After lectures by

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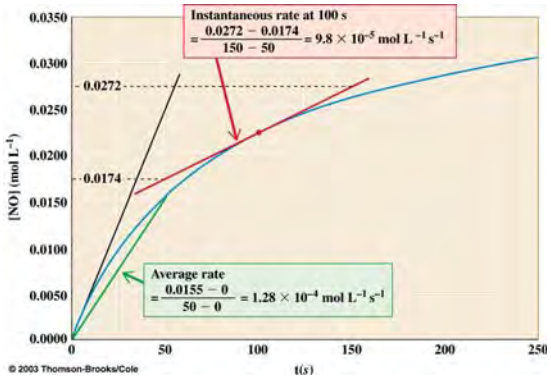
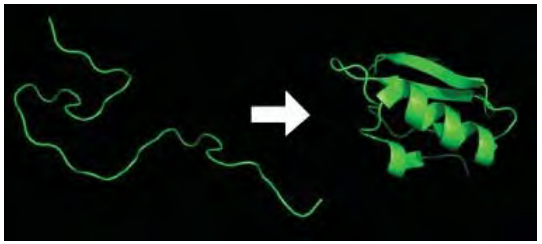
Reaction Rates (reaction velocities): To measure a reaction rate we monitor the disappearance of reactants or appearance of products.

e.g., $2NO_2 + F_2 \rightarrow 2NO_2F$

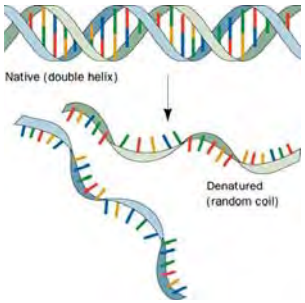


initial velocity =>
[product] = 0,
no back reaction

Protein Folding: 1st order reaction



DNA annealing: 2nd order reaction



Enzyme Kinetics

- Rates of enzyme reactions are affected by
  - Enzymes/catalysts
  - Substrates
  - Temperature
  - Concentrations

Why study enzyme kinetics?

- Quantitative description of biocatalysis
- Understand catalytic mechanism
- Find effective inhibitors
- Understand regulation of activity

Enzyme Kinetics

General Observations

- Enzymes are able to exert their influence at very low concentrations ~ [enzyme] = nM
- The initial rate (velocity) is linear with [enzyme].
- The initial velocity increases with [substrate] at low [substrate].
- The initial velocity approaches a maximum at high [substrate].

Enzyme Kinetics

The initial velocity increases with [S] at low [S].

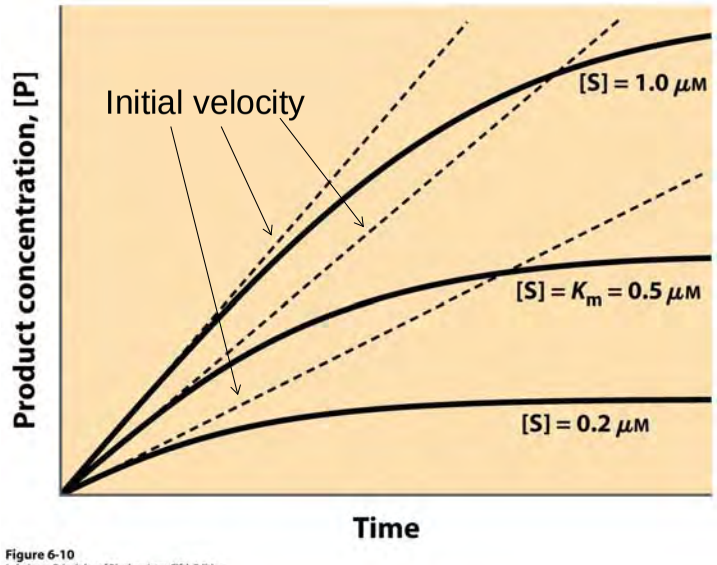


Figure 6-10
Lehninger Principles of Biochemistry, Fifth Edition

Enzyme Kinetics

The initial velocity increases with [S] at low [S].
[velocity =d[P]/dt, P=product]

The initial velocity approaches a maximum at high [S].

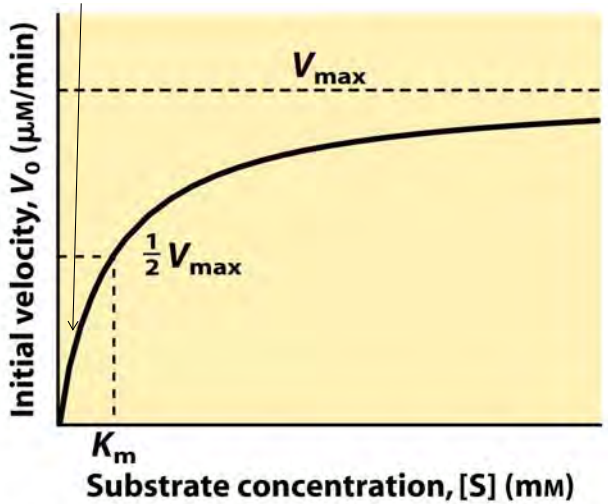


Figure 6-11
Lehninger Principles of Biochemistry, Fifth Edition

Equations describing Enzyme Kinetics

- Start with a mechanistic model
- Identify constraints and assumptions
- Solve for velocity (d[Product]/dt)

Michaelis-Menten Kinetics

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

$$ES \xrightarrow{k2} E + P$$

Simplest enzyme mechanism

- One reactant (S)
- One intermediate (ES)
- One product (P)

Michaelis-Menten Kinetics

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

$$ES \xrightarrow{k2} E + P$$

1. First step: The enzyme (E) and the substrate (S) reversibly and quickly form a non-covalent ES complex.
2. Second step: The ES complex undergoes a chemical transformation and dissociates to give product (P) and enzyme (E).
3. $v=k_2[ES]$
4. Many enzymatic reactions follow Michaelis–Menten kinetics, even though enzyme mechanisms are always more complicated than the Michaelis–Menten model.
5. For real enzymatic reactions use k_{cat} instead of k_2.

Michaelis-Menten Kinetics

The Enzyme-Substrate Complex (ES)

- The enzyme binds non-covalently to the substrate to form a non-covalent ES complex
  - the ES complex is known as the Michaelis complex.
  - A Michaelis complex is stabilized by molecular interactions (non-covalent interactions).
  - Michaelis complexes form quickly and dissociate quickly.

k_{cat} and the reaction velocity

$$E + S \rightleftarrows ES \xrightarrow{k_{cat}} E + P$$

$$\text{velocity} = v = \frac{d[P]}{dt} = k_{cat}[ES]$$

$$v_{max} = k_{cat}[E_0]$$

$$k_{cat} = \frac{v_{max}}{[E_0]}$$

- The enzyme is either free ([E]) or bound ([ES]): $[E_o] = [ES] + [E]$.
- At sufficiently high [S] all of the enzyme is tied up as ES (i.e., $[E_o] \approx [ES]$, according to Le Chatelier's Principle)
- At high [S] the enzyme is working at full capacity ($v=v_{max}$).
- The full capacity velocity is determined only by k_{cat} and $[E_o]$.
- k_{cat} = turnover #: number of moles of substrate produced per time per enzyme active site.

Michaelis-Menten Kinetics

$$E + S \rightleftarrows ES \xrightarrow{k_{cat}} E + P$$

- For any enzyme it is possible (pretty easy) to determine k_{cat}.
- To understand and compare enzymes we need to know how well the enzyme binds to S (i.e, what happens in the first part of the reaction.) k_{cat} does not tell us anything about how well the enzyme binds to the substrate.

Michaelis-Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES$$

$$ES \xrightarrow{k2} E + P$$

Assumptions

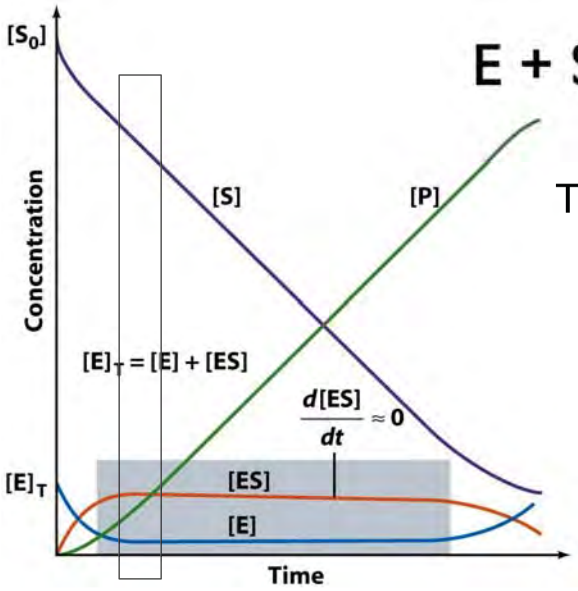
1. $k_1, k_{-1} >> k_2$ (i.e., the first step is fast and is always at equilibrium).
2. $d[ES]/dt \approx 0$ (i.e., the system is at steady state.)

$$\frac{d[ES]}{dt} = \text{rate of formation of ES} - \text{rate of breakdown of ES} \approx 0 \text{ (at steady state)}$$

3. There is a single reaction/dissociation step (i.e., $k_2=k_{cat}$).
4. $S_{Tot} = [S] + [ES] \approx [S]$
5. There is no back reaction of P to ES (i.e. $[P] \approx 0$). This assumption allows us to ignore k_{-2}. We measure initial velocities, when $[P] \approx 0$.

Michaelis-Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k2} E + P$$



The time dependence of everything (in a Michaelis-Menten reaction)

Michaelis-Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Now: we derive the Michaelis-Menten Equation

$d[ES]/dt = k_1[E][S] - k_{-1}[ES] - k_2[ES]$
$= 0$ (steady state assumption, see previous graph)

solve for [ES] (do the algebra)

$[ES] = [E][S]\ k_1/(k_{-1} + k_2)$

Define K_M (Michealis Constant)
$K_M = (k_{-1} + k_2)/k_1 \Rightarrow [ES] = [E][S]/K_M$

rearrange to give $K_M = [E][S]/[ES]$

Michaelis-Menten Kinetics

$K_M = [E][S]/[ES]$

substitute $[E] = [E]_0 - [ES]$

$$\frac{([E]_0 - [ES])[S]}{[ES]} = K_M$$

multiply both sides by [ES]

$$K_M[ES] = ([E]_0 - [ES])[S]$$

solve for [ES]

$$[ES] = \frac{[E]_0[S]}{K_m + [S]}$$

multiply both sides by k_2 (this gives get the velocity of the reaction)

$$\frac{dP}{dt} = v = k_2[ES] = \frac{k_2[E]_0[S]}{K_M + [S]}$$

and remember that $k_2[E]_0 = v_{max}$

$$v = \frac{v_{max}[S]}{K_M + [S]}$$ Michaelis Menten Equation

Michaelis-Menten Kinetics

$$v = \frac{v_{max}[S]}{K_M + [S]}$$ Michaelis Menten Equation

When $[S] = K_M$ then,

$$v = \frac{v_{max}[S]}{[S] + [S]} = \frac{v_{max}}{2}$$

This is saying that when K_M=[S], the reaction runs at half maximum velocity.

Michaelis-Menten Kinetics

K_M is the substrate concentration required to reach half-maximal velocity ($v_{max}/2$).

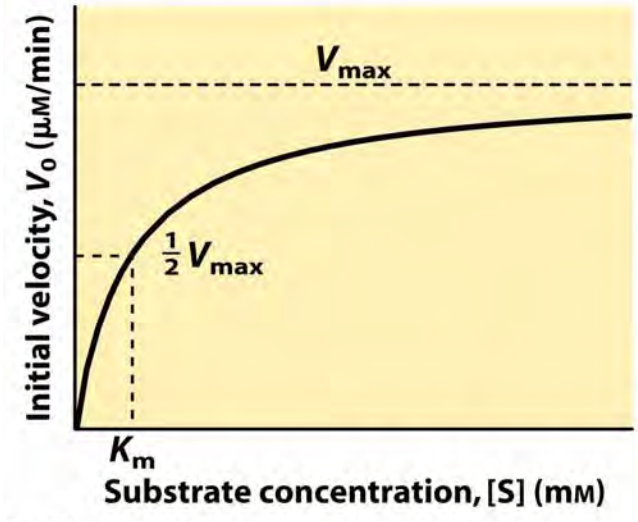


Figure 6-11
Lehninger Principles of Biochemistry, Fifth Edition

$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \quad ES \xrightarrow{k_2} E + P$

Significance of K_M

- $K_M = [E][S]/[ES]$ and $K_M = (k_{-1} + k_2)/k_1$.
- K_M is the apparent dissociation constant of the ES complex. A dissociation constant (K_D) is the reciprocal of the equilibrium constant ($K_D=K_A^{-1}$). K_M is a measure of a substrate's affinity for the enzyme (but it is the reciprocal of the affinity).
- If $k_1, k_{-1} >> k2$, the $K_M=K_D$.
- K_M is the substrate concentration required to reach half-maximal velocity ($v_{max}/2$). A small K_M means the sustrate binds tightly to the enzyme and saturates (max's out) the enzyme.
- The microscopic meaning of K_m depends on the details of the mechanism.

Michaelis-Menten Kinetics

The significance of k_{cat}

- $v_{max} = k_{cat} E_{tot}$
- k_{cat}: For the simplest possible mechanism, where ES is the only intermediate, and dissociation is fast, then $k_{cat}=k_2$, the first order rate constant for the catalytic step.
- If dissociation is slow then the dissociation rate constant also contributes to k_{cat}.
- If one catalytic step is much slower than all the others (and than the dissociation step), than the rate constant for that step is approximately equal to to k_{cat}.
- k_{cat} is the "turnover number": indicates the rate at which the enzyme turns over, i.e., how many substrate molecules one catalytic site converts to substrate per second.
- If there are multiple catalytic steps (see trypsin) then each of those rate constants contributes to k_{cat}.
- The microscopic meaning of k_{cat} depends on the details of the mechanism.

Michaelis-Menten Kinetics

Significance of k_{cat}/K_M

- k_{cat}/K_M is the catalytic efficiency. It is used to rank enzymes. A big k_{cat}/K_M means that an enzyme binds tightly to a substrate (small K_M), with a fast reaction of the ES complex.
-
- k_{cat}/K_M is an apparent second order rate constant

  $v=k_{cat}/K_M[E]_0[S]$

- k_{cat}/K_M can be used to estimate the reaction velocity from the total enzyme concentration ($[E]_0$). $k_{cat}/K_M = 10^9$ => diffusion control.
- k_{cat}/K_M is the specificity constant. It is used to distinguish and describe various substrates.

Michaelis-Menten Kinetics

Data analysis

- It would be useful to have a linear plot of the MM equation
- Lineweaver and Burk (1934) proposed the following: take the reciprocal of both sides and rearrange.
- Collect data at a fixed $[E]_0$.

Michaelis-Menten Kinetics

$$v = \frac{v_{max}[S]}{K_M + [S]}$$ Michaelis Menten Equation

take the reciprocal

$$\frac{1}{v} = \frac{K_M + [S]}{v_{max}[S]} = \frac{K_M}{v_{max}[S]} + \frac{1}{v_{max}}$$

Graph $\frac{1}{v}$ versus $\frac{1}{[S]}$

the y (1/v) intercept (1/[S] = 0) is $1/v_{max}$
the x (1/[S]) intercept (1/v = 0) is $-1/K_M$
the slope is K_M/v_{max}

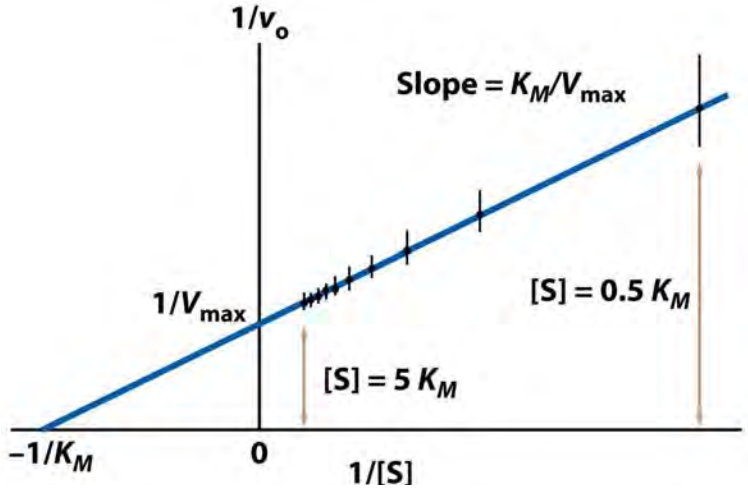
Enzyme Inhibition

Michaelis-Menten Kinetics

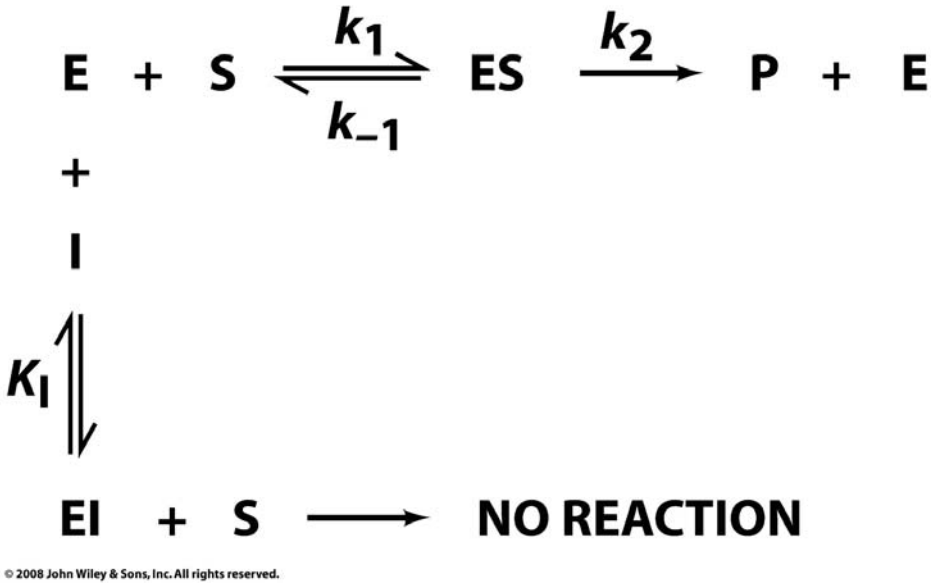
Lineweaver-Burk-Plot

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

the y (1/v) intercept (1/[S] = 0) is $1/v_{max}$
the x (1/[S]) intercept (1/v = 0) is $-1/K_M$
the slope is K_M/v_{max}



Competitive Inhibition



Competitive Inhibition

$$\frac{[E][I]}{[EI]} = K_I \qquad \text{inhibitor dissociation constant}$$

$$[E]_0 = [E] - [ES] - [EI] \qquad \text{total enzyme concentration}$$

$$\alpha = 1 + \frac{[I]}{K_I}$$

$$\frac{1}{v} = \frac{\alpha K_M + [S]}{v_{max}[S]} = \frac{\alpha K_M}{v_{max}[S]} + \frac{1}{v_{max}}$$

Competitive Inhibition

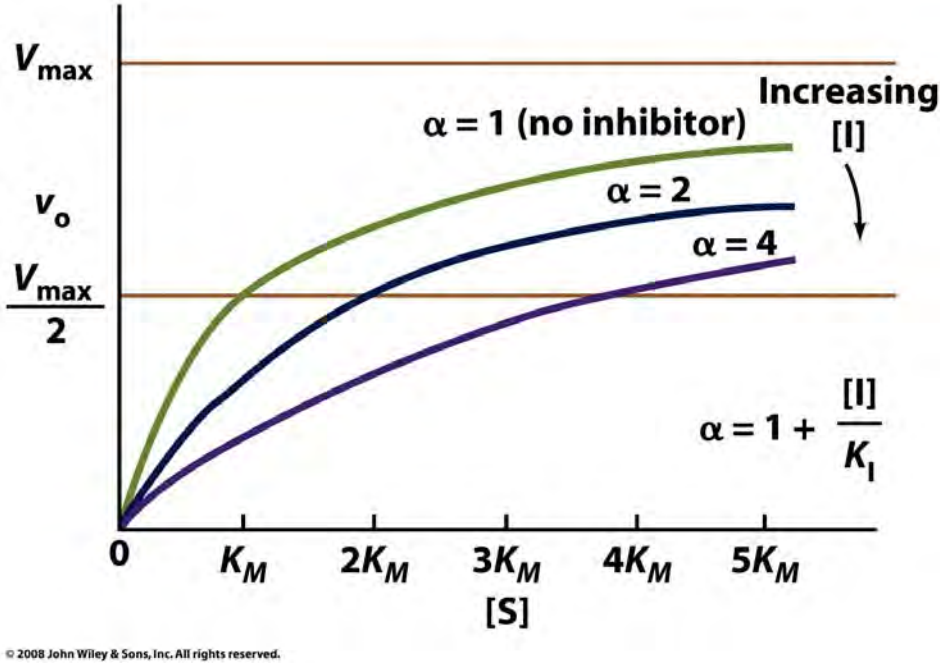


Figure 12-6

Competitive Inhibition

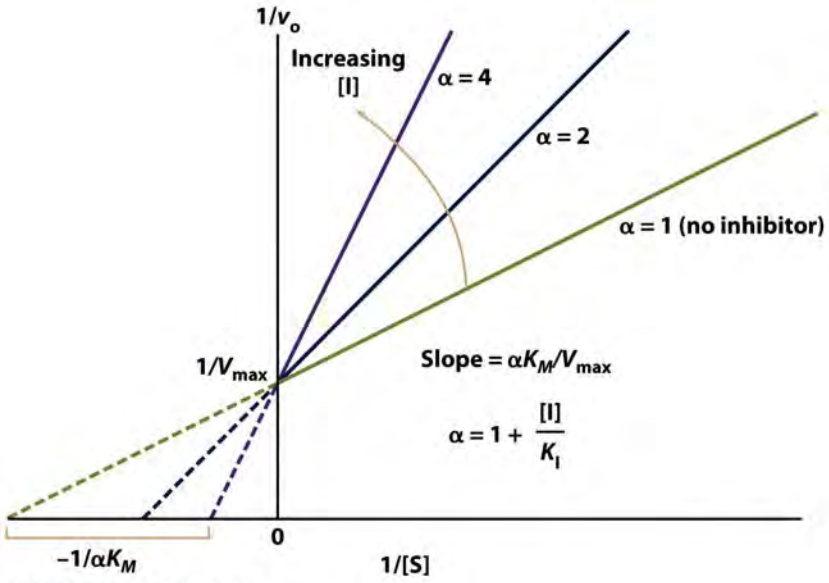
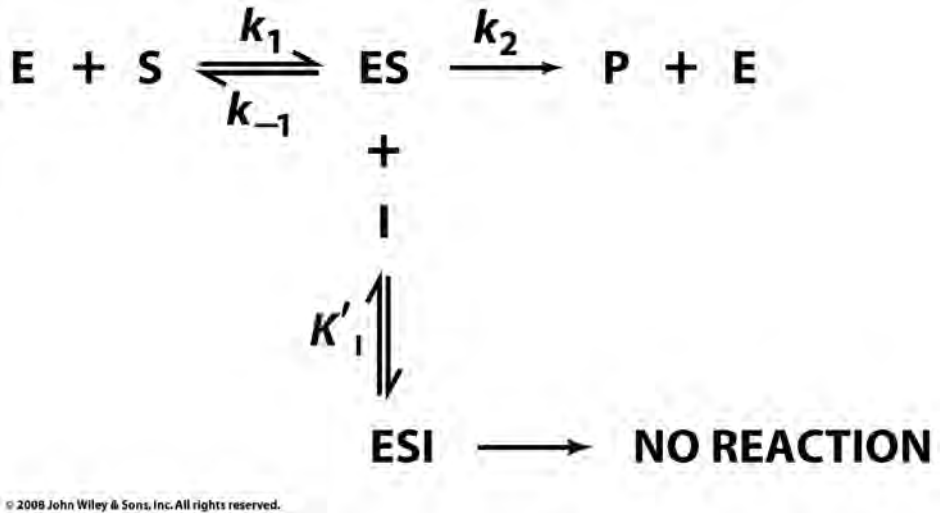


Figure 12-7

Uncompetitive Inhibition



Uncompetitive Inhibition

$$\frac{[ES][I]}{[ESI]} = K_I \qquad \text{inhibitor dissociation constant}$$

$$[E]_0 = [E] - [ES] - [ESI] \qquad \text{total enzyme concentration}$$

$$\frac{1}{v} = \frac{K_M}{v_{max}[S]} + \frac{\alpha}{v_{max}}$$

Uncompetitive Inhibition

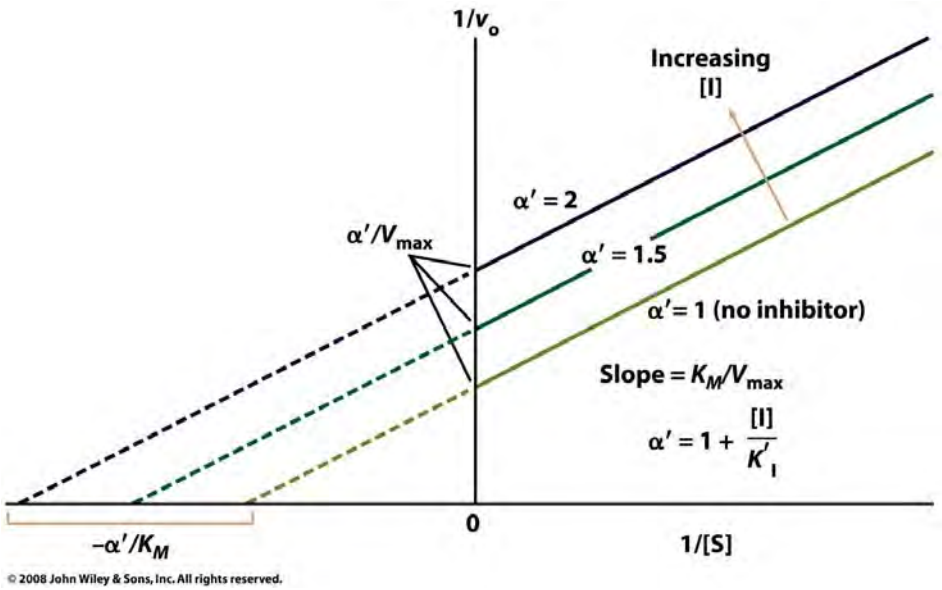
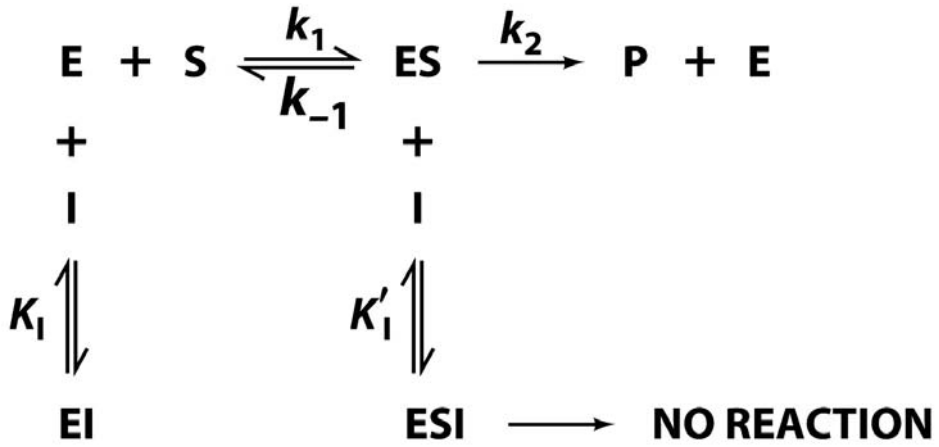


Figure 12-8

Mixed (competitive and uncompetitive) Inhibition



Mixed (competitive and uncompetitive) Inhibition