3. Enzymes

- Enzymes
  - Proteins that act as catalysts
- Ribozyme
  - RNA molecules that have catalytic properties
- Some enzymes require a nonprotein group of their activity.
  - Cofactors --- metal ions, Mg, Zn, Mn, Fe
  - Coenzymes --- NAD, FAD, CoA, or some vitamines

  - Apoenzyme
    - Protein part of enzyme
  - Holoenzyme
    - An enzyme containing a nonprotein group
    - Holoenzyme = apoenzyme + cofactor
- Isozymes
  - Enzymes having different molecular forms but catalyzing the same reaction

3.2. How enzymes work

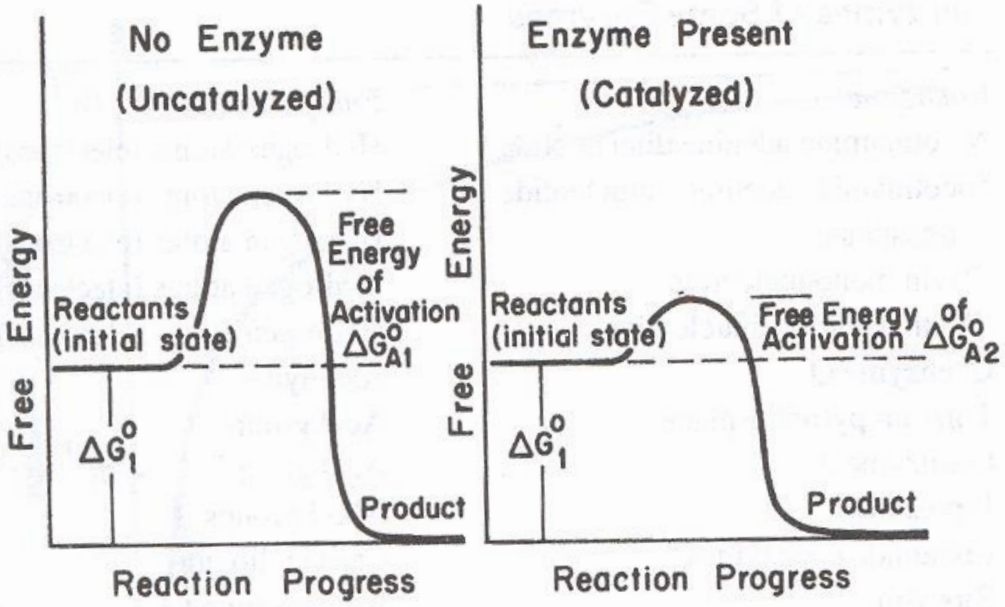


Figure 3.1. Activation energies of enzymatically catalyzed and uncatalyzed reactions. Note that $|\Delta G^{\circ}_{A_2}| < |\Delta G^{\circ}_{A_1}|$.

Lock-and-key Model

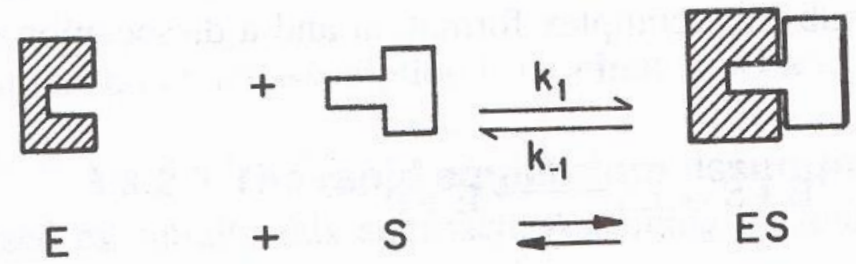
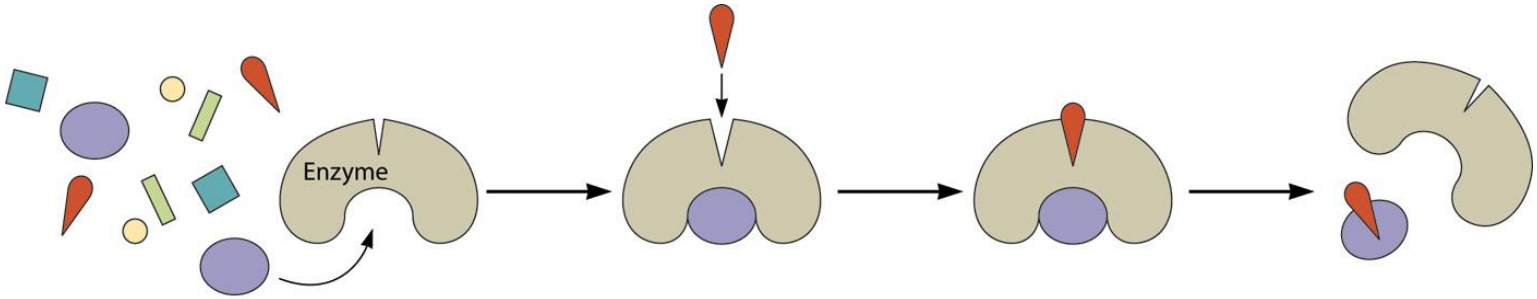


Figure 3.2. Schematic of the lock-and-key model of enzyme catalysis.

In multisubstrate enzyme-catalyzed reaction

(1) proximity effect: holding the substrates close to each other
(2) orientation effect: holding the substrate at an advantageous angle



3.3. Enzyme Kinetics

- **Mechanism**

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \qquad (3.1)$$

- **Rate of Product Formation**

$$v = \frac{d[\mathrm{P}]}{dt} = k_2[\mathrm{ES}]$$

(k_2 is also denoted as k_{cat}.)

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}]$$

$$[\mathrm{E}] = [\mathrm{E}_0] - [\mathrm{ES}]$$

(i) Rapid equilibrium assumption

The equilibrium constant is

$$K'_m = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} \tag{3.5}$$

Since $[E] = [E_0] - [ES]$ if enzyme is conserved, then

$$[ES] = \frac{[E_0][S]}{(k_{-1}/k_1) + [S]} \tag{3.6}$$

$$[ES] = \frac{[E_0][S]}{K'_m + [S]} \tag{3.7}$$

where $K'_m = k_{-1}/k_1$, which is the dissociation constant of the ES complex. Substituting eq. 3.7 into eq. 3.2 yields

$$v = \frac{d[P]}{dt} = k_2 \frac{[E_0][S]}{K'_m + [S]} = \frac{V_m[S]}{K'_m + [S]} \tag{3.8}$$

where $V_m = k_2[E_0]$.

(ii) Quasi-steady-state assumption

By applying the quasi-steady-state assumption to eq. 3.3, we find

$$[ES] = \frac{k_1[E][S]}{k_{-1} + k_2} \tag{3.9}$$

Substituting the enzyme conservation eq. 3.4 in eq. 3.9 yields

$$[ES] = \frac{k_1([E_0] - [ES])[S]}{k_{-1} + k_2} \tag{3.10}$$

Solving eq. 3.10 for [ES],

$$[ES] = \frac{[E_0][S]}{\frac{k_{-1} + k_2}{k_1} + [S]} \tag{3.11}$$

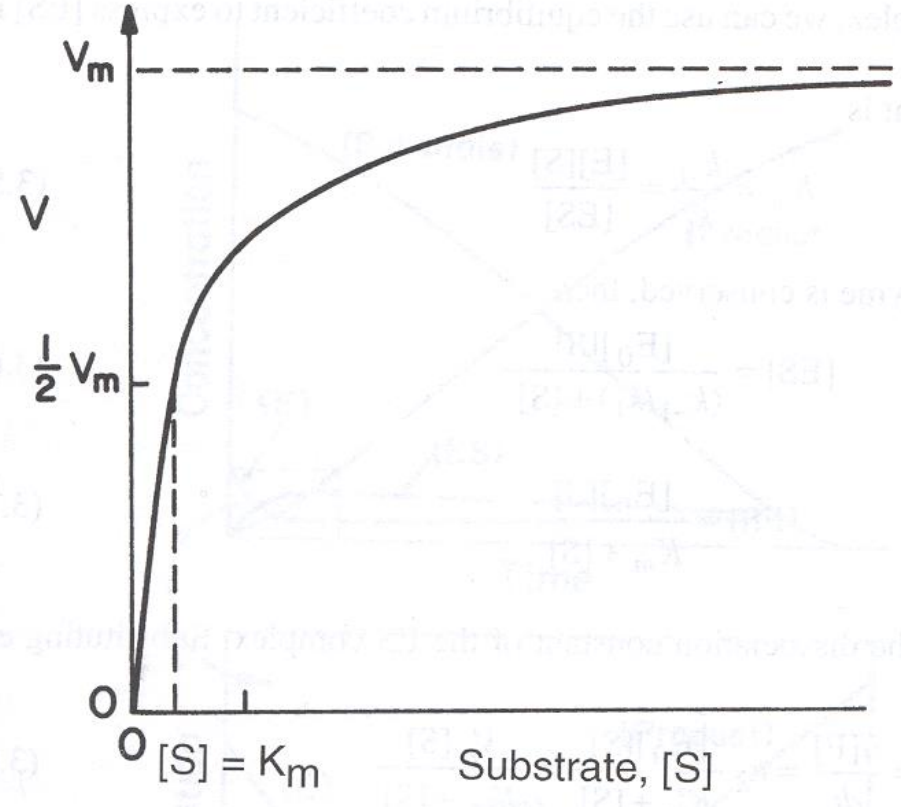
Substituting eq. 3.11 into eq. 3.2 yields

$$v = \frac{d[P]}{dt} = \frac{k_2[E_0][S]}{\frac{k_{-1} + k_2}{k_1} + [S]} \tag{3.12a}$$

$$v = \frac{V_m[S]}{K_m + [S]} \tag{3.12b}$$

where K_m is $(k_{-1} + k_2)/k_1$ and V_m is $k_2[E_0]$. Under most circumstances (simple experi-

Michaelis-Menten Kinetics



$$v = \frac{V_m[S]}{K_m + [S]}$$

Figure 3.3. Effect of substrate concentration on the rate of an enzyme-catalyzed reaction.

Profile of [ES]

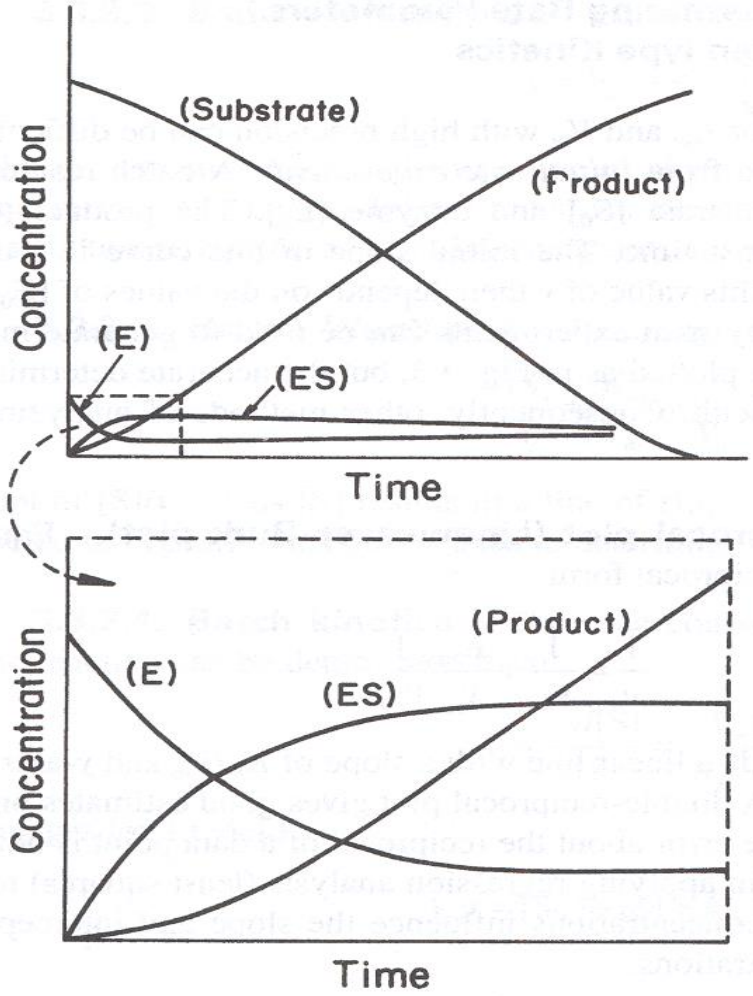


Figure 3.4. Time course of the formation of an enzyme/substrate complex and initiation of the steady state, as derived from computer solutions of data obtained in an actual experiment on a typical enzyme. The portion in the dashed box in the top graph is shown in magnified form on the lower graph. (With permission, adapted from A. Lehninger, *Biochemistry*, 2d ed., Worth Publishers, New York, 1975, p. 191.)

Determination of K_m and V_m

3.3.3.1. Double-reciprocal plot (Lineweaver–Burk plot). Equation 3.12b can be linearized in double-reciprocal form:

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{[S]} \qquad (3.13)$$

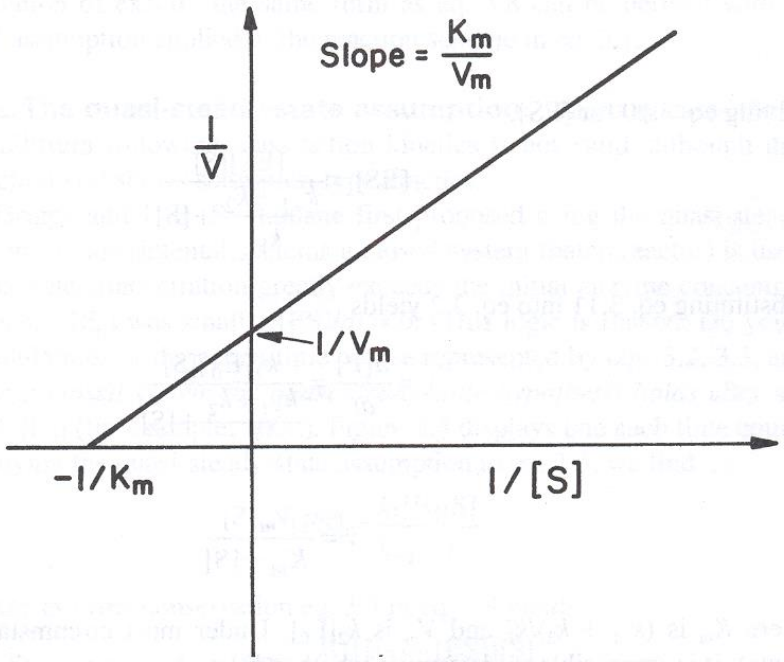


Figure 3.5. Double-reciprocal (Lineweaver–Burk) plot.

The error about the reciprocal of a data is not symmetric.

Data points at low substrate concentrations influence the slope and intercept more than those at high substrate concentrations.

3.3.3.2. Eadie–Hofstee plot.

Equation 3.12b can be rearranged as

$$v = V_m - K_m \frac{v}{[S]} \tag{3.14}$$

A plot of v versus $v/[S]$ results in a line of slope $-K_m$ and y-axis intercept of V_m, as depicted in Fig. 3.6. Eadie–Hofstee plots can be subject to large errors since both coordinates contain v, but there is less bias on points at low [S].

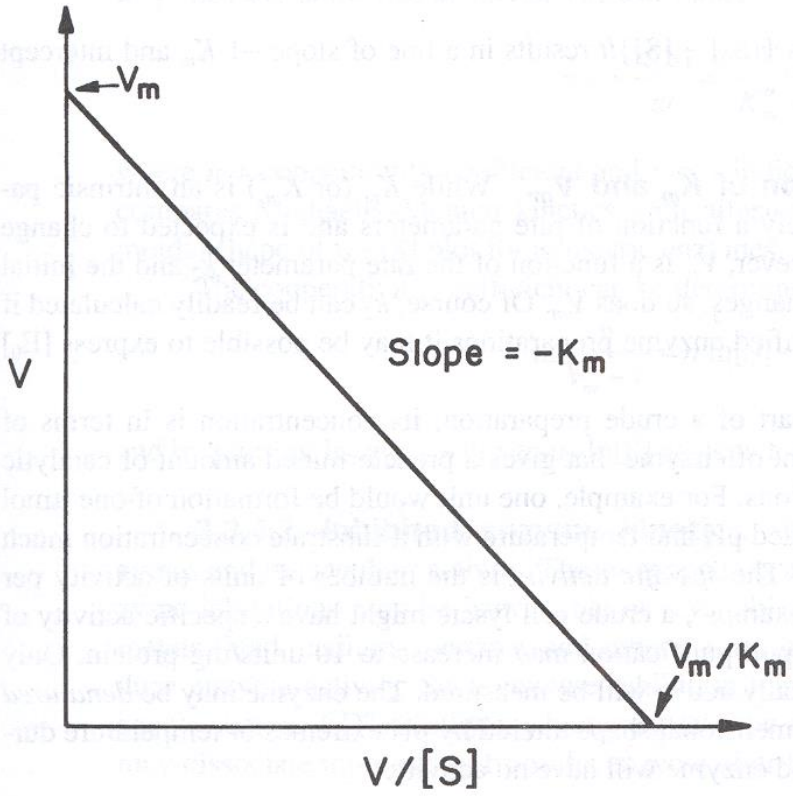


Figure 3.6. Eadie–Hofstee plot.

3.3.3.3. Hanes–Woolf plot.

Rearrangement of eq. 3.12b yields

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{1}{V_m}[S] \tag{3.15}$$

A plot of [S]/v versus [S] results in a line of slope $1/V_m$ and y-axis intercept of K_m/V_m, as depicted in Fig. 3.7. This plot is used to determine V_m more accurately.

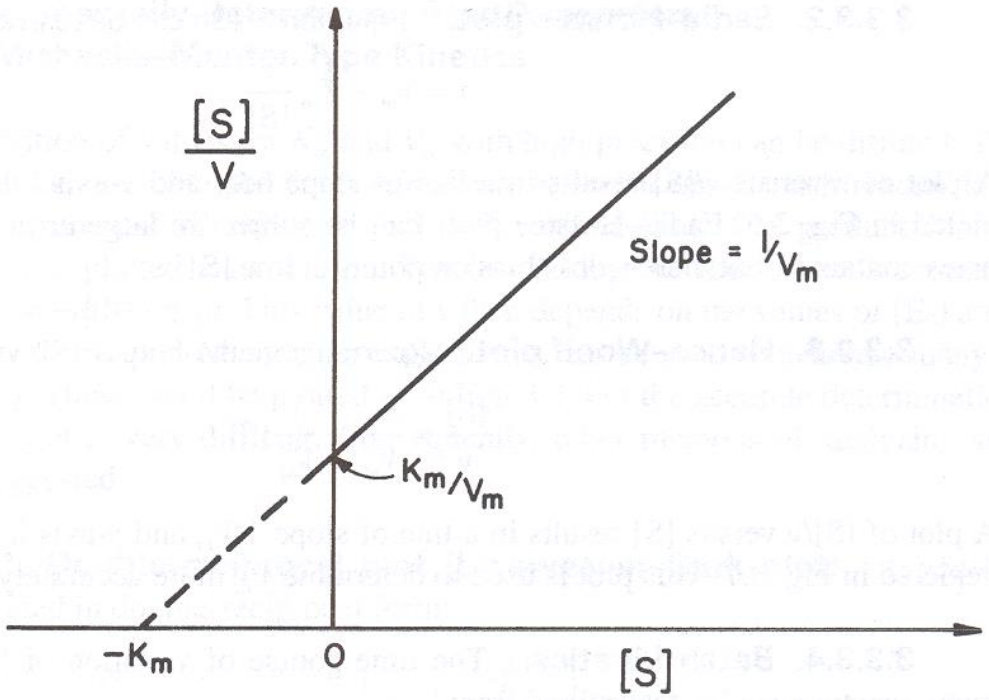


Figure 3.7. Hanes–Woolf plot.

3.3.3.4. Batch kinetics. The time course of variation of [S] in a batch enzymatic reaction can be determined from

$$v = -\frac{d[S]}{dt} = \frac{V_m[S]}{K_m + [S]} \tag{3.12b}$$

by integration to yield

$$V_m t = [S_0] - [S] + K_m \ln \frac{[S_0]}{[S]} \tag{3.16}$$

or

$$V_m - \frac{[S_0] - [S]}{t} = \frac{K_m}{t} \ln \frac{[S_0]}{[S]} \tag{3.17}$$

A plot of $1/t \ln[S_0]/[S]$ versus $\{[S_0] - [S]\}/t$ results in a line of slope $-1/K_m$ and intercept of V_m/K_m.