$$K'_{eq} = 10^{-\Delta G°'/(2.303RT)} \quad (7)$$

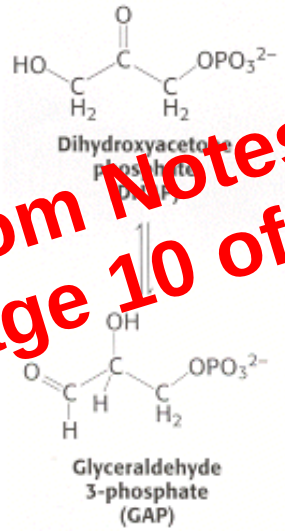
Substituting $R = 1.987 \times 10^{-3}$ kcal mol^{-1} deg^{-1} and $T = 298$ K (corresponding to 25°C) gives

$$K'_{eq} = 10^{-\Delta G°'/1.36} \quad (8)$$

where $\Delta G°'$ is here expressed in kilocalories per mole because of the choice of the units for R in equation 7. Thus, the standard free energy and the equilibrium constant of a reaction are related by a simple expression. For example, an equilibrium constant of 10 gives a standard free-energy change of -1.36 kcal mol^{-1} (-5.69 kJ mol^{-1}) at 25°C (Table 8.4). Note that, for each 10-fold change in the equilibrium constant, the $\Delta G°'$ changes by 1.36 kcal mol^{-1} (5.69 kJ mol^{-1}).

As an example, let us calculate $\Delta G°'$ and ΔG for the isomerization of dihydroxyacetone phosphate (DHAP) to glyceraldehyde 3-phosphate (GAP). This reaction takes place in glycolysis (Section 16.1.4). At equilibrium, the ratio of GAP to DHAP is 0.0475 at 25°C (298 K) and pH 7. Hence, $K'_{eq} = 0.0475$. The standard free-energy change for this reaction is then calculated from equation 6:

$$\begin{aligned}\Delta G°' &= -2.303RT \log_{10} K'_{eq} \\ &= -2.303 \times 1.987 \times 10^{-3} \times 298 \times \log_{10}(0.0475) \\ &= +1.80 \text{ kcal mol}^{-1} (7.53 \text{ kJ mol}^{-1})\end{aligned}$$



Under these conditions, the reaction is endergonic. DHAP will not spontaneously convert to GAP.

Now let us calculate ΔG for this reaction when the initial concentration of DHAP is 2×10^{-4} M and the initial concentration of GAP is 3×10^{-6} M. Substituting these values into equation 1 gives

$$\begin{aligned}\Delta G &= 1.80 \text{ kcal mol}^{-1} + 2.303RT \log_{10} \frac{3 \times 10^{-6} \text{ M}}{2 \times 10^{-4} \text{ M}} \\ &= 1.80 \text{ kcal mol}^{-1} - 2.49 \text{ kcal mol}^{-1} \\ &= -0.69 \text{ kcal mol}^{-1} (-2.89 \text{ kJ mol}^{-1})\end{aligned}$$

This negative value for the ΔG indicates that the isomerization of DHAP to GAP is exergonic and can occur spontaneously when these species are present at the aforestated concentrations. Note that ΔG for this reaction is negative, although $\Delta G°'$ is positive. *It is important to stress that whether the* Δ G *for a reaction is larger, smaller, or the same as* Δ G$°'$ *depends on the concentrations of the reactants and products*. The criterion of spontaneity for a reaction is ΔG, not $\Delta G°'$. This point is important because reactions that are not spontaneous based on $\Delta G°'$ can be made spontaneous by adjusting the concentrations of reactants and products. This principle is the basis of the coupling of

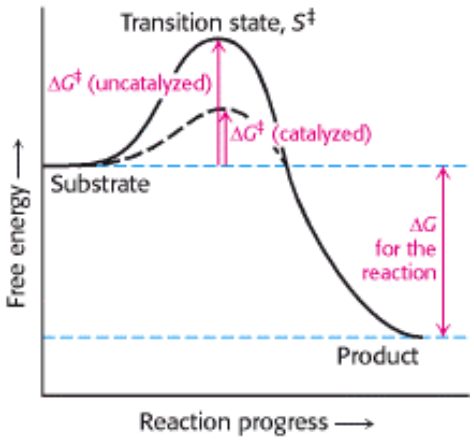


Figure 8.3. Enzymes Decrease the Activation Energy. Enzymes accelerate reactions by decreasing $\Delta G^{\ddagger}$, the free energy of activation.

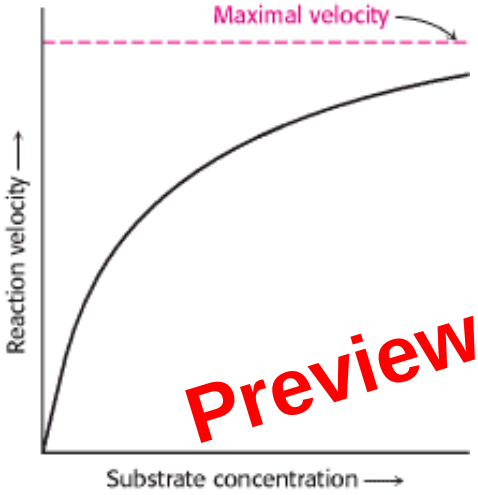


Figure 8.4. Reaction Velocity Versus Substrate Concentration in an Enzyme-Catalyzed Reaction. An enzyme-catalyzed reaction reaches a maximal velocity.

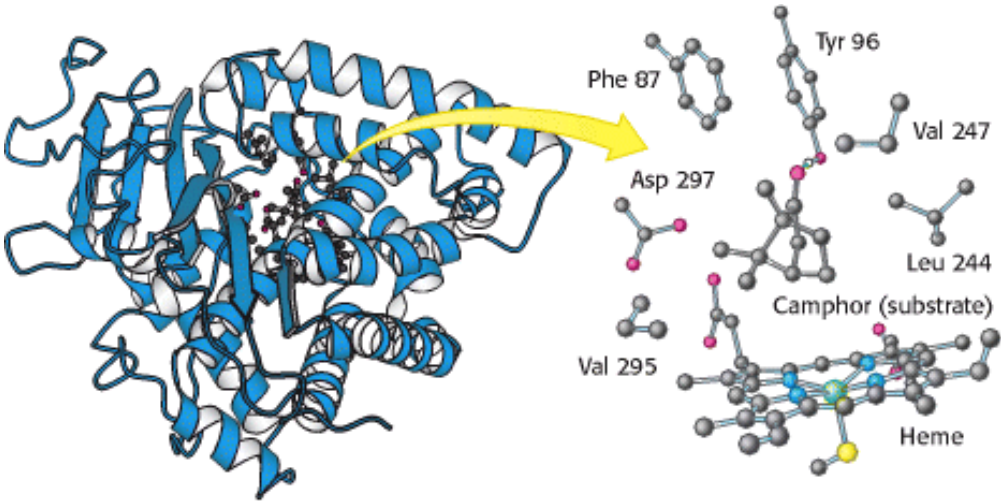


Figure 8.5. Structure of an Enzyme-Substrate Complex. (Left) The enzyme cytochrome P-450 is illustrated bound to

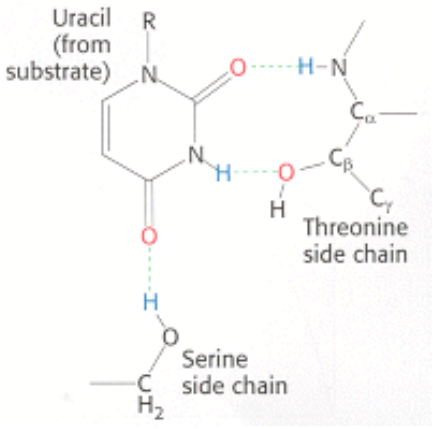


Figure 8.8. Hydrogen Bonds between an Enzyme and Substrate. The enzyme ribonuclease forms hydrogen bonds with the uridine component of the substrate. [After F. M. Richards, H. W. Wyckoff, and N. Allewel. In *The Neurosciences: Second Study Program,* F. O. Schmidt, Ed. (Rockefeller University Press, 1970), p. 970.]

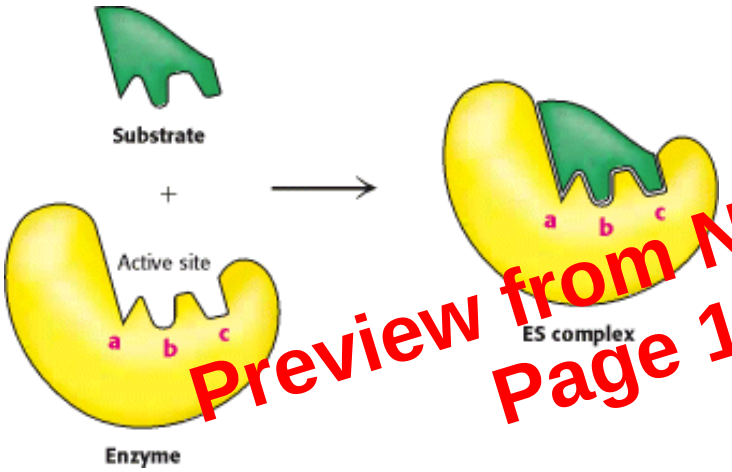


Figure 8.9. Lock-and-Key Model of Enzyme-Substrate Binding. In this model, the active site of the unbound enzyme is complementary in shape to the substrate.

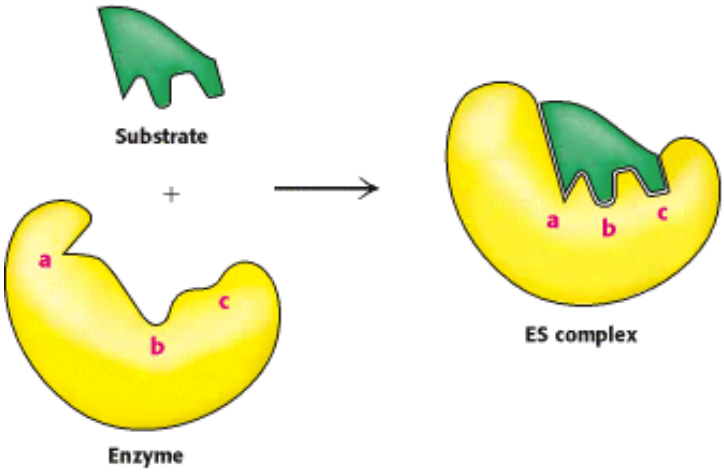


Figure 8.10. Induced-Fit Model of Enzyme-Substrate Binding. In this model, the enzyme changes shape on substrate binding. The active site forms a shape complementary to the substrate only after the substrate has been bound.

8.4. The Michaelis-Menten Model Accounts for the Kinetic Properties of Many Enzymes

The primary function of enzymes is to enhance rates of reactions so that they are compatible with the needs of the organism. To understand how enzymes function, we need a kinetic description of their activity. For many enzymes, the rate of catalysis V_0, which is defined as the number of moles of product formed per second, varies with the substrate concentration [S] in a manner shown in Figure 8.11. The rate of catalysis rises linearly as substrate concentration increases and then begins to level off and approach a maximum at higher substrate concentrations. Before we can accurately interpret this graph, we need to understand how it is generated. Consider an enzyme that catalyzes the S to P by the following pathway:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

The extent of product formation is determined as a function of time for a series of substrate concentrations (Figure 8.12). As expected, in each case, the amount of product formed increases with time, although eventually a time is reached when there is *no net change* in the concentration of S or P. The enzyme is still actively converting substrate into product and visa versa, but the reaction equilibrium has been attained. Figure 8.13A illustrates the changes in concentration observed in all of the reaction participants with time until equilibrium has been reached.

Enzyme kinetics are more easily approached if we can ignore the back reaction. We define V_0 as the rate of increase in product with time when [P] is low; that is, at times close to zero (hence, V_0) (Figure 8.13B). Thus, for the graph in Figure 8.11, V_0 is determined for each substrate concentration by measuring the rate of product formation at early times before P accumulates (see Figure 8.12).

We begin our kinetic examination of enzyme activity with the graph shown in Figure 8.11. At a fixed concentration of enzyme, V_0 is almost linearly proportional to [S] when [S] is small but is nearly independent of [S] when [S] is large. In 1913, Leonor Michaelis and Maud Menten proposed a simple model to account for these kinetic characteristics. The critical feature in their treatment is that a specific ES complex is a necessary intermediate in catalysis. The model proposed, which is the simplest one that accounts for the kinetic properties of many enzymes, is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (9)$$

An enzyme E combines with substrate S to form an ES complex, with a rate constant k_1. The ES complex has two possible fates. It can dissociate to E and S, with a rate constant k_{-1}, or it can proceed to form product P, with a rate constant k_2. Again, we assume that almost none of the product reverts to the initial substrate, a condition that holds in the initial stage of a reaction before the concentration of product is appreciable.

We want an expression that relates the rate of catalysis to the concentrations of substrate and enzyme and the rates of the individual steps. Our starting point is that the catalytic rate is equal to the product of the concentration of the ES complex and k_2.

$$V_0 = k_2[ES] \qquad (10)$$

Now we need to express [ES] in terms of known quantities. The rates of formation and breakdown of ES are given by:

Solving equation 18 for [ES] gives

$$[ES] = \frac{[E]_T[S]/K_M}{1 + [S]/K_M} \qquad (19)$$

or

$$[ES] = [E]_T \frac{[S]}{[S] + K_M} \qquad (20)$$

By substituting this expression for [ES] into equation 10, we obtain

$$V_0 = k_2[E]_T \frac{[S]}{[S] + K_M} \qquad (21)$$

The maximal rate, V_{max}, is attained when the catalytic sites on the enzyme are saturated with substrate—that is, when $[ES] = [E]_T$. Thus,

$$V_{max} = k_2[E]_T \qquad (22)$$

Substituting equation 22 into equation 21 yields the *Michaelis-Menten equation*:

$$V_0 = V_{max} \frac{[S]}{[S] + K_M} \qquad (23)$$

This equation accounts for the kinetic data given in Figure 8.11. At very low substrate concentration, when [S] is much less than K_M, $V_0 = (V_{max}/K_M)[S]$; that is, the rate is directly proportional to the substrate concentration. At high substrate concentration, when [S] is much greater than K_M, $V_0 = V_{max}$; that is, the rate is maximal, independent of substrate concentration.

The meaning of K_M is evident from equation 23. When $[S] = K_M$, then $V_0 = V_{max}/2$. Thus, K_M *is equal to the substrate concentration at which the reaction rate is half its maximal value*. K_M is an important characteristic of an enzyme-catalyzed reaction and is significant for its biological function.

The physiological consequence of K_M is illustrated by the sensitivity of some individuals to ethanol. Such persons exhibit facial flushing and rapid heart rate (tachycardia) after ingesting even small amounts of alcohol. In the liver, alcohol dehydrogenase converts ethanol into acetaldehyde.

$$CH_3CH_2OH + NAD^+ \xrightleftharpoons{\text{Alchohol dehydrogenase}} CH_3CHO + H^+ + NADH$$

Normally, the acetaldehyde, which is the cause of the symptoms when present at high concentrations, is processed to acetate by acetaldehyde dehydrogenase.

Concentration changes under (A) steady-state conditions, and (B) the pre-steady-state conditions.

Table 8.5. K_M values of some enzymes

Enzyme	Substrate	K_M (μM)
Chymotrypsin	Acetyl-l-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

Table 8.6. Maximum turnover numbers of some enzymes

Enzyme	Turnover number (per second)
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

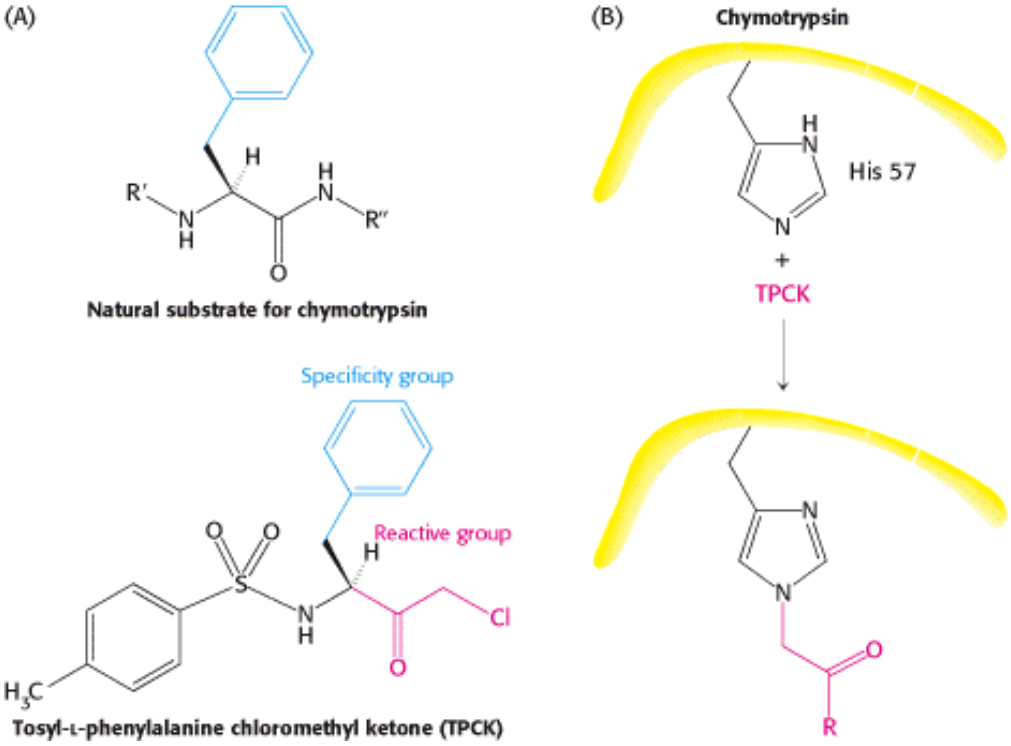


Figure 8.21. Affinity Labeling. (A) Tosyl-L-phenylalanine chloromethyl ketone (TPCK) is a reactive analog of the normal substrate for the enzyme chymotrypsin. (B) TPCK binds at the active site of chymotrypsin and modifies an essential histidine residue.

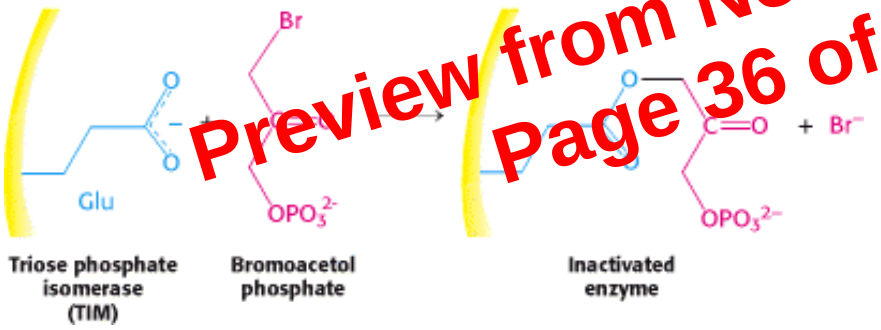


Figure 8.22. Bromoacetol Phosphate, an Affinity Label for Triose Phosphate Isomerase (TIM). Bromoacetol phosphate, an analog of dihydroxyacetone phosphate, binds at the active site of the enzyme and covalently modifies a glutamic acid residue required for enzyme activity.

Table 8.9. Water-Soluble Vitamins

Vitamin	Coenzyme	Typical reaction type	Consequences of deficiency
Thiamine (B_1)	Thiamine pyrophosphate	Aldehyde transfer	Beriberi (weight loss, heart problems, neurological dysfunction)
Riboflavin (B_2)	Flavin adenine dinucleotide (FAD)	Oxidation-reduction	Cheliosis and angular stomatitus (lesions of the mouth), dermatitis
Pyridoxine (B_6)	Pyridoxal phosphate	Group transfer to or from amino acids	Depression, confusion, convulsions
Nicotinic acid (niacin)	Nicotinamide adenine dinucleotide (NAD^+)	Oxidation-reduction	Pellagra (dermatitis, depression, diarrhea)
Pantothenic acid	Coenzyme A	Acyl-group transfer	Hypertension
Biotin	Biotin-lysine complexes (biocytin)	ATP-dependent carboxylation and carboxyl-group transfer	Rash about the eyebrows, muscle pain, fatigue (rare)
Folic acid	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis	Anemia, neural-tube defects in development
B_{12}	5′-Deoxyadenosyl cobalamin	Transfer of methyl groups; intramolecular rearrangements	Anemia, pernicious anemia, methylmalonic acidosis
C (ascorbic acid)		Antioxidant	Scurvy (swollen and bleeding gums, subdermal hemorrhages)

Table 8.10. Fat-soluble vitamins

Vitamin	Function	Deficiency
A	Roles in vision, growth, reproduction	Night blindness, cornea damage, damage to respiratory and gastrointestinal tract
D	Regulation of calcium and phosphate metabolism	Rickets (children): skeletal deformaties, impaired growth Osteomalacia (adults): soft, bending bones
E	Antioxidant	Inhibition of sperm production; lesions in muscles and nerves (rare)
K	Blood coagulation	Subdermal hemorrhaging

See answer

6. *A fresh view.* The plot of $1/V_0$ versus 1/[S] is sometimes called a Lineweaver-Burk plot. Another way of expressing the kinetic data is to plot V_0 versus V_0/[S], which is known as an Eadie-Hofstee plot.

(a) Rearrange the Michaelis-Menten equation to give V_0 as a function of V_0/[S].

(b) What is the significance of the slope, the vertical intercept, and the horizontal intercept in a plot of V_0 versus V_0/[S]?

(c) Sketch a plot of V_0 versus V_0/[S] in the absence of an inhibitor, in the presence of a competitive inhibitor, and in the presence of a noncompetitive inhibitor.

See answer

7. *Potential donors and acceptors.* The hormone progesterone contains two ketone groups. At pH 7, which side chains of the receptor might form hydrogen bonds with progesterone?

See answer

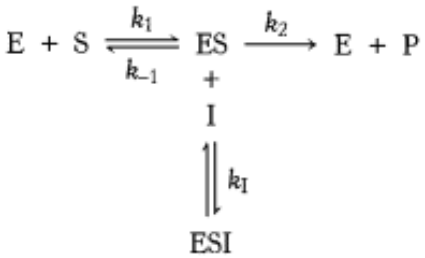
8. *Competing substrates.* Suppose that two substrates, A and B, compete for an enzyme. Derive an expression relating the ratio of the rates of utilization of A and B, V_A/V_B, to the concentrations of these substrates and their values of k_2 and K_M. (Hint: Express V_A as a function of k_2/K_M for substrate A, and do the same for V_B.) Is specificity determined by K_M alone?

See answer

9. *A tenacious mutant.* Suppose that a mutant enzyme binds a substrate 100-fold as tightly as does the native enzyme. What is the effect of this mutation on catalytic rate if the binding of the transition state is unaffected?

See answer

10. *Uncompetitive inhibition.* The following reaction represents the mechanism of action of an uncompetitive inhibitor.



(a) Draw a standard Michaelis-Menton curve in the absence and in the presence of increasing amounts of inhibitor. Repeat for a double-reciprocal plot.

(b) Explain the results obtained in part *a*.

See answer