

23. A relaxation experiment probes the homodimerization of an oligonucleotide. At 10-nM oligonucleotide, the apparent rate constant ( $k_{\text{apparent}}$ ) is  $2.06 \text{ sec}^{-1}$ . At 100-nM oligonucleotide, the apparent rate constant ( $k_{\text{apparent}}$ ) is  $6.34 \text{ sec}^{-1}$ . What are the association and dissociation rate constants?
24. A protein folding reaction has two intermediate states, each of which individually obeys Arrhenius-type behavior. At low temperatures, forming the first intermediate is rate-limiting. At high temperatures, forming the second intermediate is rate-limiting.
- Does the protein folding reaction obey Arrhenius-type behavior over all temperatures?
  - Forming which intermediate has a higher activation energy?

### Further Reading

- Atkins PW & De Paula J (2006) Atkins' Physical Chemistry, 8th ed. Oxford, UK: Oxford University Press.
- Eisenberg DS & Crothers DM (1979) Physical Chemistry: With Applications to the Life Sciences. Menlo Park, CA: Benjamin-Cummings.
- Fersht A (1999) Structure and Mechanism in Protein Science: a Guide to Enzyme Catalysis and Protein Folding. San Francisco: W.H. Freeman.
- Hammes GG (2000) Thermodynamics and Kinetics for the Biological Sciences. New York: John Wiley & Sons.
- Steinfeld JL, Francisco JS & Hase WL (1999) Chemical Kinetics and Dynamics, 2nd ed. Upper Saddle River, NJ: Prentice Hall.

# CHAPTER 16

## Principles of Enzyme Catalysis

**F**or two molecules to undergo a reaction, they must collide and be in a relative orientation that brings the reacting groups close together. The molecules must also have sufficient energy to overcome the energetic barrier to the reaction. We have noted, in Chapter 15, that catalysts can speed up the rates for reactions by bringing molecules together and by reducing the activation energy for the reaction.

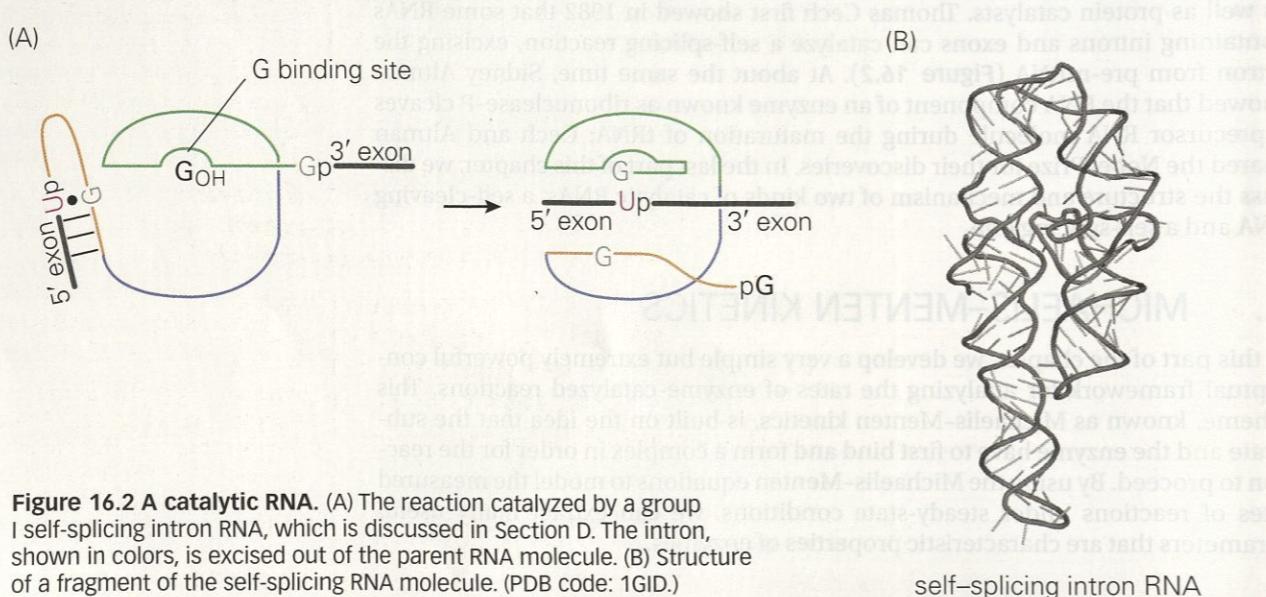
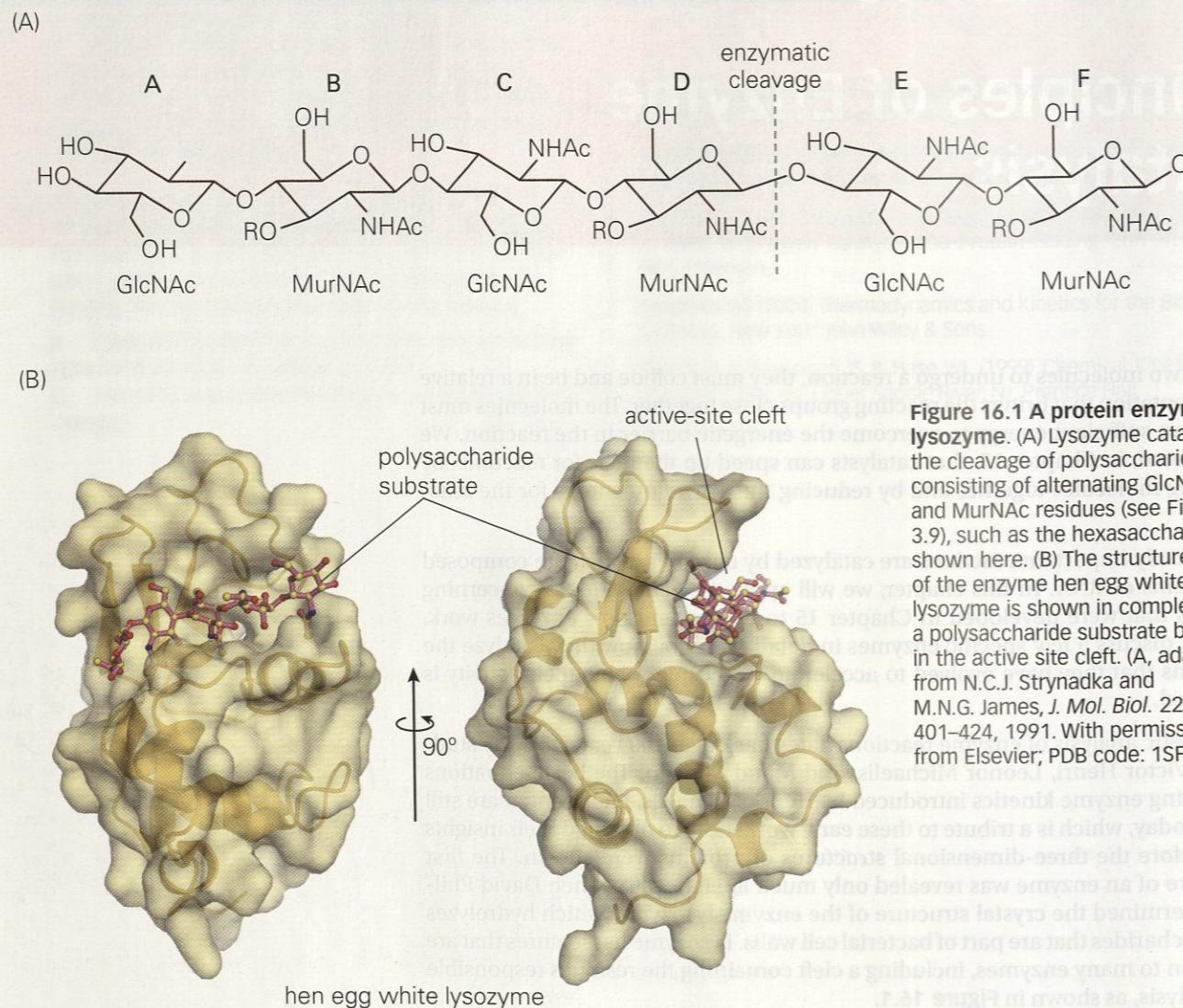
Biologically important reactions are catalyzed by enzymes, which are composed of proteins or RNA. In this chapter, we will apply the general ideas concerning kinetics that were developed in Chapter 15 to understand how enzymes work. We will discuss a few specific enzymes in detail, describe how they catalyze the reactions that they have evolved to accelerate, and explain how their activity is regulated.

The kinetic analysis of enzyme reactions dates back over 100 years, to early studies by Victor Henri, Leonor Michaelis, and Maud Menten. The basic equations describing enzyme kinetics introduced by Henri, Michaelis, and Menten are still in use today, which is a tribute to these early workers, who obtained their insights long before the three-dimensional structures of proteins were known. The first structure of an enzyme was revealed only much later, in 1965, when David Phillips determined the crystal structure of the enzyme lysozyme, which hydrolyzes polysaccharides that are part of bacterial cell walls. Lysozyme has features that are common to many enzymes, including a cleft containing the residues responsible for catalysis, as shown in **Figure 16.1**.

Long after the first protein enzymes were characterized, it was shown that some RNAs in cells also have catalytic activity. This discovery caused a conceptual shift in biochemistry, because it expanded our definition of an enzyme to include RNA as well as protein catalysts. Thomas Cech first showed in 1982 that some RNAs containing introns and exons can catalyze a self-splicing reaction, excising the intron from pre-mRNA (**Figure 16.2**). At about the same time, Sidney Altman showed that the RNA component of an enzyme known as ribonuclease-P cleaves a precursor RNA molecule during the maturation of tRNA; Cech and Altman shared the Nobel Prize for their discoveries. In the last part of this chapter, we discuss the structure and mechanism of two kinds of catalytic RNAs, a self-cleaving RNA and a self-splicing one.

### A. MICHAELIS-MENTEN KINETICS

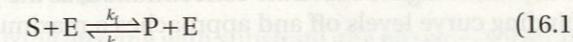
In this part of the chapter, we develop a very simple but extremely powerful conceptual framework for analyzing the rates of enzyme-catalyzed reactions. This scheme, known as Michaelis-Menten kinetics, is built on the idea that the substrate and the enzyme have to first bind and form a complex in order for the reaction to proceed. By using the Michaelis-Menten equations to model the measured rates of reactions under steady-state conditions, we can extract many useful parameters that are characteristic properties of enzymes.



## 16.1 Enzyme-catalyzed reactions can be described as a binding step followed by a catalytic step

For enzymatic reactions, the reactants are usually referred to as the **substrates** for the enzyme. Substrate, enzyme, and product will be indicated by S, E, and P, respectively. Within each enzyme there is an **active site**, which includes a region where the chemical reaction actually occurs (the **catalytic site**). The active site may also contain other regions that help hold and position the substrate at the correct location in the active site. Most of the essential elements of enzyme kinetic behavior are manifested when there is just a single substrate, and so that case will be considered first because it is the simplest to understand.

For a single substrate, the overall enzyme-catalyzed reaction can be written as:

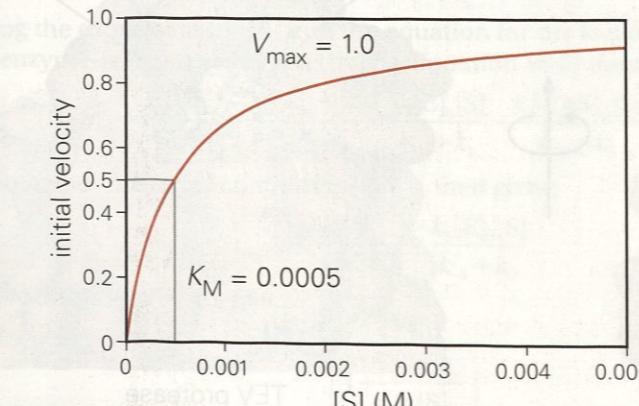
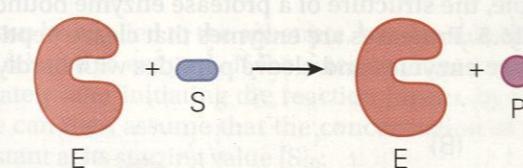


We need, in general, to consider both the forward rate constant ( $k_f$ ) and the rate constant for the reverse reaction ( $k_r$ ). When analyzing the results of experiments, the situation is simplified if the reaction is initiated by adding the substrate to the enzyme, so that there are no product molecules initially. The rate of back reaction is often very slow, and hence it can be ignored in the analysis. The rate of product formation is then given by:

$$v = \frac{d[P]}{dt} = k_f[S][E] \quad (16.2)$$

The rate of product formation is called the **velocity of the reaction**,  $v$ . If the rate of the reaction is measured only during the initial period of the reaction (that is, before substrate is depleted and product builds up), then the rate is referred to as the **initial velocity**,  $v_0$ .

Equation 16.2 predicts that the velocity of the reaction depends linearly on the concentrations of both substrate and enzyme. In actuality, if one measures the velocity of the reaction as a function of substrate concentration, holding enzyme concentration constant, results such as those shown in Figure 16.3 are found. At very low substrate concentrations the response is indeed linear, but with increasing substrate the rate levels off, reaching a maximum value termed the **maximal velocity** ( $V_{\max}$ ). A second parameter, the **Michaelis constant** ( $K_M$ ), specifies the concentration of substrate required to reach half of this maximum velocity. As a result, a more complex kinetic scheme than that described by Equation 16.1 is required to explain these data.



### Initial velocity

When substrate is added to the enzyme to initiate a reaction, there is initially little or no product present and the amount of substrate has not decreased significantly. The rate of the reaction during this period is called the **initial velocity**,  $v_0$ .

### Maximal velocity

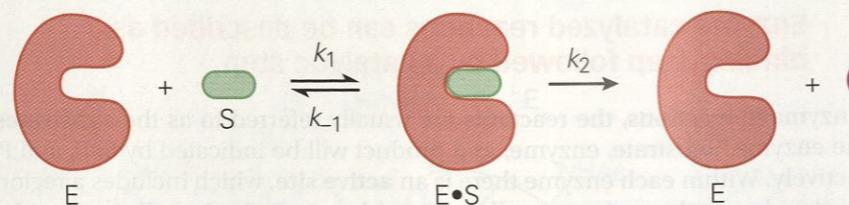
The maximal velocity,  $V_{\max}$ , is the maximum rate of reaction catalyzed by an enzyme at very high substrate concentration.

### Michaelis constant

The Michaelis constant,  $K_M$ , is the concentration of substrate required for the reaction velocity to be  $\frac{1}{2} V_{\max}$ .

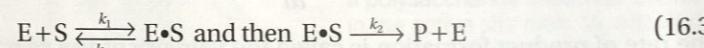
Figure 16.3 A graph of the initial velocity of reaction for different concentrations of the substrate, S. At very high concentration, the velocity approaches the value  $V_{\max}$ , and it reaches half of that value when the substrate concentration is  $K_M$ , the Michaelis constant.

**Figure 16.4** A schematic drawing of the process of an enzyme binding substrate, which reacts and then is released as product. The analysis of such a reaction scheme is known as Michaelis-Menten kinetics. An example of a real enzyme-substrate complex is shown in Figure 16.5.



The graph of reaction velocity as a function of substrate concentration (see Figure 16.3) is reminiscent of the hyperbolic binding isotherm for a ligand binding to a protein (see Figure 12.4). Recall that at low substrate concentration the fraction of the protein that is bound to ligand increases steeply with substrate concentration. But, at higher substrate concentration, as the protein becomes saturated, the binding curve levels off and approaches a maximum value asymptotically. There is, indeed, a close connection between the thermodynamics of binding and the kinetics of enzyme-catalyzed reactions, because the first step in catalysis is the binding of substrate to the enzyme. If the on- and off-rates for the substrate binding to the enzyme are fast compared to the catalytic step, then the binding can be considered to be a reversible event, as shown in Figure 16.4.

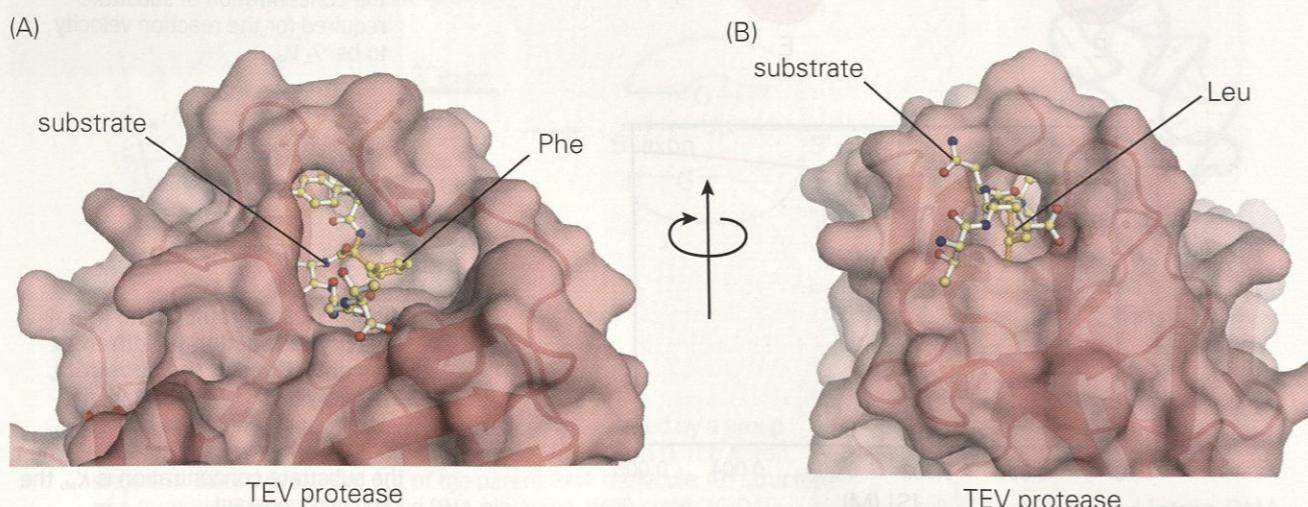
Considering binding and dissociation of the substrate as separate kinetic steps that come before the actual catalyzed reaction gives a more complicated kinetic model:



The rate constant  $k_2$  is associated with the actual chemical step. As we discussed earlier, the reverse reaction is ignored because, if the measurements are made within a short time after mixing substrate and enzyme, then not enough product is generated to drive the reverse reaction. This description of kinetic behavior is called **Michaelis-Menten kinetics**, named for the biochemists who established this model in 1913, following the slightly earlier work of Henri.

**Figure 16.5** The structure of the TEV (tobacco etch virus) protease, bound to its substrate. Flaps on the enzyme close around the substrate to enclose it. Binding pockets for a phenylalanine in the front view (A) and a leucine in the back view (B) are evident. These are just two of the many sequence-specific interactions that are apparent when the structure is examined carefully. (PDB code: 1LVB.)

One important consequence of the reaction scheme shown in Equation 16.3 is that the recognition of the substrate is separated from the catalysis of the chemical reaction. The specificity of the enzyme for the substrate often manifests itself in the first step, with the nature of the interactions between the substrate and the enzyme determining whether the substrate binds to the enzyme and whether it binds in an orientation appropriate for catalysis. This step is governed by the same set of principles that we have established in Chapters 12 and 13 for simple binding events. As an example, the structure of a protease enzyme bound to its substrate is shown in Figure 16.5. Proteases are enzymes that cleave peptide bonds. Some proteases are digestive enzymes and cleave peptides with hardly any specificity.



Others, such as the one shown in Figure 16.5, are very specific, and cleave only particular peptide bonds within a specific sequence context. As you can see from the structure shown in Figure 16.5, numerous interactions between the enzyme and the substrate ensure that only the correct target sequence binds with high affinity to the enzyme.

## 16.2 The Michaelis-Menten equation describes the kinetics of the simplest enzyme-catalyzed reactions

For the reaction scheme shown in Equation 16.3, the initial velocity,  $v_0$ , is given by:

$$v_0 = \frac{d[P]}{dt} = k_2 [E \cdot S] \quad (16.4)$$

If we initiate the reaction by mixing enzyme with substrate, the enzyme-substrate complex will begin to form, but can dissociate to release free enzyme. The complex can also generate product and then dissociate to release free enzyme. After some time, the rates of formation and dissociation of the enzyme complex will become equal, and so the concentrations of the free enzyme and the enzyme-substrate complex will reach constant values. Recall from Section 15.13 that this corresponds to a **steady-state** situation. We will use  $[E \cdot S]_{ss}$  to denote the steady state concentration of the enzyme-substrate complex. As long as there is a large amount of substrate present,  $[E \cdot S]_{ss}$  does not change with time and we can write:

$$\frac{d[E \cdot S]_{ss}}{dt} = k_1 [E][S] - k_{-1} [E \cdot S]_{ss} - k_2 [E \cdot S]_{ss} = 0 \quad (16.5)$$

Rearranging this gives:

$$[E \cdot S]_{ss} = \frac{k_1 [E][S]}{k_{-1} + k_2} \quad (16.6)$$

Using Equation 16.6 in Equation 16.4, the initial velocity,  $v_0$ , is given by:

$$v_0 = k_2 [E \cdot S]_{ss} = \frac{k_1 k_2 [E][S]}{k_{-1} + k_2} \quad (16.7)$$

In Equation 16.7,  $[E]$  is the concentration of free enzyme. The concentration of the free enzyme is not straightforward to determine experimentally or to calculate.  $[E]$  can be eliminated from the rate equations by rewriting them to use the total enzyme concentration present,  $[E]_0$ :

$$[E]_0 = [E] + [E \cdot S]_{ss} \quad \text{or} \quad [E] = [E]_0 - [E \cdot S]_{ss} \quad (16.8)$$

The same idea can be applied to the substrate, but the substrate is usually present in large excess relative to enzyme. If the rates are measured only during a brief period immediately after initiating the reaction (again, by measuring the *initial velocity*,  $v_0$ ), we can then assume that the concentration of the substrate ( $[S]$ ) is essentially constant at its starting value  $[S]_0$ :

$$[S]_0 = [S] + [E \cdot S]_{ss} \approx [S] \quad (16.9)$$

Substituting the expression for  $[E]$  into the equation for the steady-state concentration of enzyme-substrate complex (that is, Equation 16.6) then gives:

$$[E \cdot S]_{ss} = \frac{k_1 ([E]_0 - [E \cdot S]_{ss})[S]}{k_{-1} + k_2} = \frac{k_1 [E]_0 [S]}{k_{-1} + k_2} - \frac{k_1 [E \cdot S]_{ss} [S]}{k_{-1} + k_2} \quad (16.10)$$

Grouping together the terms containing  $[E \cdot S]_{ss}$  then gives:

$$[E \cdot S]_{ss} \left( 1 + \frac{k_1 [S]}{k_{-1} + k_2} \right) = \frac{k_1 [E]_0 [S]}{k_{-1} + k_2} \quad (16.11)$$

and again rearranging,

$$[E \cdot S]_{ss} = \frac{[E]_0}{\left( 1 + \frac{k_1 [S]}{k_{-1} + k_2} \right)} \quad (16.12)$$

### Michaelis-Menten kinetics

Michaelis-Menten kinetics derives from a model with reversible substrate binding to the enzyme, followed by the chemical transformation. It predicts the behavior of many enzymes very well.

We define the Michaelis constant,  $K_M$ , as follows:

$$K_M = \frac{k_{-1} + k_2}{k_1} \quad (16.13)$$

Using this definition for  $K_M$  in Equation 16.12, we get:

$$[E \cdot S]_{ss} = \frac{[E]_0}{\left(1 + \frac{K_M}{[S]}\right)} \quad (16.14)$$

We obtain an expression for the initial velocity by multiplying  $[E \cdot S]_{ss}$  by  $k_2$ :

$$v_0 = k_2 [E \cdot S]_{ss} = \frac{k_2 [E]_0}{\left(1 + \frac{K_M}{[S]}\right)} \quad (16.15)$$

The maximum velocity,  $V_{max}$ , of the enzyme-catalyzed reaction occurs when all of the enzyme is bound to substrate—that is, when  $[E \cdot S]$  is equal to  $[E]_0$ . And so the value of  $V_{max}$  is given by:

$$V_{max} = k_2 [E]_0 \quad (16.16)$$

Substituting this into Equation 16.15 gives:

$$v_0 = \frac{V_{max}}{\left(1 + \frac{K_M}{[S]}\right)} \quad (16.17)$$

Equation 16.17 is called the **Michaelis-Menten equation** and it predicts precisely the behavior shown in Figure 16.3 and Figure 16.6.

Note that when  $[S] = K_M$ , the velocity will be half of  $V_{max}$ , which is the functional definition of  $K_M$  given originally in Section 16.1. The value of the Michaelis constant can be determined experimentally by using data such as those in Figure 16.6 and equating  $K_M$  to the substrate concentration at which the velocity is half-maximal. The determination of the value of  $K_M$  in this way is only possible if the rate of the reaction can in fact be measured at a sufficiently high substrate concentration that the plateau value is observed. This may not be possible if the substrate is not sufficiently soluble, and alternate methods for determining the value of  $K_M$  are discussed below in Section 16.8.

### Michaelis-Menten equation

For the simplest enzyme-catalyzed reaction, shown in Equation 16.3, the initial rate of the reaction under steady-state conditions is given by:

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

$V_{max}$  is the maximum rate of the reaction and  $K_M$  is the Michaelis constant, which corresponds to the substrate concentration at which the rate is half-maximal.

### 16.3 The value of the Michaelis constant, $K_M$ , is related to how much enzyme has substrate bound

The value of the Michaelis constant ( $K_M$ ) determines how much of the enzyme is bound to the substrate, as can be seen from the expression for the concentration of the enzyme-substrate complex,  $[E \cdot S]_{ss}$ , given by Equation 16.14.  $[E \cdot S]_{ss}$  can be expressed as the product of the fractional occupancy of the enzyme,  $f$ , multiplied by the total enzyme concentration,  $[E]_0$ :

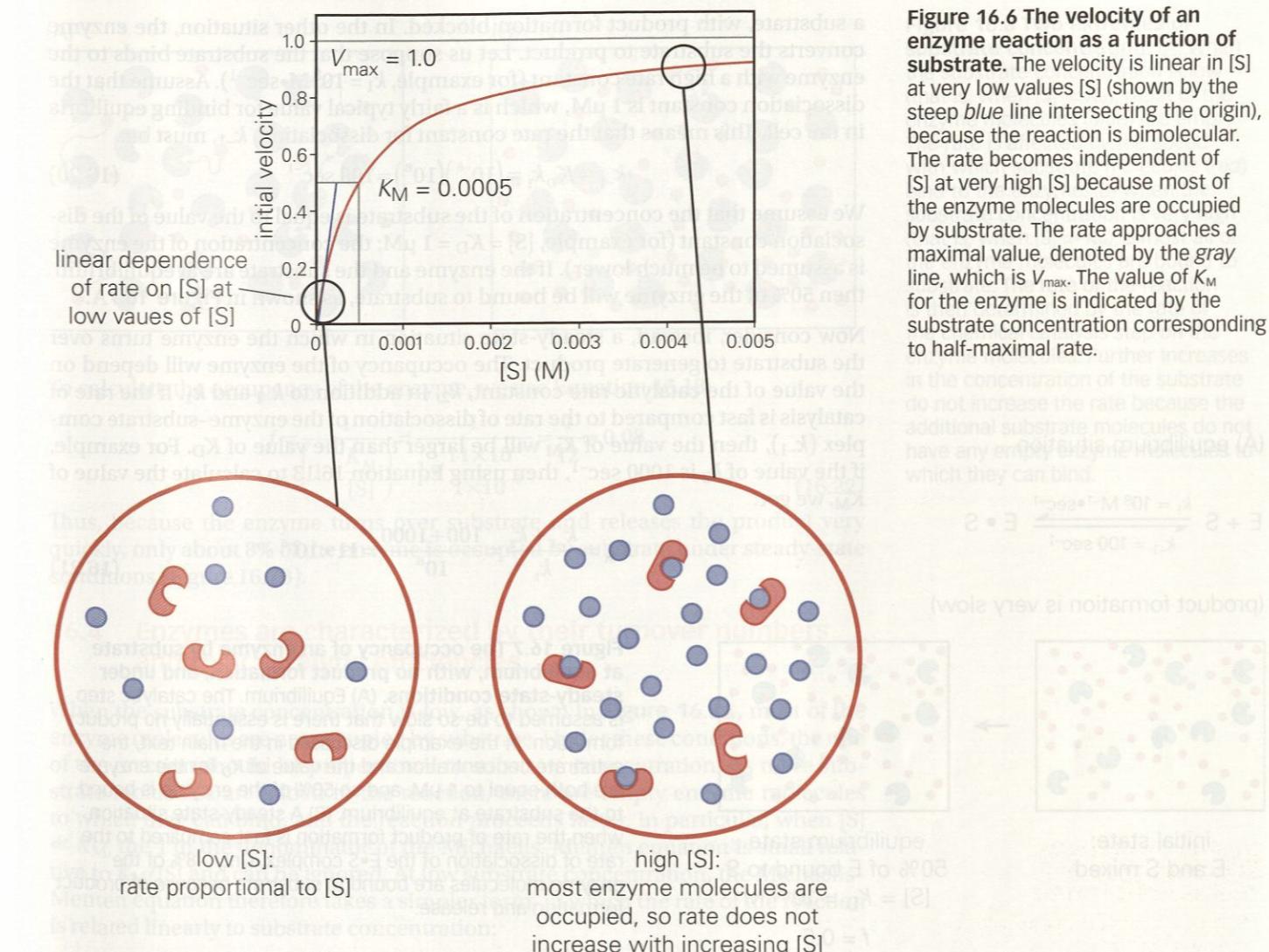
$$[E \cdot S]_{ss} = f [E]_0 \quad (16.18)$$

where

$$f = \frac{1}{\left(1 + \frac{K_M}{[S]}\right)}$$

Based on the discussion in Chapter 12 (see Equation 12.11), we know that if the enzyme and the substrate are in equilibrium, then the fraction of enzyme bound to ligand,  $f$ , is given by:

$$f = \frac{[S]}{K_D + [S]} = \frac{1}{\left(1 + \frac{K_D}{[S]}\right)} \quad (16.19)$$



It is apparent, from a comparison of Equations 16.18 and 16.19, that the Michaelis constant,  $K_M$ , plays a role analogous to that of the dissociation constant,  $K_D$ , in determining how much of the enzyme is bound to the substrate. Note, however, that  $K_M$  and  $K_D$  are fundamentally different. Equation 16.18 applies to the steady-state (nonequilibrium) situation, and the Michaelis constant is determined by a set of rate constants, including the rate constant for product formation (see Equation 16.13). Equation 16.19 applies only at equilibrium and the dissociation constant for the formation of the enzyme-substrate complex does not depend on the product at all, and is simply given by the ratio of rate constants for the dissociation of the enzyme-substrate complex and for its formation ( $K_D = \frac{k_{-1}}{k_1}$ , as explained in Section 15.18).

Looking at the reaction scheme in Equation 16.3, if  $k_2 \ll k_{-1}$  (that is, if the chemical catalysis step is slow compared to dissociation of the substrate), then the value of  $K_M$  approaches the value of the dissociation constant for the enzyme-substrate complex (that is,  $K_M \approx k_{-1}/k_1$ ). When  $k_2$  is comparable to or larger than  $k_{-1}$ , then the occupancy of the active site decreases (as though the dissociation constant was higher, corresponding to apparently weaker binding) because substrate is converted to product and is released frequently, emptying the active site.

To make these ideas more concrete, let us look at an example in which we compare two situations. In one, there is a binding equilibrium between an enzyme and

a substrate, with product formation blocked. In the other situation, the enzyme converts the substrate to product. Let us suppose that the substrate binds to the enzyme with a high rate constant (for example,  $k_1 = 10^8 \text{ M}^{-1}\text{sec}^{-1}$ ). Assume that the dissociation constant is  $1 \mu\text{M}$ , which is a fairly typical value for binding equilibria in the cell. This means that the rate constant for dissociation,  $k_{-1}$ , must be:

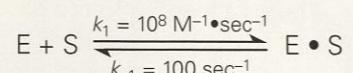
$$k_{-1} = K_D k_1 = (10^{-6})(10^8) = 100 \text{ sec}^{-1} \quad (16.20)$$

We assume that the concentration of the substrate is equal to the value of the dissociation constant (for example,  $[S] = K_D = 1 \mu\text{M}$ ; the concentration of the enzyme is assumed to be much lower). If the enzyme and the substrate are at equilibrium, then 50% of the enzyme will be bound to substrate, as shown in Figure 16.7A.

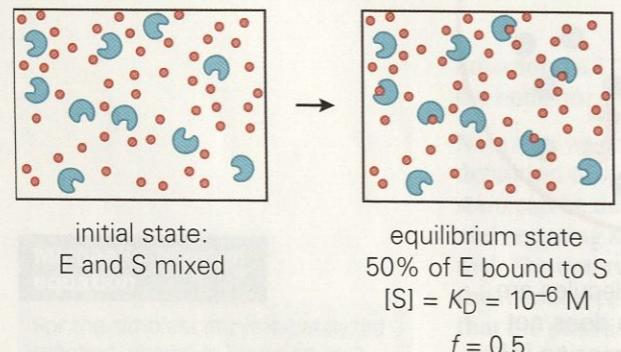
Now consider, instead, a steady-state situation in which the enzyme turns over the substrate to generate product. The occupancy of the enzyme will depend on the value of the catalytic rate constant,  $k_2$ , in addition to  $k_{-1}$  and  $k_1$ . If the rate of catalysis is fast compared to the rate of dissociation of the enzyme-substrate complex ( $k_{-1}$ ), then the value of  $K_M$  will be larger than the value of  $K_D$ . For example, if the value of  $k_2$  is  $1000 \text{ sec}^{-1}$ , then using Equation 16.13 to calculate the value of  $K_M$ , we get:

$$K_M = \frac{k_{-1} + k_2}{k_1} = \frac{100 + 1000}{10^8} = 11 \times 10^{-6} \quad (16.21)$$

(A) equilibrium situation



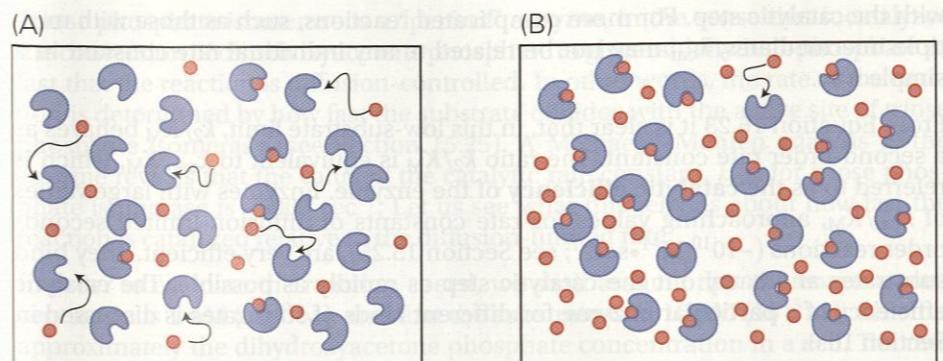
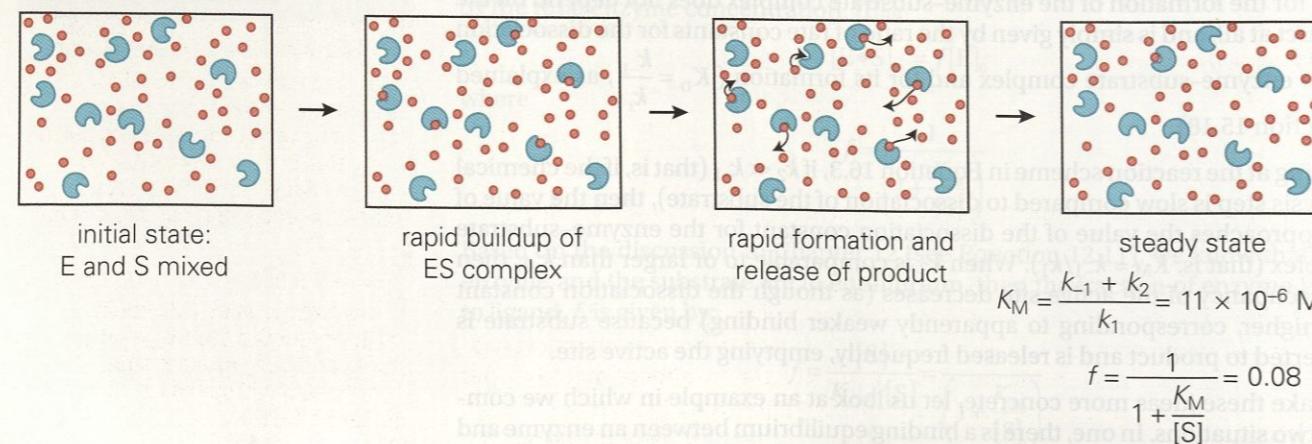
(product formation is very slow)



(B) steady-state situation



(product formation and release is fast)



To calculate the occupancy of the enzyme, we use Equation 16.18:

$$f = \frac{1}{1 + \frac{K_M}{[S]}} = \frac{1}{1 + \frac{11 \times 10^{-6}}{1 \times 10^{-6}}} = \frac{1}{12} \approx 0.08 \quad (16.22)$$

Thus, because the enzyme turns over substrate and releases the product very quickly, only about 8% of the enzyme is occupied by substrate under steady-state conditions (Figure 16.7B).

#### 16.4 Enzymes are characterized by their turnover numbers and their catalytic efficiencies

When the substrate concentration is low, as shown in Figure 16.8A, most of the enzyme molecules are not occupied by substrate. Under these conditions, the rate of the reaction should be proportional to substrate concentration. As more substrate molecules are added to the reaction, there are empty enzyme molecules to which they can bind, and the reaction proceeds faster. In particular, when  $[S] \ll K_M$ , the 1 in the denominator of the Michaelis-Menten equation is small relative to  $K_M/[S]$  and can be ignored. At low substrate concentration, the Michaelis-Menten equation therefore takes a simpler form, in which the rate of the reaction is related linearly to substrate concentration:

$$v_0 = \frac{V_{\max}}{1 + \frac{K_M}{[S]}} \approx \frac{V_{\max}}{\frac{K_M}{[S]}} = \left( \frac{k_2}{K_M} \right) [E]_0 [S] \quad (16.23)$$

In Equation 16.23, we have used Equation 16.16 to replace  $V_{\max}$  with  $k_2 [E]_0$ . Under such conditions (that is, when  $[S] \ll K_M$ ), most enzyme molecules are not occupied at any given time, so  $[E] \approx [E]_0$ .

At high substrate concentration, specifically when  $[S] \gg K_M$ , the enzyme becomes saturated with substrate (Figure 16.8B). Under such conditions,  $K_M/[S] \ll 1$ , so  $K_M/[S]$  can be ignored in the Michaelis-Menten equation. Thus, as the substrate concentration is increased, the rate equation becomes independent of substrate concentration asymptotically:

$$v_0 = \frac{V_{\max}}{1 + \frac{K_M}{[S]}} \approx \frac{V_{\max}}{1} = k_2 [E]_0 \quad (16.24)$$

This asymptotic plateau in the value of the reaction rate is exactly the behavior observed experimentally, as shown in Figure 16.6.

The apparent rate constant for an enzyme reaction at high substrate concentration (that is, when the reaction is proceeding with maximal rate) is often referred to as the **catalytic rate constant**,  $k_{\text{cat}}$ . The value of  $k_{\text{cat}}$  is also referred to as the **turnover number** for the enzyme, because it is the maximum number of reactions per second per mole of the enzyme. For a reaction obeying simple Michaelis-Menten kinetics, the value of  $k_{\text{cat}}$  is the same as the value of  $k_2$ , the rate constant associated

**Figure 16.8 Two extremes of substrate concentration.** (A) When the substrate concentration is low (that is, when  $[S] \ll K_M$ ), most of the enzyme molecules (blue) are empty. The rate is affected by the speed with which substrate molecules (red) bind to the enzyme. (B) When the substrate concentration is very high (that is, when  $[S] \gg K_M$ ), almost all of the enzyme molecules are bound to substrate. The rate of the reaction is then determined by the rate of the chemical catalysis step on the enzyme molecules. Further increases in the concentration of the substrate do not increase the rate because the additional substrate molecules do not have any empty enzyme molecules to which they can bind.

#### Catalytic rate constant, $k_{\text{cat}}$

The apparent rate constant for an enzyme-catalyzed reaction operating at maximum rate is called the catalytic rate constant,  $k_{\text{cat}}$ . The maximum rate,  $V_{\max}$ , occurs when the enzyme is saturated with substrate:

$$V_{\max} = k_{\text{cat}} [E]_0$$

If the enzyme obeys Michaelis-Menten kinetics then  $k_{\text{cat}}$  is the same as  $k_2$ , the rate constant for the catalytic step (see Equation 16.16).