WHY ANALYZE THE KINETICS OF ENZYMES?

Enzymes are the most selective and powerful catalysts known. An understanding of their detailed mechanisms provides a critical tool for the discovery of new drugs, for the large-scale industrial synthesis of useful chemicals, and for appreciating the chemistry of cells and organisms. A detailed study of the rates of the chemical reactions that are catalyzed by a purified enzyme—more specifically how these rates change with changes in conditions such as the concentrations of substrates, products, inhibitors, and regulatory ligands—allows biochemists to figure out exactly how each enzyme works. For example, this is the way that the ATP-producing reactions of glycolysis, shown previously in Figure 2–72, were deciphered—allowing us to appreciate the rationale for this critical enzymatic pathway.

In this Panel, we introduce the important field of enzyme kinetics, which has been indispensable for deriving much of the detailed knowledge that we now have about cell chemistry.

STEADY-STATE ENZYME KINETICS

Many enzymes have only one substrate, which they bind and then process to produce products according to the scheme outlined in Figure 3–50A. In this case, the reaction is written as

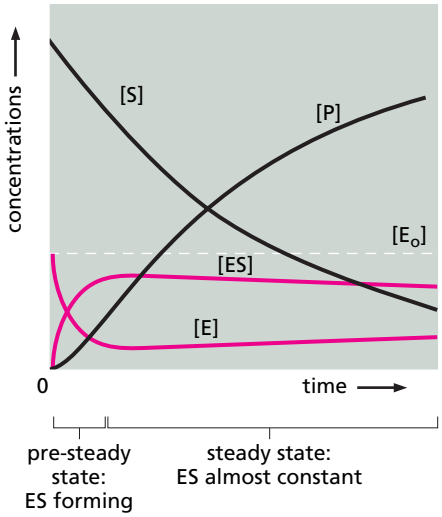
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

Here we have assumed that the reverse reaction, in which E + P recombine to form EP and then ES, occurs so rarely that we can ignore it. In this case, EP need not be represented, and we can express the rate of the reaction — known as its velocity, V, as

$$V = k_{cat}\,[ES]$$

where [ES] is the concentration of the enzyme–substrate complex, and k_{cat} is the turnover number, a rate constant that has a value equal to the number of substrate molecules processed per enzyme molecule each second.

But how does the value of [ES] relate to the concentrations that we know directly, which are the total concentration of the enzyme, $[E_o]$, and the concentration of the substrate, [S]? When enzyme and substrate are first mixed, the concentration [ES] will rise rapidly from zero to a so-called steady-state level, as illustrated below.



At this steady state, [ES] is nearly constant, so that

$$\text{rate of ES breakdown } k_{-1}\,[ES] + k_{cat}\,[ES] = \text{rate of ES formation } k_1\,[E][S]$$

or, since the concentration of the free enzyme, [E], is equal to $[E_o] - [ES]$,

$$[ES] = \left(\frac{k_1}{k_{-1} + k_{cat}}\right)[E][S] = \left(\frac{k_1}{k_{-1} + k_{cat}}\right)([E_o] - [ES])[S]$$

Rearranging, and defining the constant K_m as

$$\frac{k_{-1} + k_{cat}}{k_1}$$

we get

$$[ES] = \frac{[E_o][S]}{K_m + [S]}$$

or, remembering that $V = k_{cat}\,[ES]$, we obtain the famous Michaelis–Menten equation

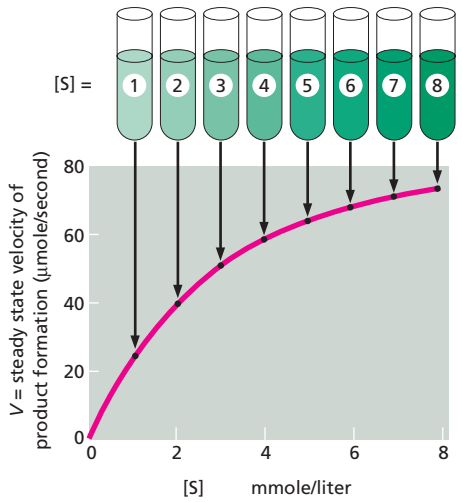
$$V = \frac{k_{cat}\,[E_o][S]}{K_m + [S]}$$

As [S] is increased to higher and higher levels, essentially all of the enzyme will be bound to substrate at steady state; at this point, a maximum rate of reaction, V_{max}, will be reached where $V = V_{max} = k_{cat}\,[E_o]$. Thus, it is convenient to rewrite the Michaelis–Menten equation as

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

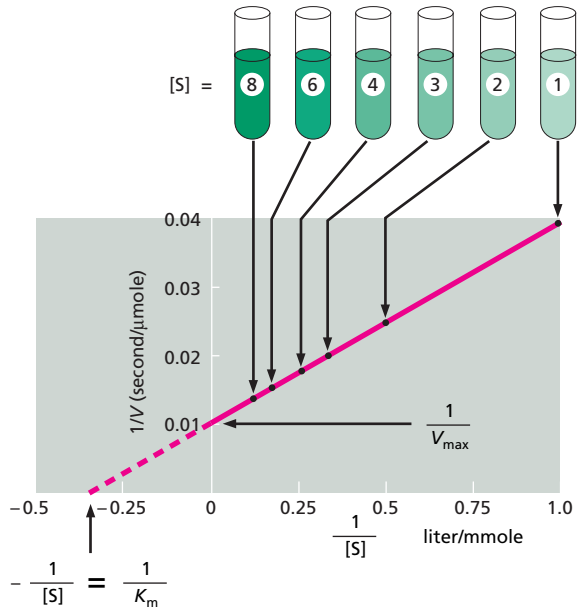
THE DOUBLE-RECIPROCAL PLOT

A typical plot of V versus [S] for an enzyme that follows Michaelis–Menten kinetics is shown below. From this plot, neither the value of V_{max} nor of K_m is immediately clear.



To obtain V_{max} and K_m from such data, a double-reciprocal plot is often used, in which the Michaelis–Menten equation has merely been rearranged, so that $1/V$ can be plotted versus $1/[S]$.

$$1/V = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + 1/V_{max}$$



THE SIGNIFICANCE OF K_m, k_{cat}, and k_{cat}/K_m

As described in the text, K_m is an approximate measure of substrate affinity for the enzyme: it is numerically equal to the concentration of [S] at $V = 0.5\ V_{max}$. In general, a lower value of K_m means tighter substrate binding. In fact, for those cases where k_{cat} is much smaller than k_{-1}, the K_m will be equal to K_d, the dissociation constant for substrate binding to the enzyme ($K_d = 1/K_a$).

We have seen that k_{cat} is the turnover number for the enzyme. At very low substrate concentrations, where $[S] << K_m$, most of the enzyme is free. Thus we can think of $[E] = [E_o]$, so that the Michaelis–Menten equation becomes $V = k_{cat}/K_m\ [E][S]$. Thus, the ratio k_{cat}/K_m is equivalent to the rate constant for the reaction between free enzyme and free substrate.

A comparison of k_{cat}/K_m for the same enzyme with different substrates, or for two enzymes with their different substrates, is widely used as a measure of enzyme effectiveness.

For simplicity, in this Panel we have discussed enzymes that have only one substrate, such as the lysozyme enzyme described in the text (see p. 164). Most enzymes have two substrates, one of which is often an active carrier molecule—such as NADH or ATP.

A similar, but more complex, analysis is used to determine the kinetics of such enzymes—allowing the order of substrate binding and the presence of covalent intermediates along the pathway to be revealed.

SOME ENZYMES ARE DIFFUSION LIMITED

The values of k_{cat}, K_m, and k_{cat}/K_m for some selected enzymes are given below:

enzyme	substrate	k_{cat} (sec^{-1})	K_m (M)	k_{at}/K_m ($sec^{-1}M^{-1}$)
acetylcholinesterase	acetylcholine	$1.4x10^4$	$9x10^{-5}$	$1.6x10^8$
catalase	H_2O_2	$4x10^7$	1	$4x10^7$
fumarase	fumarate	$8x10^2$	$5x10^{-6}$	$1.6x10^8$

Because an enzyme and its substrate must collide before they can react, k_{cat}/K_m has a maximum possible value that is limited by collision rates. If every collision forms an enzyme–substrate complex, one can calculate from diffusion theory that k_{cat}/K_m will be between 10^8 and 10^9 $sec^{-1}M^{-1}$, in the case where all subsequent steps proceed immediately. Thus, it is claimed that enzymes like acetylcholinesterase and fumarase are "perfect enzymes," each enzyme having evolved to the point where nearly every collision with its substrate converts the substrate to a product.