sufficiently high that essentially all the free enzyme has been converted to the ES form. After the ES complex breaks down to yield the product P, the enzyme is free to catalyze the reaction of another molecule of substrate (and will do so rapidly under saturating conditions). The saturation effect is a distinguishing characteristic of enzymatic catalysts and is responsible for the plateau observed in Figure 6-11, and the pattern seen in the figure is sometimes referred to as saturation kinetics.

When the enzyme is first mixed with a large excess of substrate, there is an initial transient period, the **pre–steady state**, during which the concentration of ES builds up. For most enzymatic reactions, this period is very brief. It is often too short to be easily observed, lasting just microseconds, and is not evident in Figure 6-10. (We return to the pre–steady state later in this section.) The reaction quickly achieves a **steady state** in which [ES] (and the concentrations of any other intermediates) remains approximately constant over time. The concept of a steady state, introduced by G. E. Briggs and Haldane in 1925, is an approximation based on a simple reality. As noted earlier, enzymes are powerful catalysts that are typically present at concentrations orders of magnitude lower than the concentration of substrate. Once the transient phase or pre–steady state has passed (often after only one enzymatic turnover; that is, conversion of one molecule of substrate to one molecule of product on each molecule of enzyme), P is generated at the same rate that S is consumed only if the concentration of the intermediate ES remains steady. The measured V_0 generally reflects the steady state, even though V_0 is limited to the early part of the reaction, and analysis of these initial rates is referred to as **steady-state kinetics**.

The Relationship between Substrate Concentration and Reaction Rate Can Be Expressed Quantitatively

The curve expressing the relationship between [S] and V_0 (Fig. 6-11) has the same general shape for most enzymes (it approaches a rectangular hyperbola), which can be expressed algebraically by the Michaelis-Menten equation. Michaelis and Menten derived this equation starting from their basic hypothesis that the rate-limiting step in enzymatic reactions is the breakdown of the ES complex to product and free enzyme. The equation is

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \qquad (6\text{-}9)$$

All these terms—[S], V_0, V_{max}, and a constant, K_m, called the Michaelis constant—are readily measured experimentally.

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 → 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 [E] 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 to reactants and products, respectively), according to the expressions

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

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

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

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

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

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

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

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

We then solve this equation for [ES]:

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

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

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

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

$$[ES] = \frac{[E_t][S]}{K_m + [S]} \qquad (6\text{-}19)$$

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

$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]} \qquad (6\text{-}20)$$

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

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

This is the **Michaelis-Menten equation**, the **rate equation** for a one-substrate enzyme-catalyzed reaction. It is a statement of the quantitative relationship between the initial velocity V_0, the maximum velocity V_{max}, and the initial substrate concentration [S], all related through the Michaelis constant K_m. Note that K_m has units of molar concentration. Does the equation fit experimental observations? Yes; we can confirm this by considering the limiting situations where [S] is very high or very low, as shown in **Figure 6-12**.

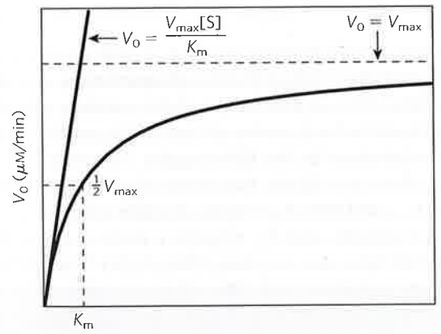

FIGURE 6-12 Dependence of initial velocity on substrate concentration. This graph shows the kinetic parameters that define the limits of the curve at high and low [S]. At low [S], $K_m \gg [S]$, and the [S] term in the denominator of the Michaelis-Menten equation (Eqn 6-9) becomes insignificant. The 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 $[S] \gg K_m$, 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].

An important numerical relationship emerges from the Michaelis-Menten equation in the special case when V_0 is exactly one-half V_{max} (Fig. 6-12). Then

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]} \qquad (6\text{-}21)$$

On dividing by V_{max}, we obtain

$$\frac{1}{2} = \frac{[S]}{K_m + [S]} \qquad (6\text{-}22)$$

Solving for K_m, we get $K_m + [S] = 2[S]$, or

$$K_m = [S], \quad \text{when} \quad V_0 = \tfrac{1}{2}V_{max} \qquad (6\text{-}23)$$

This is a very useful, practical definition of K_m: K_m is equivalent to the substrate concentration at which V_0 is one-half V_{max}.

The Michaelis-Menten equation (Eqn 6-9) can be algebraically transformed into versions that are useful in the practical determination of K_m and V_{max} (Box 6-1) and, as we describe later, in the analysis of inhibitor action (see Box 6-2).

Kinetic Parameters Are Used to Compare Enzyme Activities

It is important to distinguish between the Michaelis-Menten equation and the specific kinetic mechanism on which it was originally based. The equation describes the kinetic behavior of a great many enzymes, and all enzymes that exhibit a hyperbolic dependence of V_0 on [S] are said to follow **Michaelis-Menten kinetics**. The practical rule that $K_m = [S]$ when $V_0 = \frac{1}{2}V_{max}$ (Eqn 6-23) holds for all enzymes that follow Michaelis-Menten kinetics. (The most important exceptions to Michaelis-Menten kinetics are the regulatory enzymes, discussed in Section 6.5.) However, the Michaelis-Menten equation does not depend on the relatively simple two-step reaction mechanism proposed by Michaelis and Menten (Eqn 6-10). Many enzymes that follow Michaelis-Menten kinetics have quite different reaction mechanisms, and enzymes that catalyze reactions with six or eight identifiable steps often exhibit the same steady-state kinetic behavior. Even though Equation 6-23 holds true for many enzymes, both the magnitude and the real meaning of V_{max} and K_m can differ from one enzyme to the next. This is an important limitation of the steady-state approach to enzyme kinetics. The parameters V_{max} and K_m can be obtained experimentally for any given enzyme, but by themselves they provide little information about the number, rates, or chemical nature of discrete steps in the reaction. Steady-state kinetics nevertheless is the standard language through which biochemists compare and characterize the catalytic efficiencies of enzymes.

Interpreting V_{max} and K_m Figure 6-12 shows a simple graphical method for obtaining an approximate value for K_m. A more convenient procedure, using a **double-reciprocal plot**, is presented in Box 6-1. The K_m can