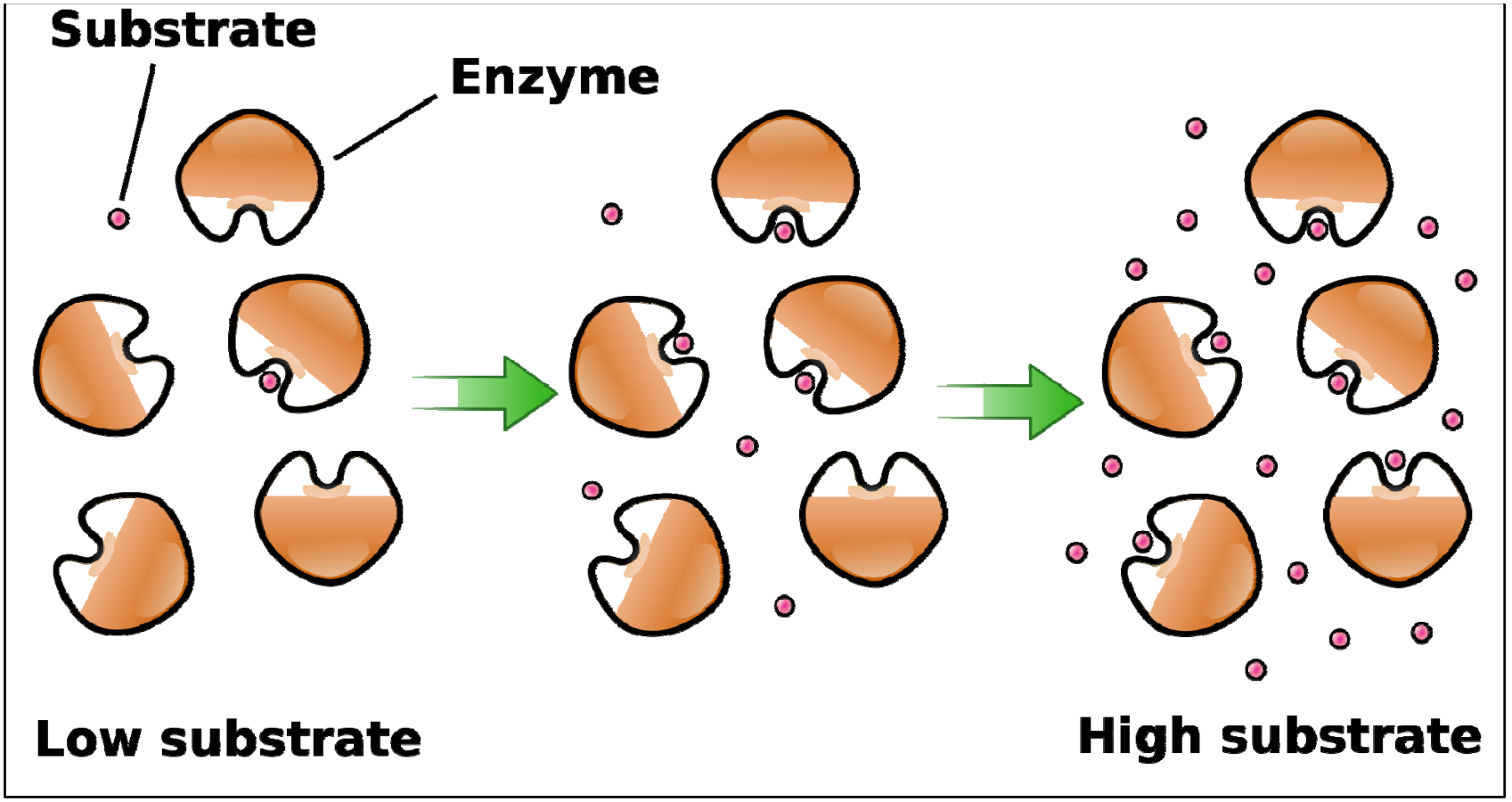
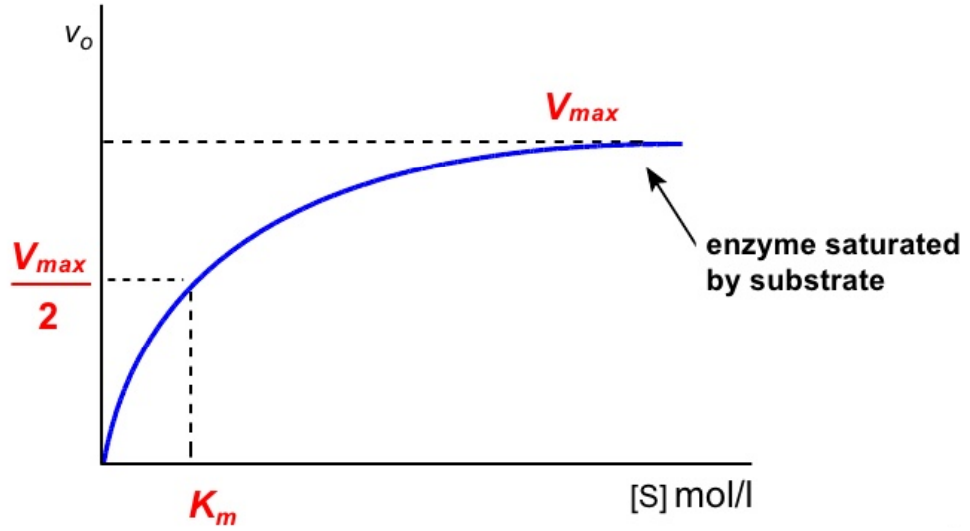
Enzyme kinetics:

The study of the rates of enzymatic reactions


Substrate
Enzyme
Low substrate
High substrate


V_o
V_{max}
$\frac{V_{max}}{2}$
enzyme saturated
by substrate
K_m
[S] mol/l

Let us assume that A⟶P *is* an elementary reaction and that it is spontaneous and essentially irreversible. Irreversibility is easily assumed if the rate of P conversion to A is very slow *or* the concentration of P (expressed as [P]) is negligible under the conditions chosen. The **velocity,** v, or **rate,** of the reaction A⟶P is the amount of P formed or the amount of A consumed per unit time, t. That is,

$$v = \frac{d[\mathrm{P}]}{dt} \quad \text{or} \quad v = \frac{-d[\mathrm{A}]}{dt} \tag{13.1}$$

The mathematical relationship between reaction rate and concentration of reactant(s) is the **rate law.** For this simple case, the rate law is

$$v = \frac{-d[\mathrm{A}]}{dt} = k[\mathrm{A}] \tag{13.2}$$

From this expression, it is obvious that the rate is proportional to the concentration of A, and k is the proportionality constant, or **rate constant.** k has the units of $(\text{time})^{-1}$, usually sec^{-1}. v is a function of [A] to the first power, or in the terminology of kinetics, v is first-order with respect to A. For an elementary reaction, the **order** for any reactant is given by its exponent in the rate equation. The number of molecules that must simultaneously interact is defined as the **molecularity** of the reaction. Thus, the simple elementary reaction of A⟶P is a **first-order reaction.**

unimolecular reactions (the molecularity equals 1).

Bimolecular Reactions Are Reactions Involving Two Reactant Molecules

Consider the more complex reaction, where two molecules must react to yield products:

$$A + B \longrightarrow P + Q$$

Assuming this reaction is an elementary reaction, its molecularity is 2; that is, it is a **bimolecular reaction.** The velocity of this reaction can be determined from the rate of disappearance of either A or B, or the rate of appearance of P or Q:

$$v = \frac{-d[A]}{dt} = \frac{-d[B]}{dt} = \frac{d[P]}{dt} = \frac{d[Q]}{dt} \tag{13.3}$$

The rate law is

$$v = k[A][B] \tag{13.4}$$

Since A and B must collide in order to react, the rate of their reaction will be proportional to the concentrations of both A and B. Because it is proportional to the product of two concentration terms, the reaction is **second-order** overall, first-order with respect to A and first-order with respect to B. (Were the elementary reaction $2A \longrightarrow P + Q$, the rate law would be $v = k[A]^2$, second-order overall and second-order with respect to A.) Second-order rate constants have the units of $(\text{concentration})^{-1}(\text{time})^{-1}$, as in $M^{-1}\,\text{sec}^{-1}$.

Molecularities greater than 2 are rarely found (and greater than 3, never). (The likelihood of simultaneous collision of three molecules is very, very small.) When the overall stoichiometry of a reaction is greater than two (for example, as in $A + B + C \longrightarrow$ or $2A + B \longrightarrow$), the reaction almost always proceeds via unimolecular or bimolecular elementary steps, and the overall rate obeys a simple first- or second-order rate law.

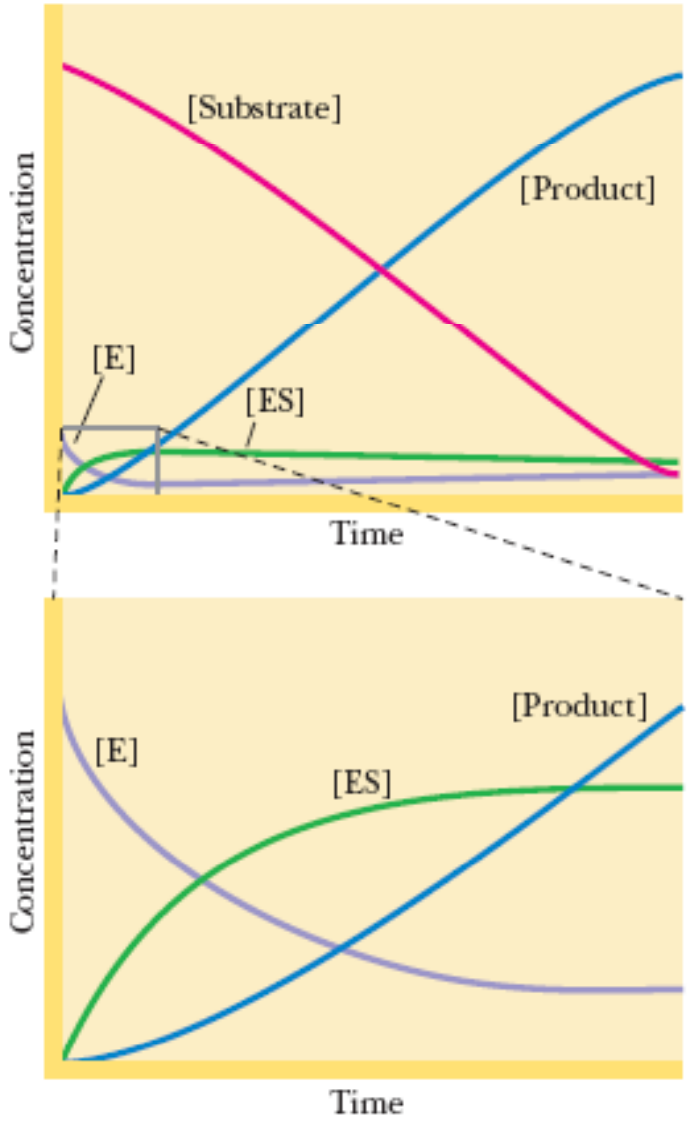


FIGURE 13.8 Time course for a typical enzyme-catalyzed reaction obeying the Michaelis–Menten, Briggs–Haldane models for enzyme kinetics. The early stage of the time course is shown in greater magnification in the bottom graph.

Here we develop the basic logic and the algebraic steps in a modern derivation of the Michaelis-Menten equation, which includes the steady-state assumption introduced by Briggs and Haldane. The derivation starts with the two basic steps of the formation and breakdown of ES (Eqns 6–7 and 6–8). Early in the reaction, the concentration of the product, [P], is negligible, and we make the simplifying assumption that the reverse reaction, $P \rightarrow S$ (described by k_{-2}), can be ignored. This assumption is not critical but it simplifies our task. The overall reaction then reduces to

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (6\text{–}10)$$

V_0 is determined by the breakdown of ES to form product, which is determined by [ES]:

$$V_0 = k_2[ES] \qquad (6\text{–}11)$$

Because [ES] in Equation 6–11 is not easily measured experimentally, we must begin by finding an alternative expression for this term. First, we introduce the term $[E_t]$, representing the total enzyme concentration (the sum of free and substrate-bound enzyme). Free or unbound enzyme can then be represented by $[E_t] - [ES]$. Also, because [S] is ordinarily far greater than $[E_t]$, the amount of substrate bound by the enzyme at any given time is negligible compared with the total [S]. With these conditions in mind, the following steps lead us to an expression for V_0 in terms of easily measurable parameters.

Step 1 The rates of formation and breakdown of ES are determined by the steps governed by the rate constants k_1 (formation) and $k_{-1} + k_2$ (breakdown), according to the expressions

$$\text{Rate of ES formation} = k_1([\text{E}_\text{t}] - [\text{ES}])[\text{S}] \qquad (6\text{–}12)$$

$$\text{Rate of ES breakdown} = k_{-1}[\text{ES}] + k_2[\text{ES}] \qquad (6\text{–}13)$$

Step 2 We now make an important assumption: that the initial rate of reaction reflects a steady state in which [ES] is constant—that is, the rate of formation of ES is equal to the rate of its breakdown. This is called the **steady-state assumption.** The expressions in Equations 6–12 and 6–13 can be equated for the steady state, giving

$$k_1([\mathrm{E_t}] - [\mathrm{ES}])[\mathrm{S}] = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}] \qquad (6\text{–}14)$$

Step 3 In a series of algebraic steps, we now solve Equation 6–14 for [ES]. First, the left side is multiplied out and the right side simplified to give

$$k_1[\mathrm{E_t}][\mathrm{S}] - k_1[\mathrm{ES}][\mathrm{S}] = (k_{-1} + k_2)[\mathrm{ES}] \qquad (6\text{–}15)$$

Adding the term $k_1[\mathrm{ES}][\mathrm{S}]$ to both sides of the equation and simplifying gives

$$k_1[\mathrm{E_t}][\mathrm{S}] = (k_1[\mathrm{S}] + k_{-1} + k_2)[\mathrm{ES}] \qquad (6\text{–}16)$$

We then solve this equation for [ES]:

$$[\mathrm{ES}] = \frac{k_1[\mathrm{E_t}][\mathrm{S}]}{k_1[\mathrm{S}] + k_{-1} + k_2} \qquad (6\text{–}17)$$

This can now be simplified further, combining the rate constants into one expression:

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{[\mathrm{S}] + (k_2 + k_{-1})/k_1} \qquad (6\text{–}18)$$

The term $(k_2 + k_{-1})/k_1$ is defined as the **Michaelis constant, K_m.** Substituting this into Equation 6–18 simplifies the expression to

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]} \qquad (6\text{–}19)$$

Step 4 We can now express V_0 in terms of [ES]. Substituting the right side of Equation 6–19 for [ES] in Equation 6–11 gives

$$V_0 = \frac{k_2[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]} \tag{6–20}$$

This equation can be further simplified. Because the maximum velocity occurs when the enzyme is saturated (that is, with [ES] = [$\mathrm{E_t}$]) $V_{\max}$ can be defined as $k_2[\mathrm{E_t}]$. Substituting this in Equation 6–20 gives Equation 6–9:

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

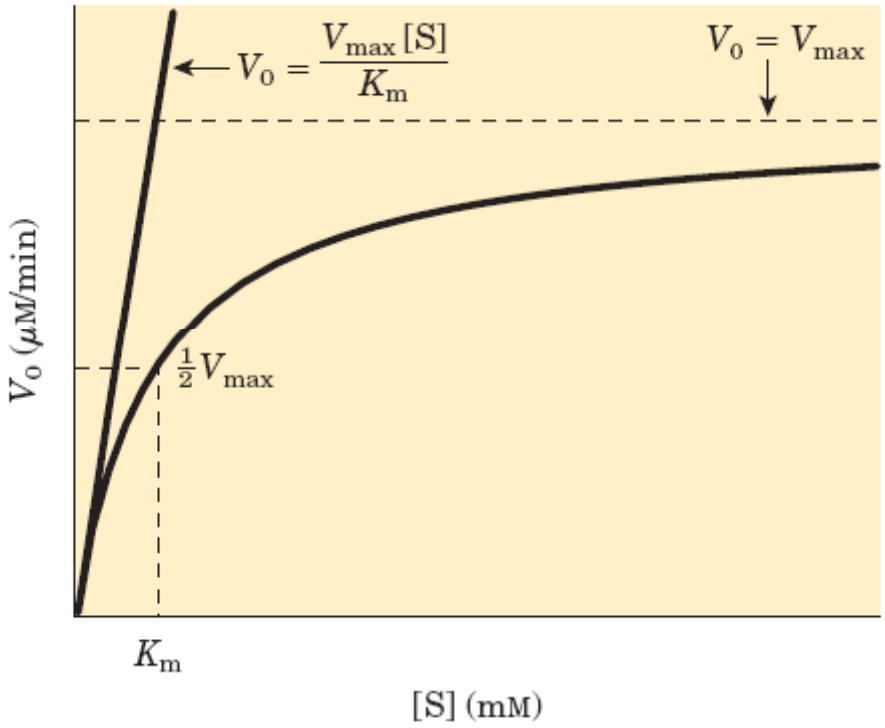


FIGURE 6–12 Dependence of initial velocity on substrate concentration. This graph shows the kinetic parameters that define the limits of the curve at high and low [S]. At low [S], $K_m >>$ [S] and the [S] term in the denominator of the Michaelis-Menten equation (Eqn 6–9) becomes insignificant. The equation simplifies to $V_0 = V_{max}[S]/K_m$ and V_0 exhibits a linear dependence on [S], as observed here. At high [S], where [S] $>> K_m$, the K_m term in the denominator of the Michaelis-Menten equation becomes insignificant and the equation simplifies to $V_0 = V_{max}$; this is consistent with the plateau observed at high [S]. The Michaelis-Menten equation is therefore consistent with the observed dependence of V_0 on [S], and the shape of the curve is defined by the terms V_{max}/K_m at low [S] and V_{max} at high [S].

Transformations of the Michaelis-Menten Equation: The Double-Reciprocal Plot

The Michaelis-Menten equation

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

can be algebraically transformed into equations 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:

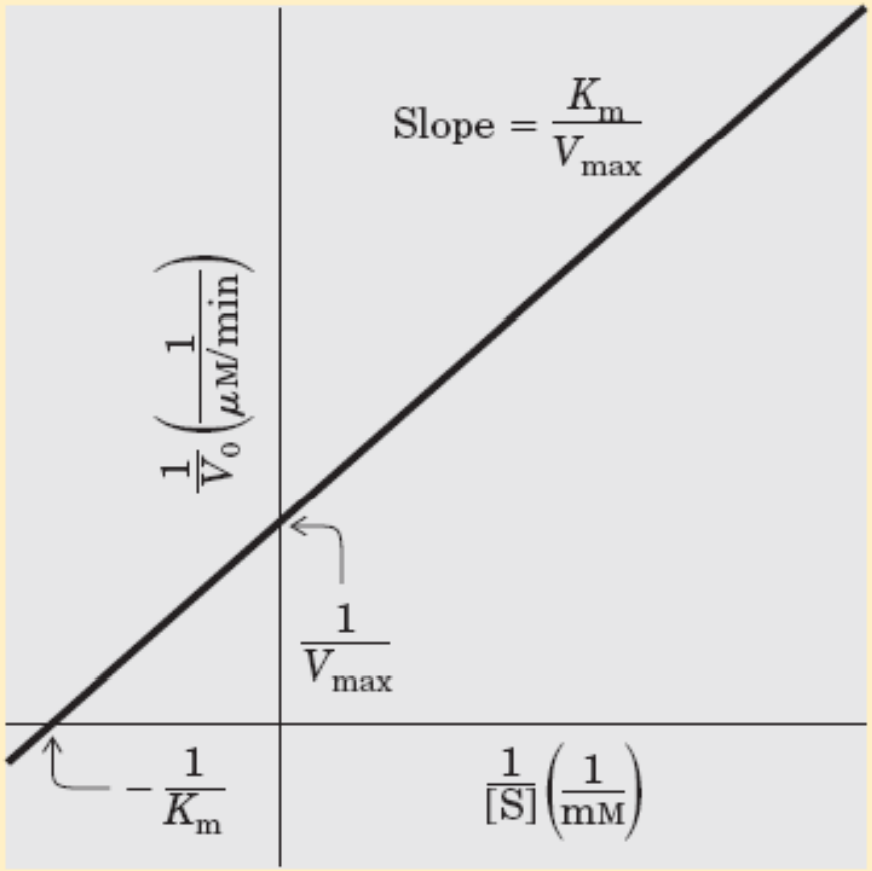
$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

Separating the components of the numerator on the right side of the equation gives

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

which simplifies to

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



A double-reciprocal or Lineweaver-Burk plot.

TABLE 13.5 Enzymes Whose k_{cat}/K_m Approaches the Diffusion-Controlled Rate of Association with Substrate

Enzyme	Substrate	k_{cat} (sec^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}\ sec^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^{4}	9×10^{-5}	1.6×10^{8}
Carbonic anhydrase	CO_2	1×10^{6}	0.012	8.3×10^{7}
	HCO_3^-	4×10^{5}	0.026	1.5×10^{7}
Catalase	H_2O_2	4×10^{7}	1.1	4×10^{7}
Crotonase	Crotonyl-CoA	5.7×10^{3}	2×10^{-5}	2.8×10^{8}
Fumarase	Fumarate	800	5×10^{-6}	1.6×10^{8}
	Malate	900	2.5×10^{-5}	3.6×10^{7}
Triosephosphate isomerase	Glyceraldehyde-3-phosphate*	4.3×10^{3}	1.8×10^{-5}	2.4×10^{8}
β-Lactamase	Benzylpenicillin	2×10^{3}	2×10^{-5}	1×10^{8}

*K_m for glyceraldehyde-3-phosphate is calculated on the basis that only 3.8% of the substrate in solution is unhydrated and therefore reactive with the enzyme.

Adapted from Fersht, A., 1985. *Enzyme Structure and Mechanism*, 2nd ed. New York: W. H. Freeman.

Turnover Number Defines the Activity of One Enzyme Molecule

The **turnover number** of an enzyme, k_{cat}, is a measure of its maximal catalytic activity. k_{cat} is defined as the number of substrate molecules converted into product per enzyme molecule per unit time when the enzyme is saturated with substrate. The turnover number is also referred to as the **molecular activity** of the enzyme. For the simple Michaelis–Menten reaction (Equation 13.9) under conditions of initial velocity measurements, $k_2 = k_{cat}$. Provided the concentration of enzyme, $[E_T]$, in the reaction mixture is known, k_{cat} can be determined from V_{max}. At saturating [S], $v = V_{max} = k_2 [E_T]$. Thus,

$$k_2 = \frac{V_{max}}{[E_T]} = k_{cat} \tag{13.25}$$

The term k_{cat} represents the kinetic efficiency of the enzyme. Table 13.4 lists turnover numbers for some representative enzymes. Catalase has the highest turnover number known; each molecule of this enzyme can degrade 40 million molecules of H_2O_2 in 1 second! At the other end of the scale, lysozyme requires 2 seconds to cleave a glycosidic bond in its glycan substrate.

In many situations, the actual molar amount of the enzyme is not known. However, its amount can be expressed in terms of the activity observed. The International Commission on Enzymes defines **one international unit** *as the amount that catalyzes the formation of 1 micromole of product in 1 minute.* (Because enzymes are very sensitive to factors such a pH, temperature, and ionic strength, the conditions of assay must be specified.) In the process of purifying enzymes from cellular sources, many extraneous proteins may be present. Then, the units of enzyme activity are expressed as enzyme units per mg protein, a term known as **specific activity** (see Table 5.1).

"Specific Activity"

Definition:
Units of enzyme activity per mg protein

1 Unit = amount of enzyme that will convert one µmole of substrate to product in one minute at a given pH (optimum value) and temperature (usually 25°C or 37°C).

Specific activity is used as an estimate of enzyme purity.

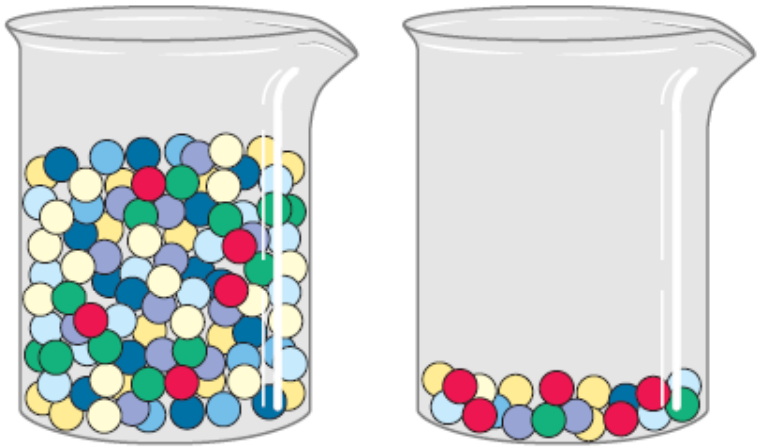


FIGURE 3–23 **Activity versus specific activity.** The difference between these two terms can be illustrated by considering two beakers of marbles. The beakers contain the same number of red marbles, but different numbers of marbles of other colors. If the marbles represent proteins, both beakers contain the same *activity* of the protein represented by the red marbles. The second beaker, however, has the higher *specific activity* because here the red marbles represent a much higher fraction of the total.

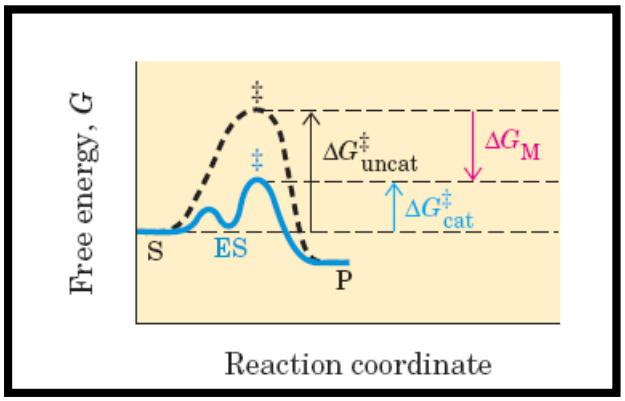
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad V_0 = k_2[ES] \qquad \frac{d[ES]}{dt} = 0 \qquad K_m = \frac{(k_{-1} + k_2)}{k_1}$$

Rate of ES formation = k1 [E][S]

Rate of ES breakdown $= k_{-1}[ES] + k_2[ES]$

k1 [E][S] $= k_{-1}[ES] + k_2[ES]$

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



Total enzyme, $[E_T] = [E] + [ES]$

[Et] = [E] + 1/Km[E][S]

….

[E] = [Et]Km / (Km+[S])

➡

k2[ES] = k2[E][S]/Km

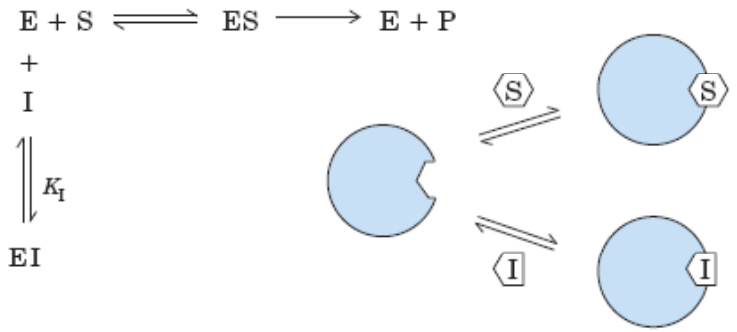
….

k2[ES] = k2[Et]Km[S] / Km (Km+ [S]

….

Vo = Vmax [S] / Km + [S]

(a) Competitive inhibition



$$[E_T] = [E] + [ES] + [EI].$$

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

Assuming that E + I⇌EI reaches rapid equilibrium, the rate of EI formation, $v_f' = k_3[E][I]$, and the rate of disappearance of EI, $v_d' = k_{-3}[EI]$, are equal. So,

$$k_3[E][I] = k_{-3}[EI] \qquad (13.35)$$

Therefore,

$$[EI] = \frac{k_3}{k_{-3}}[E][I] \qquad (13.36)$$

$$[EI] = \frac{[E][I]}{K_I}$$

$$[E] = \frac{K_I K_m [E_T]}{(K_I K_m + K_I[S] + K_m[I])}$$

$$v = \frac{V_{max}[S]}{[S] + K_m\left(1 + \frac{[I]}{K_I}\right)}$$

The Equations of Competitive Inhibition

Given the relationships between E, S, and I described previously and recalling the steady-state assumption that $d[ES]/dt = 0$, from Equations (13.14) and (13.16) we can write

$$ES = \frac{k_1[E][S]}{(k_2 + k_{-1})} = \frac{[E][S]}{K_m} \tag{13.34}$$

Assuming that E + I$\rightleftharpoons$EI reaches rapid equilibrium, the rate of EI formation, $v_f' = k_3[E][I]$, and the rate of disappearance of EI, $v_d' = k_{-3}[EI]$, are equal. So,

$$k_3[E][I] = k_{-3}[EI] \tag{13.35}$$

Therefore,

$$[EI] = \frac{k_3}{k_{-3}}[E][I] \tag{13.36}$$

If we define K_I as k_{-3}/k_3, an enzyme-inhibitor dissociation constant, then

$$[EI] = \frac{[E][I]}{K_I} \tag{13.37}$$

knowing $[E_T] = [E] + [ES] + [EI]$. Then

$$[E_T] = [E] + \frac{[E][S]}{K_m} + \frac{[E][I]}{K_I} \tag{13.38}$$

Solving for [E] gives

$$[E] = \frac{K_I K_m[E_T]}{(K_I K_m + K_I[S] + K_m[I])} \tag{13.39}$$

Because the rate of product formation is given by $v = k_2[ES]$, from Equation 13.34 we have

$$v = \frac{k_2[E][S]}{K_m} \tag{13.40}$$

So,

$$v = \frac{(k_2 K_I[E_T][S])}{(K_I K_m + K_I[S] + K_m[I])} \tag{13.41}$$

Because $V_{max} = k_2[E_T]$,

$$v = \frac{V_{max}[S]}{K_m + [S] + \dfrac{K_m[I]}{K_I}} \tag{13.42}$$

or

$$v = \frac{V_{max}[S]}{[S] + K_m\left(1 + \dfrac{[I]}{K_I}\right)} \tag{13.43}$$

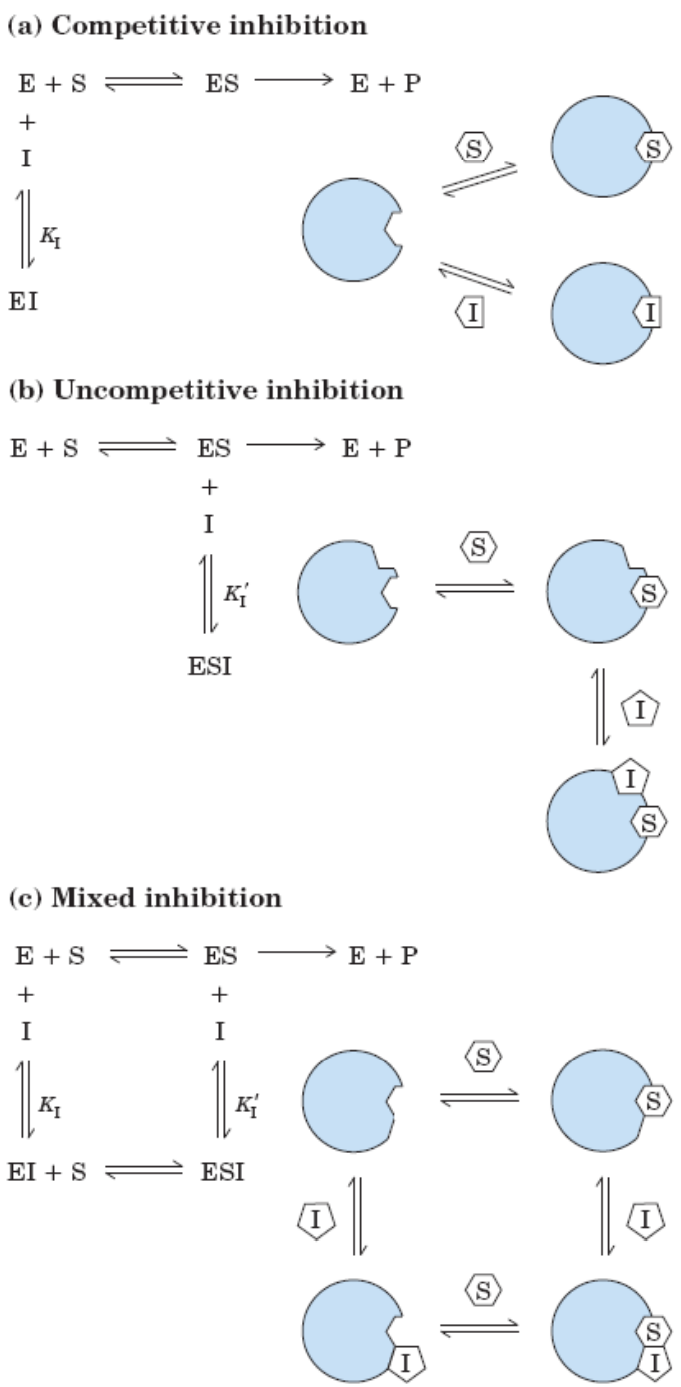


FIGURE 6–15 Three types of reversible inhibition. **(a)** Competitive inhibitors bind to the enzyme's active site. **(b)** Uncompetitive inhibitors bind at a separate site, but bind only to the ES complex. K_I is the equilibrium constant for inhibitor binding to E; K_I' is the equilibrium constant for inhibitor binding to ES. **(c)** Mixed inhibitors bind at a separate site, but may bind to either E or ES.

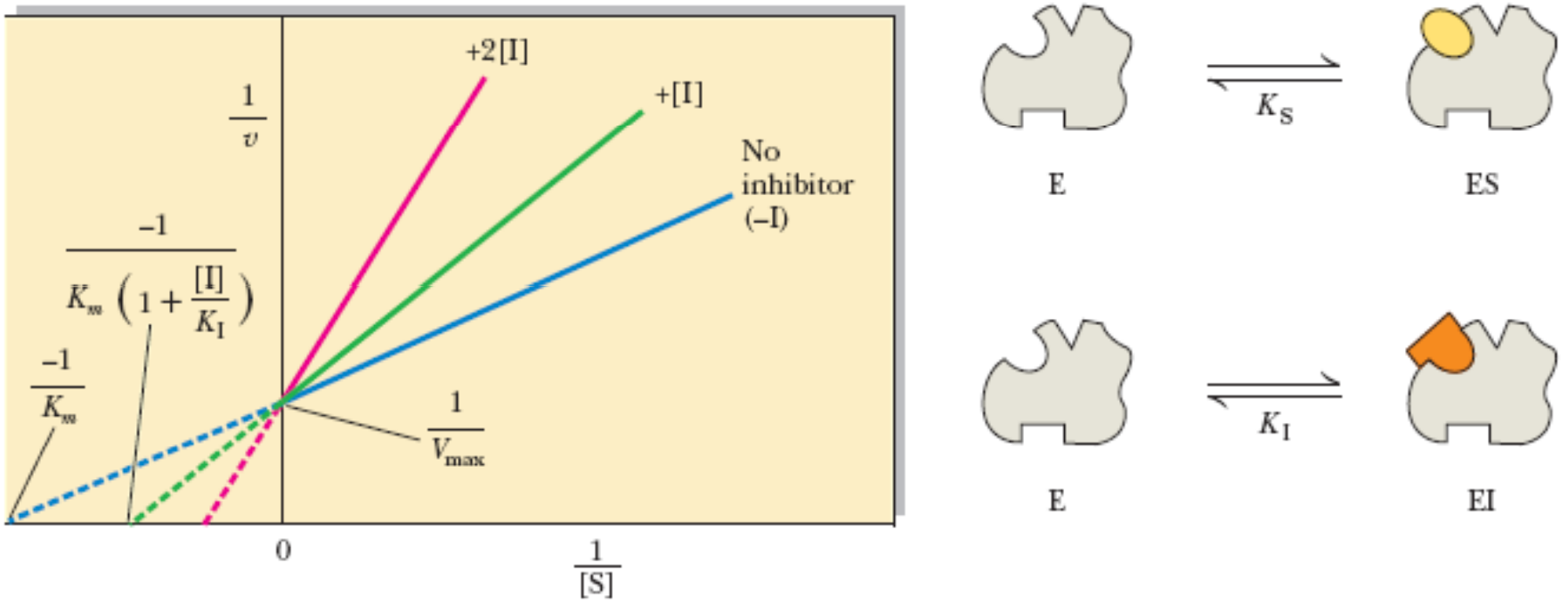


FIGURE 13.13 Lineweaver–Burk plot of competitive inhibition, showing lines for no I, [I], and 2[I]. Note that when [S] is infinitely large ($1/[S] \approx 0$), V_{max} is the same, whether I is present or not.

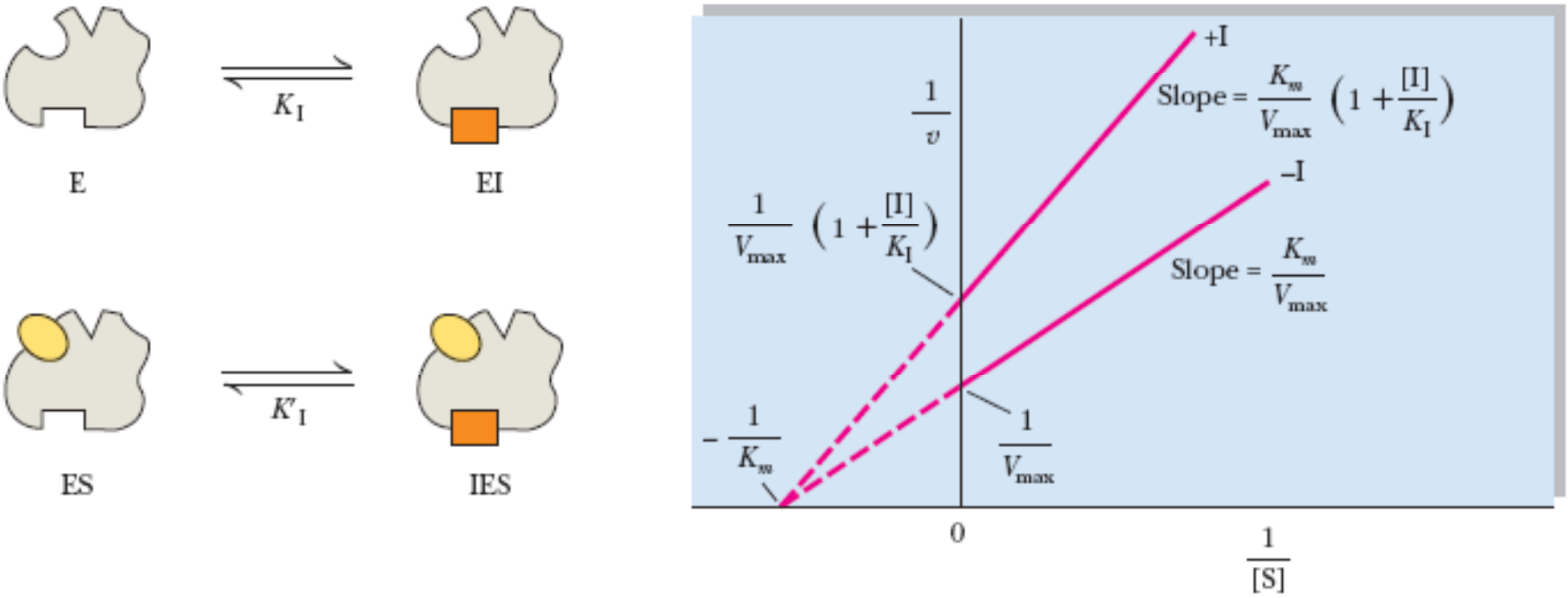


FIGURE 13.15 Lineweaver–Burk plot of pure noncompetitive inhibition. Note that I does not alter K_m but that it decreases V_{max}.

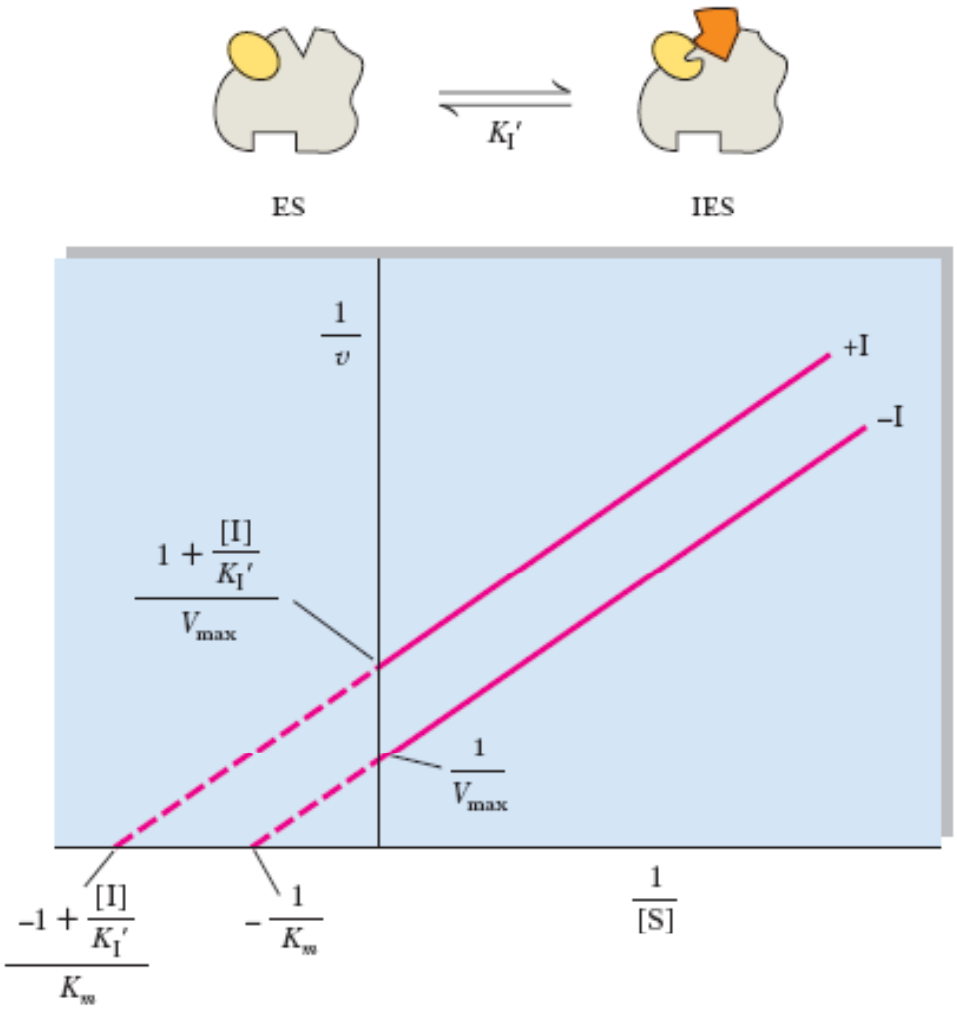


FIGURE 13.17 Lineweaver–Burk plot of uncompetitive inhibition. Note that both intercepts change but the slope (K_m/V_{max}) remains constant in the presence of I.

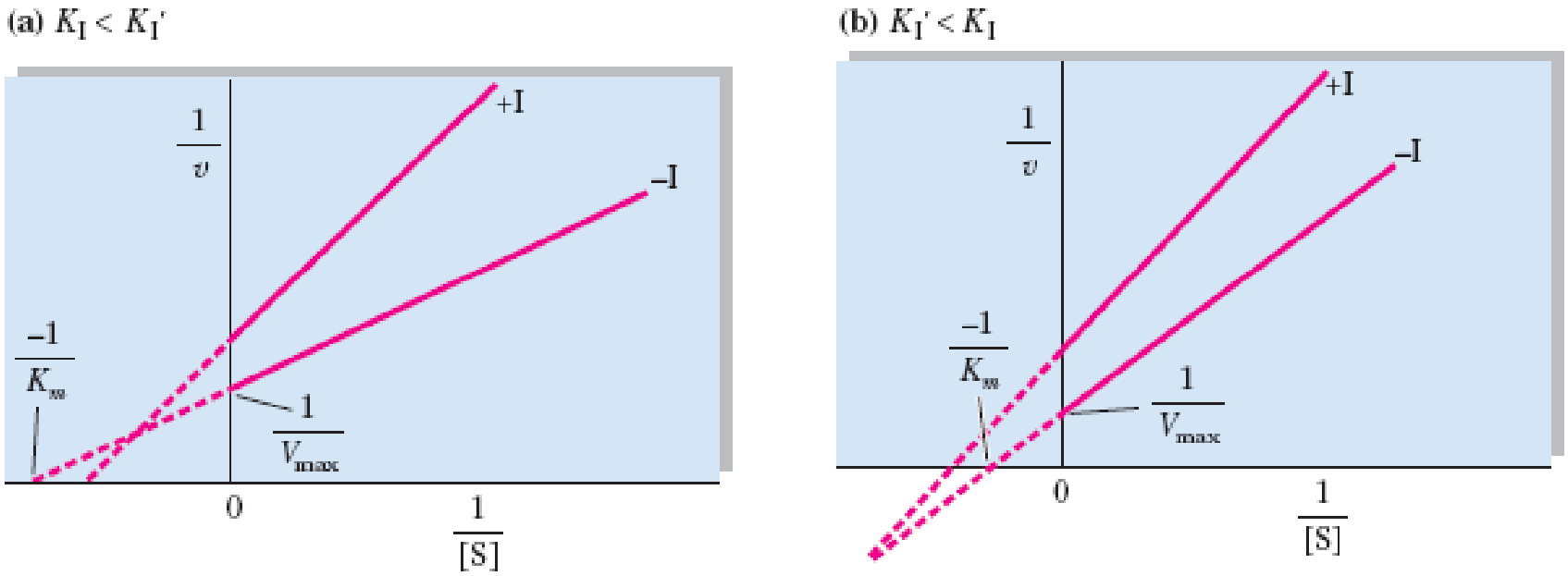


FIGURE 13.16 Lineweaver–Burk plot of mixed noncompetitive inhibition. Note that both intercepts and the slope change in the presence of I. (a) When K_I is less than K_I'; (b) when K_I is greater than K_I'.

TABLE 13.6 The Effect of Various Types of Inhibitors on the Michaelis–Menten Rate Equation and on Apparent K_m and Apparent V_{max}

Inhibition Type	Rate Equation	Apparent K_m	Apparent V_{max}
None	$v = V_{max}[S]/(K_m+[S])$	K_m	V_{max}
Competitive	$v = V_{max}[S]/([S]+K_m(1+[I]/K_I))$	$K_m(1+[I]/K_I)$	V_{max}
Noncompetitive	$v = (V_{max}[S]/(1+[I]/K_I))/(K_m+[S])$	K_m	$V_{max}/(1+[I]/K_I)$
Mixed	$v = V_{max}[S]/((1+[I]/K_I)K_m+(1+[I]/K_I'[S]))$	$K_m(1+[I]/K_I)/(1+[I]/K_I')$	$V_{max}/(1+[I]/K_I')$
Uncompetitive	$v = V_{max}[S]/(K_m+[S](1+[I]/K_I'))$	$K_m/(1+[I]/K_I')$	$V_{max}/(1+[I]/K_I')$

K_I is defined as the enzyme : inhibitor dissociation constant K_I=[E][I]/[EI]; K_I' is defined as the enzyme–substrate complex : inhibitor dissociation constant K_I'=[ES][I]/[IES].

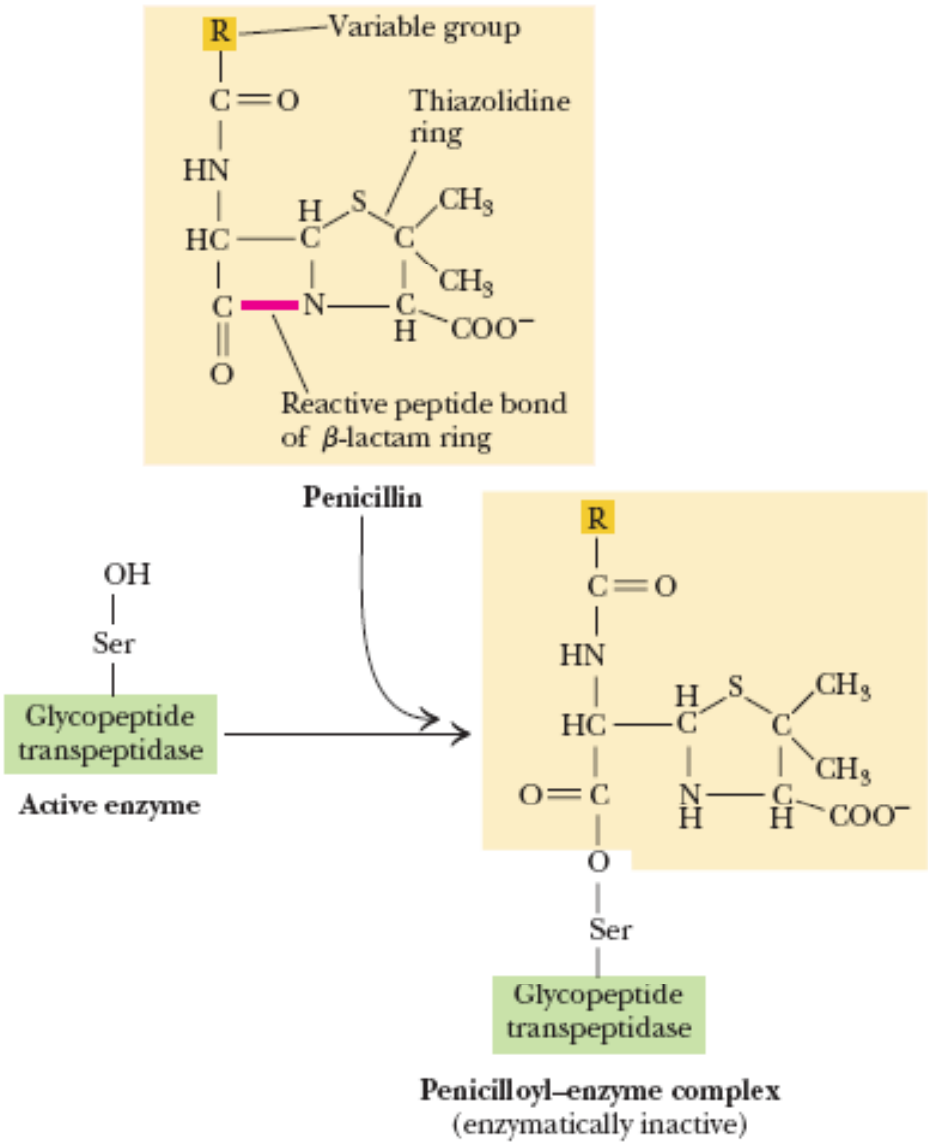


FIGURE 13.18 Penicillin is an irreversible inhibitor of the enzyme *glycopeptide transpeptidase*, also known as *glycoprotein peptidase*, which catalyzes an essential step in bacterial cell wall synthesis.