In this chapter some of the essential terminology and conceptual background relating to the topic "enzyme kinetics" are reviewed. The study of enzyme kinetics is often regarded simply as a description of the way a substrate or product interacts with an enzyme. As illustrated in later chapters, the various experimental approaches employed in the study of enzyme kinetics can give detailed insight into almost every phase of an enzyme-catalyzed reaction and its regulation.

CHEMICAL KINETICS

If we consider the reaction $S \rightleftharpoons P$, the rate of the reaction (which can be followed either as a decrease in $[S]$ or as an increase in $[P]$) is proportional to the concentration of S (i.e., $[S]$). This rate, v , at any given time, T , is given by

$$\frac{-d[S]}{dt} = \frac{d[P]}{dt} = k[S] = v \quad (13-1)$$

where k is the *rate constant* of the reaction. Integration of Eq. (13-1) gives

$$\ln \frac{[S_0]}{[S]} = kt \quad (13-2)$$

where $[S_0]$ is the initial concentration of S at $t = 0$. A half-time for the reaction, $t_{1/2}$, can be defined as the time taken for the concentration of S to decrease to half its value at any given point in time. From Eq. (13-2) we can obtain an expression for $t_{1/2}$ if we allow S_0 to decrease to $S_0/2$. Hence

$$\log 2 = \frac{kt}{2.303} \quad (13-3)$$

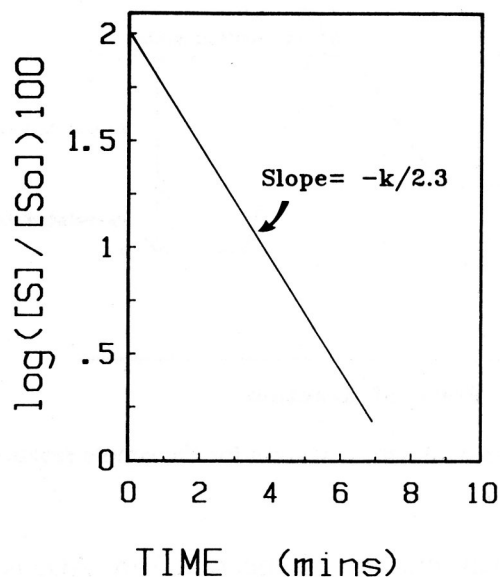


Figure 13-2 Determination of the rate constant k for a simple process where the concentration of S can be determined at various times during the course of the reaction.

and t (which is $t_{1/2}$) is equal to $0.693/k$. The half-time of a process is obtained directly from the rate constant of the process.

$$t_{1/2} = \frac{0.693}{k} \quad (13-4)$$

Figure 13-2 graphically summarizes these points and indicates how k may be calculated for such a process from a plot of $\log ([S]/[S_0])$ versus t .

The reaction $S \rightleftharpoons P$ does not continue past a finite point: Not *all* of S is converted to P. The final concentrations of S and P are the *equilibrium concentrations* and are related by the equilibrium constant (K_{eq}) for the reaction

$$K_{eq} = \frac{P_{eq}}{S_{eq}} \quad (13-5)$$

Thus we have two parameters that can be used to describe the reaction $S \rightleftharpoons P$: the rate constant, k , and the equilibrium constant, K_{eq} .

A chemical reaction proceeds through a *transition state*, and the reaction can be visualized in terms of an energy diagram. For the reaction we are considering, an energy diagram might resemble Fig. 13-3. Several important thermodynamic quantities are defined in this figure:

Standard Free-Energy Change

ΔG is related to the equilibrium constant, K_{eq} , for the reaction by

$$\Delta G^\circ = -RT \ln K_{eq} \quad (13-6)$$

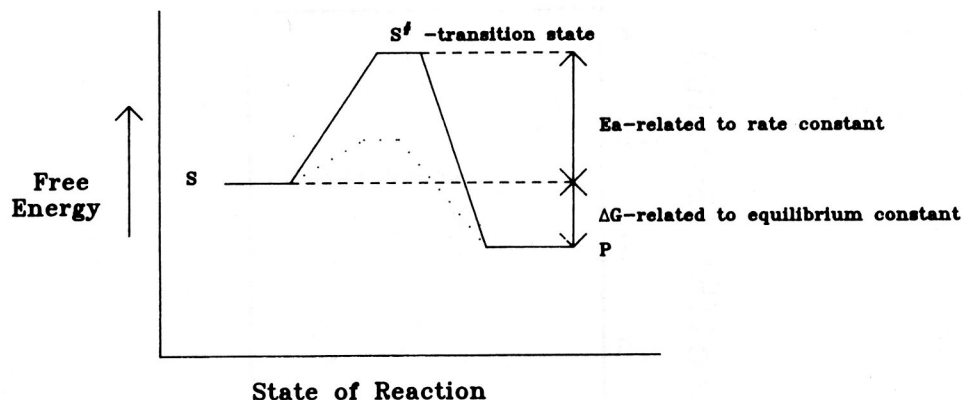


Figure 13-3 Energy diagram for the simple reaction $S \leftrightarrow P$.

The actual free-energy change of the reaction, ΔG , is related to ΔG° and the concentration of S and P by

$$\Delta G = \Delta G^\circ + RT \ln \frac{[P]}{[S]} \quad (13-7)$$

Thus a reaction can have a positive ΔG° but still proceed because the actual ΔG is negative. Since $\Delta G = \Delta H - T \Delta S$ the equilibrium position of a reaction can be regarded as a tendency toward a minimum of energy and a maximum of entropy.

Arrhenius Equation

This equation relates the rate constant, k , to the *activation energy*, E_a (defined in Fig. 13-4):

$$k = \frac{Ae^{-E_a}}{RT} \quad (13-8)$$

which gives

$$\ln k = \frac{-E_a}{RT} + \ln A \quad (13-9)$$

A plot of $\ln k$ versus $1/RT$ enables the activation energy, E_a , to be calculated, as illustrated in Fig. 13-4.

Transition-State Theory

Consider that an enzyme-catalyzed reaction proceeds through a transition state in much the same way as does the simple chemical reaction we just considered. The energy diagram for a simple one substrate–one product reaction, however, is more complex than that shown in Fig. 13-3. Apart from the chemical steps involved in converting substrate to product, one has also to consider the binding of substrate

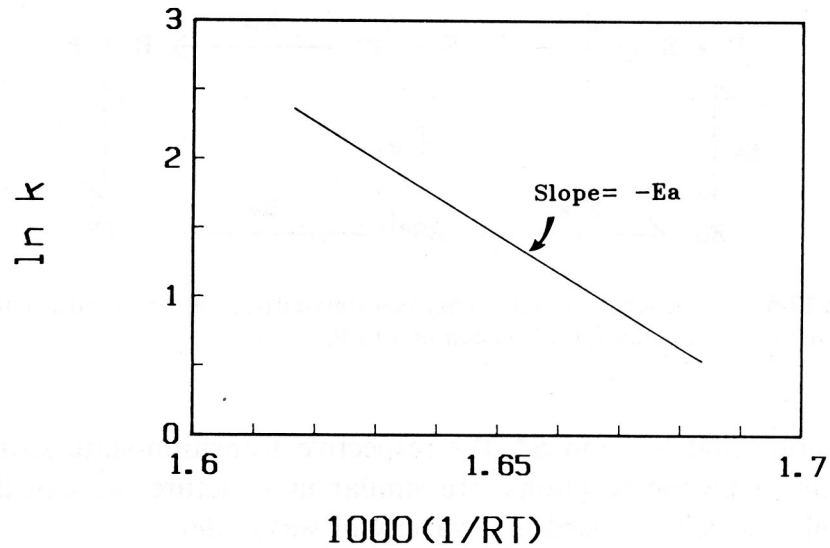


Figure 13-4 Determination of the activation energy, E_a , from the temperature dependence of the rate constant, k .

and product to the enzyme. Figure 13-5 shows a schematic energy diagram for such an enzyme system. As indicated, various thermodynamic parameters are associated with substrate binding and product release as well as the overall chemical equilibrium. Also shown is the hypothetical energy profile for the same reaction in the absence of enzyme. If the enzyme is acting as a true catalyst, it does not alter the chemical equilibrium position between substrate and product: It simply lowers the activation energy, allowing the equilibrium position to be attained more rapidly.

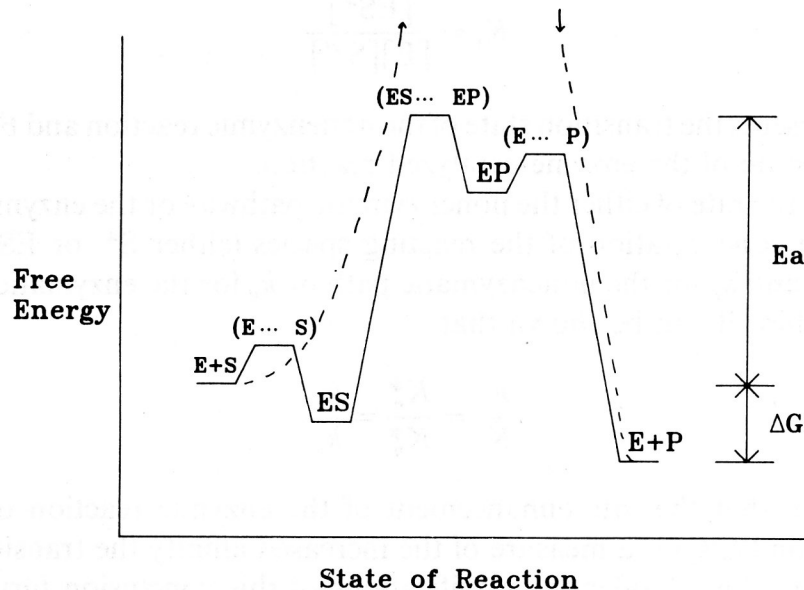


Figure 13-5 Energy diagram for a simple enzyme-catalyzed reaction. The dashed line shows the energy diagram for the non-enzyme-catalyzed reaction.

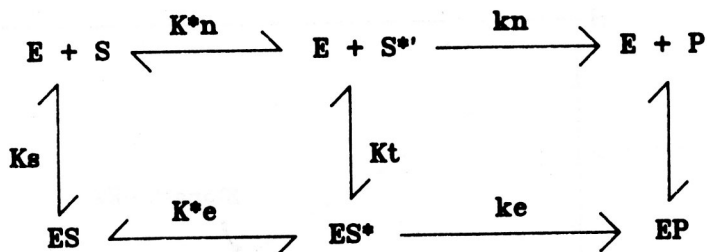


Figure 13-6 Simple scheme of a non-enzyme-catalyzed (top pathway) and an enzyme-catalyzed (bottom pathway) conversion of S to P.

If we assume that $S^{*'}$ and S^* , the respective transition-state structures of the nonenzymic and enzymic reactions, are similar in structure, we can derive several important points which are used in a variety of ways later.

Since the enzyme is acting as a catalyst (i.e., it is speeding a possible reaction), one must consider that the transition-state structure is in equilibrium with the reactant and can therefore write an equilibrium equation of the type $K^* = [S^*]/[S]$.

For the scheme shown in Fig. 13-6, we write a series of equilibrium equations,

$$K_s = \frac{[ES]}{[E][S]} \quad (13-10)$$

$$K_n^* = \frac{[S^{*'}]}{[S]} \quad (13-11)$$

$$K_e^* = \frac{[ES^*]}{[ES]} \quad (13-12)$$

$$K_t = \frac{[ES^*]}{[E][S^{*'}]} \quad (13-13)$$

where $S^{*'}$ represents the transition state of the nonenzymic reaction and ES^* represents the transition state of the enzyme-catalyzed reaction.

As before, the rate of either the nonenzymatic pathway or the enzymatic pathway is equal to the concentration of the reacting species (either $S^{*'}$ or ES^*) multiplied by a rate constant: k_n for the nonenzymatic path or k_e for the enzymatic path. From these relationships it can be shown that

$$\frac{K_t}{K_s} = \frac{K_e^*}{K_n^*} = \frac{k_e}{k_n} \quad (13-14)$$

and we can see that the rate enhancement of the enzymic reaction over the non-enzymic reaction (k_e/k_n) is a measure of the increased affinity the transition state has for the enzyme. We consider the ramifications of this conclusion further when we consider the chemistry of enzyme action.

INTRODUCTORY ENZYME KINETICS

For the simple reaction



one can derive an equation using either the steady-state assumption or the equilibrium assumption.

The *steady-state* assumption used by Briggs and Haldane is that the intermediates, in this case the ES complex, are always present in very small quantities compared to the reactants and products. Thus after an initial “*pre-steady-state*” phase, the concentrations of the intermediates remain constant, that is,

$$\frac{d[ES]}{dt} = 0 \quad (13-16)$$

The initial velocity, V_0 , is defined by

$$V_0 = k_{\text{cat}}[ES] \quad (13-17)$$

Combining Eqs. (13-16) and (13-17), we find

$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - [ES](k_2 + k_{\text{cat}}) \quad (13-18)$$

However, since the total enzyme concentration, e , is equal to $E + ES$ (the *enzyme conservation equation*), we get

$$E = e - ES \quad (13-19)$$

Substituting into Eq. (13-18) and rearranging, we find

$$k_1[E][S] = [ES](k_2 + k_{\text{cat}}) \quad (13-20)$$

Therefore,

$$k_1[e][S] = (k_1[S] + k_2 + k_{\text{cat}})[ES] \quad (13-21)$$

and

$$[ES] = \frac{k_1[e][S]}{k_1[S] + k_2 + k_{\text{cat}}} \quad (13-22)$$

Combining Eqs. (13-22) and (13-17) results in the following equations:

$$V_0 = \frac{k_{\text{cat}}k_1[e][S]}{k_1[S] + k_2 + k_{\text{cat}}} \quad (13-23)$$

$$V_0 = \frac{k_{\text{cat}}[e][S]}{[S] + \left(\frac{k_2 + k_{\text{cat}}}{k_1}\right)} \quad (13-24)$$

and

$$V_0 = \frac{V_{\max}[S]}{[S] + K_m} \quad (13-25)$$

where $V_{\max} = k_{\text{cat}}[e]$ and is the maximum rate of the enzyme-catalyzed reaction,

$$K_m = \frac{k_2 + k_{\text{cat}}}{k_1} \quad (13-26)$$

where K_m is the Michaelis constant for the substrate.

In the *equilibrium assumption*, which can replace the steady-state assumption, k_{cat} is very much slower than k_2 , and as a result the ES complex is in an effective equilibrium with E and S. Thus we can define an equilibrium constant

$$K = \frac{k_1}{k_2} = \frac{[ES]}{[E][S]} \quad (13-27)$$

Substituting, the enzyme conservation equation [Eq. (13-19)], into Eq. (13-27), we get

$$K = \frac{[ES]}{[e][S] - [ES][S]} \quad (13-28)$$

Rearranging Eq. (13-28), we find the equations

$$K[e][S] - K[ES][S] = [ES] \quad (13-29)$$

and

$$K[e][S] = [ES] + K[ES][S] = [ES](1 + K[S]) \quad (13-30)$$

Equation (13-30) can be put in terms of [ES]:

$$[ES] = \frac{K[e][S]}{1 + K[S]} \quad (13-31)$$

As before, $V_0 = k_{\text{cat}}[ES]$, and using Eq. (13-31) we get

$$V_0 = \frac{k_{\text{cat}}K[e][S]}{1 + K[S]} \quad (13-32)$$

Dividing by K we get an equation of similar form to Eq. (13-24):

$$V_0 = \frac{k_{\text{cat}}[e][S]}{[S] + 1/K} \quad (13-33)$$

As in Eq. (13-25), $V_{\max} = k_{\text{cat}}[e]$; thus

$$V_0 = \frac{V_{\max}[S]}{[S] + K_m} \quad (13-34)$$

where $K_m = 1/K = k_2/k_1$.

Equations (13-25) and (13-34) are the same: The difference lies in the definitions of K_m . In the earlier, steady-state case, K_m is equal to $(k_2 + k_{\text{cat}})/k_1$, whereas in the equilibrium case K_m is a true dissociation constant. This points to an important difference between steady-state and equilibrium assumptions. In the equilibrium assumption the Michaelis constant K_m is a true equilibrium constant, whereas in the steady-state assumption this is not the case. In steady-state mechanisms as k_{cat} gets slower (relative to k_2), we approach the *equilibrium* situation. At this point the difference may seem inconsequential; however, as is pointed out in Chaps. 14 and 15, this is important in establishing the formal kinetic mechanism of a multi-subunit enzyme.

The formula given in Eqs. (13-25) and (13-34) is the so-called Michaelis–Menten equation and describes the dependence of the initial velocity, V_0 , on the substrate concentration. From this dependence two parameters, V_{max} and K_m , can be determined, as shown in Fig. 13-7.

In many cases it is not experimentally practical to plot kinetic data directly according to the Michaelis–Menten equation, and several linear transformations are popular. If we take the reciprocal of Eq. (13-34), we get

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_m[S]} \quad (13-35)$$

$$\frac{1}{V_0} = \frac{K_m}{V_{\text{max}}[S]} + \frac{[S]}{V_{\text{max}}[S]} \quad (13-36)$$

$$\frac{1}{V_0} = \frac{K_m}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}} \quad (13-37)$$

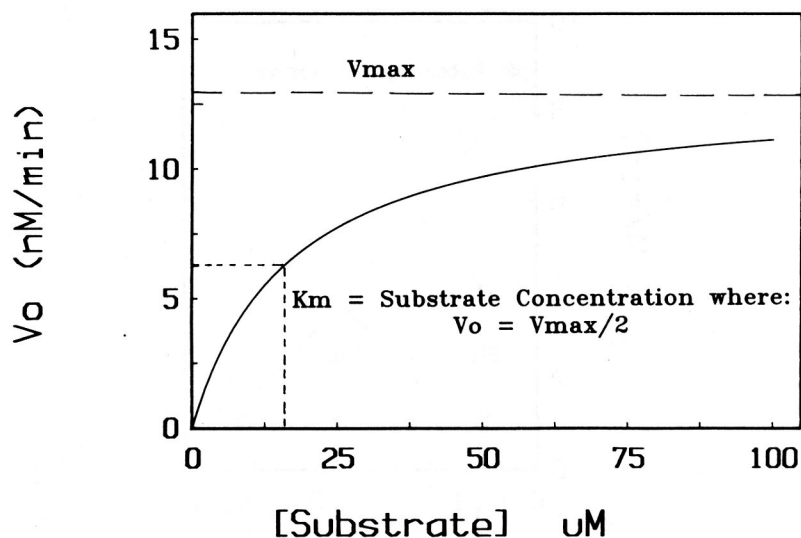


Figure 13-7 V_0 versus $[S]$ plot of the Michaelis–Menten equation, indicating how V_{max} and K_m can be determined.

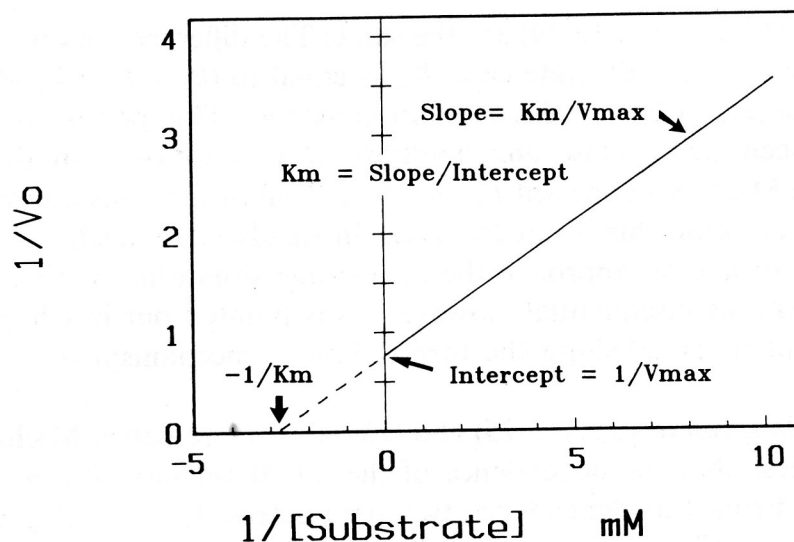


Figure 13-8 Double-reciprocal plot of kinetic data according to Eq. (13-37).

A plot of $1/V_0$ versus $1/[S]$ is linear, as illustrated in Fig. 13-8. This plot, which is the Lineweaver–Burk plot, allows extrapolation to the $1/V_0$ axis to give $1/V_{max}$ and extrapolation to the $1/[S]$ axis to give $-(1/K_m)$. Alternatively, K_m can be determined by dividing the slope of the plot by the intercept on the $1/V_0$ axis.

Alternatively, multiplying both sides by V_{max} and rearranging, we get

$$V_0 = -K_m \frac{V_0}{[S]} + V_{max} \quad (13-38)$$

and a plot of V_0 against $V_0/[S]$ is linear. This is the Eadie–Hofstee plot. As shown in Fig. 13-9, the two parameters V_{max} and K_m are obtained from the intercept on the V_0 axis or from the slope, respectively.

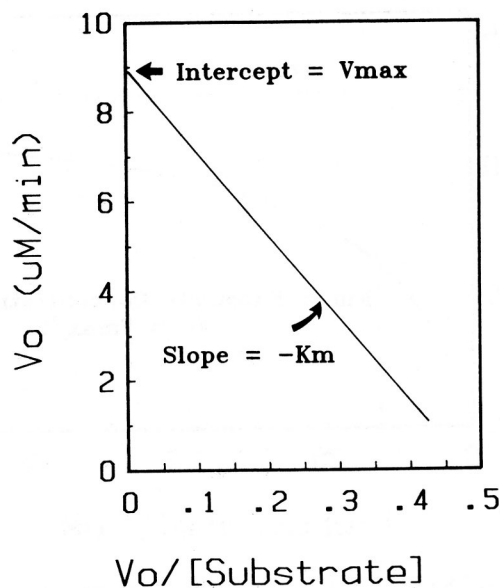


Figure 13-9 Eadie–Hofstee plot of enzyme kinetic data.

REVERSIBLE INHIBITION

The activity of an enzyme (or the binding activity of a protein) can be altered in a number of ways. In the realm of reversible inhibitors there are *two*. In the first, A, the inhibitor is considered to be a structural analog of the substrate and as such has an affinity for the active site. Once the active site has bound the inhibitor, subsequent binding of substrate is blocked. The interaction of the inhibitor, shown diagrammatically in Fig. 13-10A, can be described by a dissociation constant, K_i , where

$$K_i = \frac{[E][I]}{[EI]} \quad (13-39)$$

and where $[I]$ is the concentration of *free* inhibitor and $[EI]$ the concentration of the complex.

The second mode of interaction of an inhibitor with an enzyme, B, involves a site other than the active site (see Fig. 13-10B). As in the first case, interaction of inhibitor with protein can be described by a dissociation constant, K'_i , which has a similar form to that of K_i . Since this does not directly involve the active site, we must consider how this type of inhibitor elicits its effect: (1) after inhibitor binding there may be a conformational change in the protein that blocks the active site from binding substrate; (2) as a result of a conformational change the ability of the active site to bind substrate may be altered but not prevented; (3) as a result of a conformational change the ability of the enzyme to *bind* the substrate may not be affected, but the ability of the enzyme to *catalyze* the reaction is affected; and (4) the site of inhibitor binding, although not at the active site, may be so close to it that there is

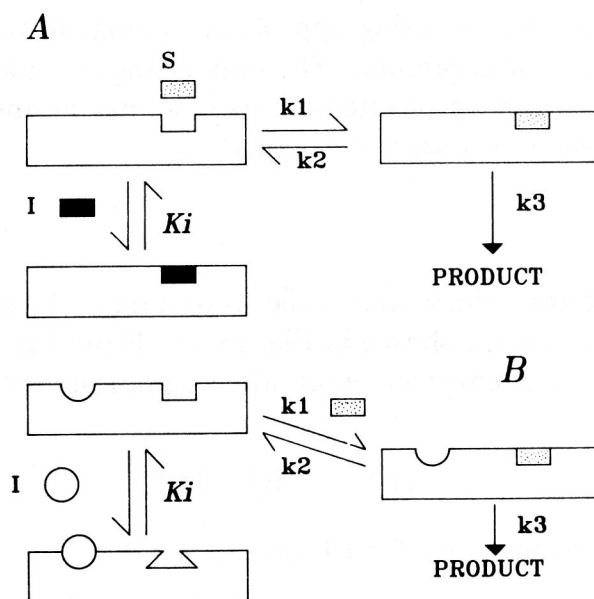


Figure 13-10 Modes of interaction of an inhibitor with an enzyme: (A) the inhibitor binds at the active site; (B) the inhibitor binds at a site other than the active site.

direct physical interaction between inhibitor and substrate binding, leading to distortion of the enzyme–substrate complex. Although all these mechanisms can lead to decreased activity (inhibition), the fourth type is conceptually quite different from the other three—no conformational change induced by inhibitor binding is required.

At this point it is appropriate to divide the modes of inhibitor interaction with enzyme into two categories; in category I is type A and type B-1. In both of these, binding of inhibitor *prevents* binding of substrate. Because inhibitor binding is reversible it follows that substrate binding in this instance can prevent inhibitor binding. Inhibition in these cases is therefore called *competitive inhibition*. In the presence of a fixed amount of inhibitor, the inhibition is completely overcome by saturating concentrations of substrate.

In the other types of inhibitor interaction that we have examined so far, the inhibitor *and* the substrate can be bound to the enzyme at the same time, giving an ESI complex. Where an ESI complex can be formed, the inhibitor is referred to as a category II inhibitor. In this complex the presence of the inhibitor can affect either the ability of the enzyme to bind substrate or the ability of the enzyme to catalyze the reaction once the enzyme has bound substrate. From the earlier discussion of basic kinetics in the absence of inhibitors, it is apparent that either K_m or V_{max} can be affected in these cases, whereas only K_m is affected in category I situations.

So far we have considered only cases where the inhibitor binds to free enzyme and has some effect on either substrate binding or catalysis. It is entirely possible, however, that the inhibitor may bind only *after* substrate has bound to the enzyme, giving an ESI complex (as in several of the situations already examined) that undergoes a conformational change leading to a decreased rate of product formation. As with the cases where an ESI complex is formed, in this instance the inhibition cannot be overcome by saturating substrate concentrations.

Rate equations describing the expected velocity in the presence of inhibitor in these cases can easily be derived using approaches similar to those described earlier for cases where no inhibitor is present. The only changes made are the inclusion of the appropriate terms describing the interaction of the enzyme and the inhibitor, which are manifested in the *enzyme conservation* equation.

Category I Inhibitors

This type of inhibitor, which causes the formation of EI at the expense of ES, can be described by the scheme shown in Fig. 13-11. From Eq. (13-39) it is apparent that $[EI] = [E][I]/K_i$. The enzyme conservation equation for this type of inhibition becomes

$$e = [ES] + [E] + [EI] \quad (13-40)$$

which, using the expression above for EI, becomes

$$e = [ES] + [E] + \frac{[E][I]}{K_i} = [ES] + [E] \left(1 + \frac{I}{K_i} \right) \quad (13-41)$$

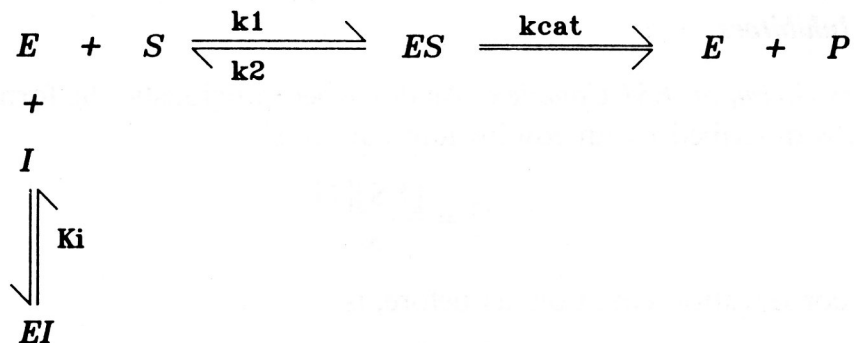


Figure 13-11 Equilibria and catalytic steps involved with a category I type of inhibitor.

Applying the steady-state assumption, we get

$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - [ES](k_2 + k_{cat}) \quad (13-42)$$

and it is apparent that

$$[E] = \frac{[ES](k_2 + k_{cat})}{k_1[S]} \quad (13-43)$$

Substituting this expression for $[E]$ into the enzyme conservation equation for this case [Eq. (13-40)], we get

$$e = [ES] + \frac{[ES](k_2 + k_{cat})}{k_1[S]} \left(1 + \frac{I}{K_i} \right) \quad (13-44)$$

which becomes

$$e = [ES] \left[\frac{1 + (k_2 + k_{cat})}{k_1[S]} \left(1 + \frac{[I]}{K_i} \right) \right] \quad (13-45)$$

As before, $V_0 = k_{cat}[ES]$, and from Eq. (13-45),

$$[ES] = \frac{e}{[1 + (k_2 + k_{cat})/k_1[S]][1 + [I]/K_i]} \quad (13-46)$$

and

$$V_0 = \frac{k_{cat}e}{[1 + (k_2 + k_{cat})/k_1[S]][1 + [I]/K_i]} \quad (13-47)$$

which, as before, can be rearranged to give

$$V_0 = \frac{V_{max}[S]}{[S] + K_m[1 + ([I]/K_i)]} \quad (13-48)$$

where K_m and V_{max} have the same meanings as previously.

Category II Inhibitors

Inhibitors Giving an ESI Complex. As described previously, the formation of an ESI complex is described by an equilibrium equation:

$$[\text{ESI}] = \frac{[\text{ES}][\text{I}]}{K'_i} \quad (13-49)$$

The enzyme conservation equation, as before, is

$$e = [\text{ES}] + [\text{ESI}] + [\text{E}] \quad (13-50)$$

Combining Eqs. (13-49) and (13-50), we get

$$e = [\text{ES}] \left(1 + \frac{[\text{I}]}{K'_i} \right) + [\text{E}] \quad (13-51)$$

which gives

$$e = [\text{ES}] \left\{ \left(1 + \frac{[\text{I}]}{K'_i} \right) + \frac{k_2 + k_{\text{cat}}}{k_1[\text{S}]} \right\} \quad (13-52)$$

and

$$[\text{ES}] = \frac{e}{\left(1 + [\text{I}]/K'_i \right) + (k_2 + k_{\text{cat}})/k_1[\text{S}]} \quad (13-53)$$

Since, as before, $V_0 = k_{\text{cat}}[\text{ES}]$, we get

$$V_0 = \frac{k_{\text{cat}}e}{\left(1 + [\text{I}]/K'_i \right) + (k_2 + k_{\text{cat}})/k_1[\text{S}]} \quad (13-54)$$

Thus

$$V_0 = \frac{V_{\text{max}}[\text{S}]/(1 + [\text{I}]/K'_i)}{[\text{S}] + K_m/(1 + [\text{I}]/K'_i)} \quad (13-55)$$

Inhibitors Giving EI and ESI Complexes. In this case we have two equilibria involving the inhibitor: those involving EI and ESI. The equilibrium constants for these, K_i and K'_i , respectively, are as described earlier. The enzyme conservation equation becomes

$$e = [\text{E}] + [\text{ES}] + [\text{EI}] + [\text{ESI}] \quad (13-56)$$

which, expressing each term in terms of ES gives

$$e = [\text{ES}] \left(1 + \frac{k_2 + k_{\text{cat}}}{k_1[\text{S}]} + \frac{k_2 + k_{\text{cat}}}{k_1[\text{S}]} \frac{[\text{I}]}{K_i} + \frac{[\text{I}]}{K'_i} \right) \quad (13-57)$$

which, in conjunction with $V_0/k_{\text{cat}}[\text{ES}]$, gives

$$V_0 = \frac{V_{\text{max}}[\text{S}]/(1 + [\text{I}]/K'_i)}{[\text{S}] + K_m \left(\frac{1 + [\text{I}]/K'_i}{1 + [\text{I}]/K_i} \right)} \quad (13-58)$$

and when $K_i = K'_i$, this becomes

$$V_0 = \frac{(V_{\max}[S])/(1 + [I]/K'_i)}{[S] + K_m} \quad (13-59)$$

Linear Transformations

As with the equations derived in the absence of inhibitors, it is convenient to express these equations in double-reciprocal (Lineweaver–Burk) form and consider the effects in Lineweaver–Burk plots. Each of the three equations is shown in Lineweaver–Burk format in Table 13-1 and the appropriate plots in Fig. 13-12. From these double reciprocal equations it is apparent that in terms of Lineweaver–Burk plots, three types of inhibitors can be distinguished. Category I inhibitors affect *only* the slope, while category II inhibitors affect the intercept. The slope may also be affected in cases where an EI *and* an ESI complex are formed.

From Table 13-1 it is apparent that K_i or K'_i can be obtained from a plot of either slope or intercept from the Lineweaver–Burk plot versus $[I]$, which gives a negative intercept on the abscissa of K_i or K'_i , respectively. In instances where EI *and* ESI can exist, slope and intercept plots against I must be made; these are also illustrated in Fig. 13-12.

Since the enzyme kinetic data are also frequently represented by the Eadie–Hofstee plot, the effects of the three types of inhibitors on these plots are illustrated in Fig. 13-13. These three types of inhibition are known as competitive [Eq. (13-48)], uncompetitive [Eq. (13-55)], and noncompetitive or mixed [Eq. (13-59)]. In the latter case K_i and K'_i are not necessarily equal in Eq. (13-59). Equal values for K_i and K'_i indicate that the inhibitor binds to free enzyme and to the enzyme–substrate complex with equal affinity. It is quite possible that the inhibitor binds to either the free enzyme or the ES complex with higher affinity, and K_i is either smaller than or greater than K'_i , respectively.

TABLE 13-1 Lineweaver–Burk equations^a

inhibition	$1/V_0 = \text{slope} \times 1/[S] +$	intercept
None	$\frac{K_m}{V_{\max}}$	$\frac{1}{V_{\max}}$
Category I	$\frac{K_m}{V_{\max}} \left(1 + \frac{I}{K_i}\right)$	$\frac{1}{V_{\max}}$
Category II (ESI)	$\frac{K_m}{V_{\max}}$	$\frac{1}{V_{\max} \left(1 + \frac{I}{K'_i}\right)}$
Category II (EI + ESI)	$\frac{K_m}{V_{\max}} \left(1 + \frac{I}{K_i}\right)$	$\frac{1}{V_{\max} \left(1 + \frac{I}{K'_i}\right)}$

^a K_i values calculated from uninhibited parameter and the parameter obtained in the presence of a known concentration of I.

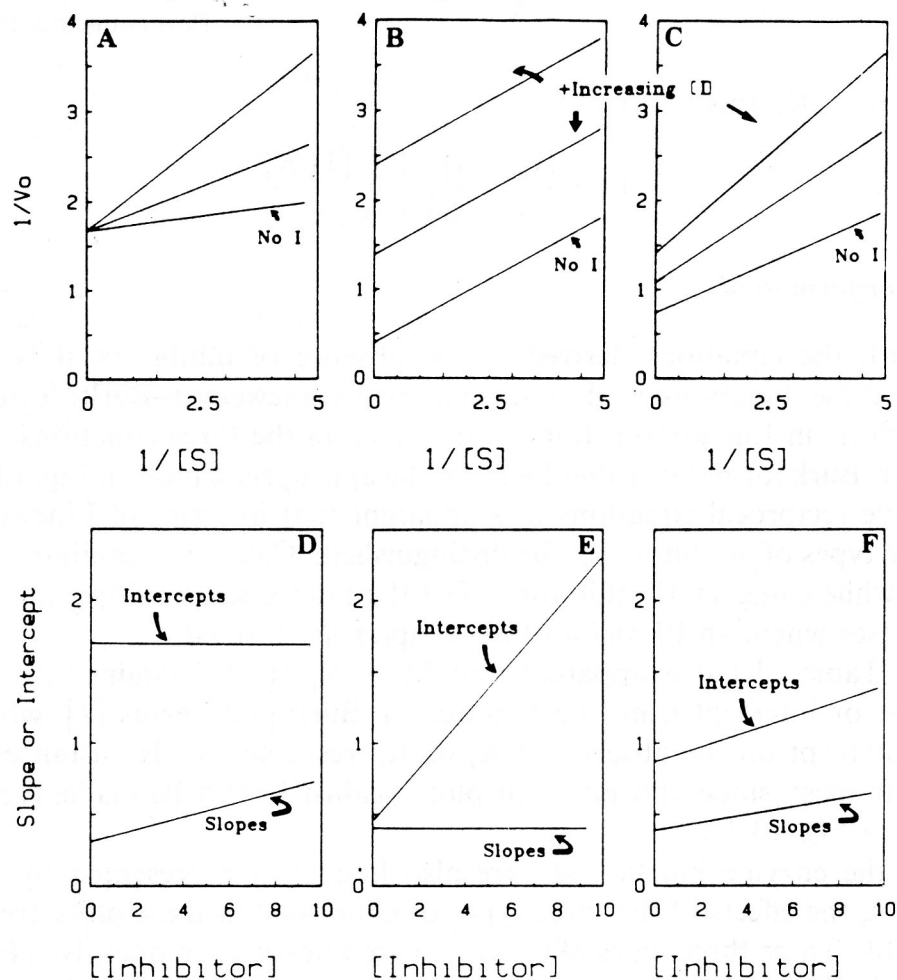


Figure 13-12 Lineweaver–Burk plots of various types of inhibition represented by (A) Eq. (13-48), (B) Eq. (13-55), and (C) Eq. (13-59). Also shown in parts (D) to (F) are the secondary plots of slope or intercept from the primary plot versus $[I]$.

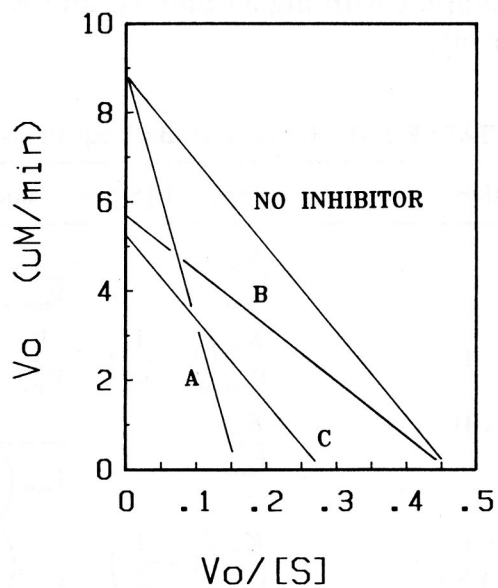


Figure 13-13 Effects of inhibitors on Eadie–Hofstee plots, according to (A) Eq. (13-48), (B) Eq. (13-55), and (C) Eq. (13-59).

The Dixon Plot

In an alternative approach to analyzing inhibition data to obtain a value for K_i , Dixon proposed the use of plots of $1/V_0$ versus $[I]$ at several fixed concentrations of the substrate. Analysis using such Dixon plots requires a somewhat different experimental design to that used in Lineweaver–Burk or Eadie–Hofstee plot analysis, as the emphasis is on using a number of inhibitor concentrations at several discrete substrate concentrations. From Eq. (13-48) it is apparent that for a category I inhibitor, where only EI can exist, a plot of $1/V_0$ versus $[I]$ at different values of $[S]$ gives linear plots with an intersection point of $-K_i$ (see Fig. 13-14). Similarly, for category II inhibitors when K_i is equal to K'_i , such plots can be used. When K_i does not equal K'_i , or when only ESI complexes are formed, Dixon plots do not intersect in this manner.

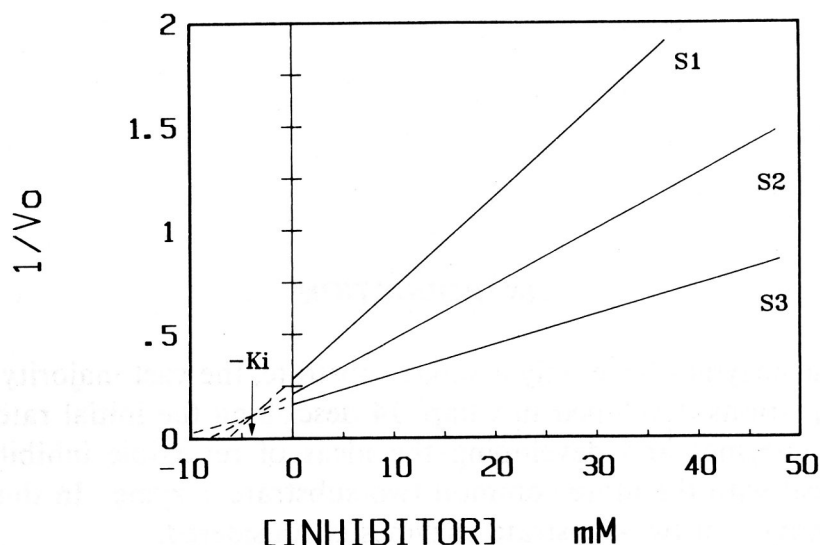


Figure 13-14 Use of a “Dixon” plot for the determination of K_i for a category I (competitive) inhibitor.

In this chapter we examined the basis for enzyme kinetics and demonstrated that for a simple one-substrate system, two quite distinct derivatives of the Michaelis–Menton equation [Eqs. (13-25) and (13-34)] can be employed. These derivatives lead to quite different physical significance for the Michaelis–Menton constant, K_m . Although this difference seems inconsequential when considering one-substrate systems, it is of much greater importance when dealing with multisubstrate systems.