Enzyme Kinetics

The overall reaction is: \( S \xrightleftharpoons{E} P \)

where
- \( S \equiv \) substrate
- \( P \equiv \) product
- \( E \equiv \) enzyme

A typical experimental observation is hyperbolic behavior:

![Figure 6-1](image)

Michaelis-Menten Model

(a) Enzyme and substrate form an enzyme-substrate complex (ES).
(b) \( ES \) is converted to enzyme-product (EP).
(c) \( EP \) decays rapidly to product and regenerated enzyme.

Hyperbolic or saturation behavior arises from saturation of enzyme by substrate.
**Michaelis-Menten Mechanism**

step 1: \( E + S \xrightleftharpoons[k_{-1}]{k_1} ES \)

step 2: \( ES \xrightleftharpoons[k_{-2}]{k_2} EP \)

step 3: \( EP \xrightleftharpoons[k_{-3}]{k_3} E + P \)

where \( \frac{k_1}{k_{-1}} \equiv \) equilibrium constant for formation of \( ES \)

\( k_2 \equiv \) rate constant for catalytic conversion

\( k_3, k_{-3} \equiv \) rate constants for dissociation and association

of enzyme - product complexes

**Initial Rates**

It is customary in enzyme kinetics to observe initial velocities. Under these conditions, the back conversion of \( EP \) to \( ES \) can be ignored. That is, the reaction is effectively irreversible in the initial reaction stages before the concentration of product has had time to accumulate. We assert this mathematically as follows:

\[ k_2 [ES] \gg k_{-2} [EP] \quad \text{and} \quad k_3 [EP] \gg k_{-3} [E][P] \]

**Reaction Velocity**

\[
\text{rate} = \text{reaction velocity} = -\frac{d[S]}{dt} = +\frac{d[P]}{dt} \approx k_2 [ES]
\]

under initial rate conditions.

Now

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

We need to solve for \([ES]\).
**Assumption**

Invoke steady state approximation for $[ES]$; that is, assume that $[ES]$ is formed at the same rate as it is converted to $P$ so that $[ES]$ is not accumulating or being depleted or

$$\frac{d[ES]}{dt} \approx 0$$

Figure 6-2

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES]_{ss} - k_2[ES]_{ss} \approx 0$$

**steady state approximation**

or

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

Now

$$[E] = E_0 - [ES]_{ss} - [EP] \approx E_0 - [ES]_{ss}$$

Substituting this result into the expression for $[ES]_{ss}$ above, we obtain

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

where $E_0 \equiv$ stoichiometric enzyme concentration
So \[ \text{rate} = \text{velocity} = v = k_2 [ES]_{ss} \]

\[ = \frac{k_2 k_1 E_0[S]}{k_{-1} + k_2 + k_1 [S]} = \frac{k_2 E_0[S]}{k_{-1} + k_2 + [S]} \]

\[ v = \frac{k_2 E_0[S]}{K_M + [S]} \] \( \Leftarrow \) Michaelis Menten equation

where \( K_M \equiv \frac{k_{-1} + k_2}{k_1} \equiv \text{Michaelis constant} \)

**Limiting Conditions**

\[ [S] \gg K_M \quad \text{velocity} = k_2 E_0 = V_{\text{max}} \]

\[ [S] \ll K_M \quad \text{velocity} = \frac{k_2 E_0[S]}{K_M} \quad (\text{first order in } [S]) \]

\[ [S] = K_M \quad \text{velocity} = \frac{1}{2} V_{\text{max}} \]

![Figure 6-3](image)
**Linear Plots**

For practical applications, the Michaelis-Menten equation is transformed into a linear equation.

\[
\frac{v}{V_{\text{max}}} = \frac{[S]}{K_M + [S]} \quad \text{Michaelis-Menten}
\]

1) **Lineweaver-Burk**

\[
\frac{V_{\text{max}}}{v} = \frac{K_M}{[S]} + 1
\]

or

\[
\frac{1}{v} = \frac{K_M}{V_{\text{max}} [S]} + \frac{1}{V_{\text{max}}}
\]

![Lineweaver-Burk double reciprocal plot](image)

**Figure 6-4**

2) **Eadie-Hofster**

\[
\frac{v}{V_{\text{max}}} = \frac{[S]}{K_M + [S]}
\]

\[
v ([S] + K_M) = V_{\text{max}} [S]
\]

\[
v [S] = V_{\text{max}} [S] - K_M v
\]

or

\[
\frac{v}{[S]} = \frac{V_{\text{max}} v - v}{K_M}
\]
\[
\frac{v}{[S]} = \frac{V_{max}}{K_M} - \frac{1}{K_M}
\]

Slope \(= -\frac{1}{K_M}\) (for normal \(K_M\)'s)

(a) Eadie plot (single reciprocal)

Figure 6-5

\[
\frac{v}{[S]} = \frac{V_{max}}{K_M}
\]

Slope \(= -\frac{1}{K_M}\) (for large \(K_M\)'s)

(b) Figure 6-6

\[
\frac{v}{[S]} = \frac{V_{max}}{K_M}
\]

Slope \(= -\frac{1}{K_M}\) (for very small \(K_M\)'s)

(c) Figure 6-7
(3) **Dixon**

\[
\frac{v}{V_{\text{max}}} = \frac{[S]}{K_M + [S]}
\]

\[
\frac{[S]}{v} = \frac{K_M}{V_{\text{max}}} + \frac{[S]}{V_{\text{max}}}
\]

![Dixon plot](image)

Figure 6-8

From one of these plots, we can obtain $K_M$ and $V_{\text{max}}$.

From $V_{\text{max}}$, we can obtain $k_2$ if $E_0$ is known.

From $K_M$ and $k_2$, we may infer $K_D$.

Recall

\[
\frac{[E][S]}{[ES]_{ss}} = \frac{k_{-1} + k_2}{k_1} = K_M \quad \text{and} \quad \frac{k_{-1}}{k_1} = K_D
\]
**Enzyme Inhibition**

Studies of the inhibition of enzyme-catalyzed reactions have contributed to our understanding of enzyme mechanisms and to the elucidation of the molecular basis for many biological mechanisms.

**Several Kinds of Inhibition**

(1) Competitive Inhibition — An inhibitor binds to the enzyme at the site normally occupied by the substrate molecule.

(2) Noncompetitive Inhibition — An inhibitor alters the activity of the enzyme without actually blocking the active site.

**Kinetics of Competitive Inhibition**

\[
E + S \xrightleftharpoons[k_1]{k_\text{-1}} ES \\
E + I \xrightleftharpoons[k_3]{k_\text{-3}} EI \\
ES \xrightarrow{k_2} E + P
\]

\[
K_I = \frac{[E][I]}{[EI]} = \frac{k_\text{-3}}{k_3}
\]

enzyme-inhibitor dissociation constant

As before,

\[
\text{velocity} = k_2 [ES]
\]

and

\[
[ES]_{ss} = \frac{k_{-1} + k_2}{k_1} = K_M
\]

But some of the enzyme is tied up with inhibitor and hence rendered inactive.

Therefore,

\[
[E] = E_0 - [ES] - [EI]
\]

and

\[
v = \frac{V_{\text{max}}[S]}{[S] + K_M\left(1 + \frac{[I]}{K_I}\right)}
\]
Note that the maximum rate $V_{\text{max}}$ is the same as in the absence of inhibition, but the rate at any given substrate concentration is reduced.

In Lineweaver-Burk form, the velocity is given by

$$\frac{1}{v} = \frac{K_M}{V_{\text{max}}} \left( 1 + \frac{[I]}{K_I} \right) \cdot \frac{1}{[S]} + \frac{1}{V_{\text{max}}}$$

Good diagnostic test for competitive inhibition!

\[ \text{Slope} = \frac{K_M}{V_{\text{max}}} \left[ 1 + \frac{[I]}{K_I} \right] \]

Figure 6-9
**Kinetics of Noncompetitive Inhibition**

Noncompetitive inhibition occurs when an inhibitor $I$ combines with $ES$ and renders it inactive.

$$
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P
$$

$$
ES + I \xrightleftharpoons{K_{SI}} ES \cdot I
$$

$$
E + I \xrightleftharpoons{K_I} EI
$$

dissociation constants

Suppose that $K_{SI} \cong K_I$, that is, the affinity of the inhibitor site for inhibitor does not depend on whether $E$ is bound to $S$. Then, it can be shown that

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

In Lineweaver-Burk form

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

Here, both the slope and y-intercept are multiplied by the same factor, but the x-intercept remains as $-K_M^{-1}$.

![Figure 6-10](image)