BCMB 3100 – Chapters 6,7,8

Enzyme Basics

• Six Classes (IUBMB)
• Kinetics
• Michaelis-Menten Equation
• Vo, Km, Vmax, Kcat
• Lineweaver-Burk Plot

Six major groups of enzymes (pgs. 94-95)

Oxidoreductases: (oxidation-reduction reactions)
Transferases: (group transfer reactions)
Hydrolases: (hydrolysis)
Lyases: (nonhydrolytic and nonoxidative lysis → double bond)
Isomerases: (isomerization)
Ligases: (ligation reaction requiring energy from NTP (nucleoside triphosphate)

YOU MUST KNOW THESE 6 GROUPS!
For more information on the types of enzymes see

Recommendations of the Nomenclature Committee of
the International Union of Biochemistry and Molecular
Biology

on the Nomenclature and Classification of Enzymes by
the Reactions they Catalyse

http://www.chem.qmul.ac.uk/iubmb/enzyme/

1. Oxidoreductases (dehydrogenases)

Catalyze oxidation-reduction reactions

\[
\text{HO} + \text{C} - \text{H} + \text{NAD}^+ \overset{\text{Lactate dehydrogenase}}{\rightleftharpoons} \text{C} = \text{O} + \text{NADH} + \text{H}^+ \quad (5.1)
\]

Oxidation can be identified as:
* Addition of oxygen
* Removal of hydrogen
* Increase in valency of metal ion (e.g. Fe^{2+} → Fe^{3+})

See also the index of your book to find more examples;
e.g. pg. 332
The Oxidation States of Carbon

**Most reduced**

- CH$_3$-CH$_3$  Alkane (ethane)
- CH$_3$-HC$_2$OH  Alcohol (ethanol)
- CH$_3$-COH  Aldehyde (acetaldehyde)
- CH$_3$-COOH  Carboxylic acid (acetic acid)
- CO$_2$  Carbon dioxide

**Most oxidized**

Each of the arrows indicates an oxidation reaction. All except the last reaction are oxidations brought about by dehydrogenation.

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2. Transferases

- Catalyze group transfer reactions

\[
\text{H}_3\text{N}-\text{C}^{\text{+}}\text{H} + \text{COO}^- \xrightarrow{\text{Alanine transaminase}} \text{C}=\text{O} + \alpha\text{-Ketoglutarate} \rightarrow \text{COO}^- \xrightarrow{\text{H}_3\text{N}-\text{C}^{\text{+}}\text{H}} \text{CH}_3 \text{Pyruvate} \]

(5.2)

See also pages?

Other examples?
3. Hydrolases

• Catalyze hydrolysis reactions where water is the acceptor of the transferred group

\[ \text{Pyrophosphate} + \text{H}_2\text{O} \xrightarrow{\text{Pyrophosphatase}} 2 \text{Phosphate} \quad (5.3) \]

4. Lyases

• Catalyze lysis of a substrate, generating a double bond in a nonhydrolytic, nonoxidative elimination (i.e. nonhydrolytic & nonoxidative lysis that yields a double bond)

\[ \text{Pyruvate} + \text{H}^+ \xrightarrow{\text{Pyruvate dehydrogenase}} \text{Acetaldehyde} + \text{Carbon dioxide} \quad (5.4) \]
5. Isomerases

- Catalyze isomerization reactions

\[
\text{L-Alanine} \quad \xrightarrow{\text{Alanine racemase}} \quad \text{D-Alanine}
\]

6. Ligases (synthetases)

- Catalyze ligation, or joining of two substrates
- Require chemical energy (e.g. ATP)
Challenge of the Weekend,  Due September 9, 2012

Find a minimum of three examples of enzymes and their reactions for each of the 6 classes of enzymes. (You should be able to find all or most of these in your book)

Label an individual page with one of each of the names of the 6 classes of enzymes. On each page for that particular class of enzymes, give a list of at least 3 full reactions, including substrate(s), products and enzyme name. A completed assignment will be six pages with a total of 18 reactions.

For credit, these must be turned in before the start of Breakout Session on Tuesday, September 9.

BCMB 3100 - Chapter 5 Lecture

• Enzymes
• Six Classes (IUBMB)
• **Kinetics**
• Michaelis-Menten Equation
• Vo, Km, Vmax, Kcat
• Lineweaver-Burk Plot
Principles of Kinetics
(for non-enzyme-catalyzed reactions) (1)

**first-order reaction**

\[
\begin{align*}
k & \quad \text{S} \rightarrow \text{P} \\
\Delta [P] & = \frac{\Delta v}{\Delta t} = k[S] \\
v & = \text{velocity, } \text{P} = \text{product, } \text{S} = \text{substrate,} \\
k & = \text{rate constant (s}^{-1})
\end{align*}
\]

Principles of Kinetics
(for non-enzyme-catalyzed reactions) (2)

**second-order reaction (bimolecular)**

\[
\begin{align*}
\text{S}_1 + \text{S}_2 & \rightarrow \text{P}_1 + \text{P}_2 \\
v & = k[\text{S}_1]^1[\text{S}_2]^1 \\
k & = \text{rate constant M}^{-1} \text{ s}^{-1}
\end{align*}
\]

**pseudo first-order reaction**

If concentration of a reactant is so high that it remains constant during reaction = zero-order

\[
v = k[\text{S}_1]^1[\text{S}_2]^0 = k[\text{S}_1]^1
\]

Important for enzyme assays
Emil Fischer  1894

_________ model for enzyme:substrate interactions

![Diagram of enzyme-substrate interactions]

**Enzyme Kinetics**

- **Enzyme-substrate complex (ES)** - complex formed when specific substrates fit into the enzyme active site

  \[ E + S \rightarrow ES \rightarrow E + P \]

- When \([S] >> [E]\), every enzyme binds a molecule of substrate (enzyme is **saturated** with substrate)

- Under these conditions the rate depends only upon \([E]\), and the reaction is **pseudo-first order**
**Enzyme kinetics**: study of the rates of enzyme-catalyzed reactions

**Why study enzyme kinetics?** - enzyme kinetics gives information about:

- enzyme catalysis
- enzyme mechanisms
- regulation of enzyme activity (i.e. inhibitors & activators)
- basis of enzyme assays

The kinetics of an enzyme are affected by:

- [enzyme]
- [substrate]
- pH & temperature
- coenzymes, activators, inhibitors

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**Enzyme Kinetics**

\[ E + S \rightarrow ES \rightarrow E + P \]

Effect of enzyme concentration \([E]\) on velocity \((v)\)

- Fixed, saturating \([S]\)
- Pseudo-first order enzyme-catalyzed reaction
Progress curve for an enzyme-catalyzed reaction

\[ E + S \rightarrow ES \rightarrow E + P \]  
See Fig. 6.2

- The initial velocity \((v_0)\) is the slope of the initial __________ of the curve
- Rate of the reaction doubles when twice as much enzyme is used

Plots of initial velocity \((v_0)\) versus \([S]\)

See Fig. 7.1

(a) Each \(v_0\) vs [S] point is from one kinetic run
(b) Michaelis constant \((K_m)\) equals the concentration of substrate needed for 1/2 maximum velocity
BCMB 3100 - Chapter 5 Lecture

- Enzymes
- Six Classes (IUBMB)
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- Vo, Km, Vmax, Kcat
- Lineweaver-Burk Plot

Michaelis-Menten Kinetics apply to many enzymes

(1913) Leonor Michaelis & Maud Menten proposed simple model for enzyme kinetics  
(See pages 107-108)

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow[k_{-2}]{k_2} E + P
\]

Assumption #1: (no product reverts to S, true in initial stage of reaction, \( k_{-2} = 0 \)) (initial velocity = Vo)

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

\( k_{\text{cat}} \) = catalytic constant = turnover number
number of catalytic events per second per enzyme

**Goal:** expression that relates rate of catalysis to concentration of \( S \) and \( E \) and rate constants for individual steps.

\[ v = k_2[ES] \]

\([ES]\) can be described by \([E]\) & \([S]\) by considering individual rate constants
rate of formation of \( ES \) = \( k_1[E][S] \)
rate of breakdown of \( ES \) = \( (k_{-1} + k_2) [ES] \)

**Assumption #2:** Steady state conditions (i.e. concentration of \( ES \) stays the same while concentration of \( E \) and \( S \) changes). Occurs when formation of \( ES \) = breakdown of \( ES \).

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

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

**Define constant** \(K_m\) (Michaelis constant)

\(K_m = (k_{-1} + k_2) / k_1\)

Therefore \([ES] = [E] [S] / K_m\)

Solve for \([ES]\) ……

(see your text. You do NOT need to know how to solve for \([ES]\))

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

Velocity of an enzyme-catalyzed reaction (depends upon rate of conversion of ES to E + P)

\[v_o = k_2[ES]\]

Recall \(k_2 = k_{cat}\)

\(V_{max}\) occurs when \([S]\) much > than \(K_m\) so

\(V_{max} = k_2[E_T]\)
The Michaelis-Menten equation

• Equation describes $v_o$ versus $[S]$ plots
• $K_m$ is the ______________

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

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

At low $[S]$, $[S] \ll K_m$ & $V_o = \frac{V_{max}[S]}{K_m}$
thus the rate is proportional to $[S]$

At high $[S]$, $[S] \gg K_m$ & $V = V_{max}$
Thus rate is independent of $[S]$

When $[S] = K_m$ $V = 1/2 V_{max}$

Thus $K_m = [S]$ at which reaction is half its maximal value
**Turnover number**: number of S molecules converted into P by E per second when E is fully saturated with S.

**Turnover number** = $k_2 = \text{kcat} = \text{s}^{-1}$

**Turnover number** can be calculated from $V_{\text{max}} = k_2[\text{ET}]$

**Turnover number** of most enzymes is from 1 to 10,000 per sec

\[
\begin{array}{c}
E + S \underset{k_{-1}}{\xrightarrow{k_1}} ES \underset{k_2}{\rightarrow} E + P \\
\end{array}
\]

For most reactions $K_m$ is a measure of the stability of the ES complex and $K_{\text{cat}}$ is the rate constant for conversion of ES to P.

For most reactions $K_m$ is a measure of the affinity of E for S. Thus, the lower the $K_m$ value, the greater the affinity of E for S.

For most cases, $K_{\text{cat}}$ is a measure of the catalytic activity of the enzyme. Thus, the greater $K_{\text{cat}}$, the faster the reaction.
Under physiological conditions enzymes are not normally saturated with S.

\[ [S]/K_m \text{ is usually between } 0.01 \text{ and } 1 \]

(K\(m\) usually \(10^{-1}\) to \(10^{-7}\)M)

How do you characterize enzyme kinetics under these low [S]?

\[ V = [E][S] \frac{k_2}{K_m} \]

When \([S] \llllll K_m \quad [E] \cong [E_T] \text{ so} \]

\[ V = [E_T][S] \frac{k_2}{K_m} \]

Thus, \(V\) depends on value of \([S]\) & \(k_2 / K_m\)

\(k_2 / K_m\) is limited by \(k_1\) which is limited < \(10^9\)M\(^{-1}\) s\(^{-1}\) (due to limits of diffusion). A few enzymes catalyze reactions at this upper physical rate = diffusion controlled reactions.

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**Fig 7.3** \(K_m\) and physiological substrate concentrations

- \(K_m\) values for enzymes are typically just above [S], so that the enzyme rate is sensitive to small changes in [S]
Meanings of \( k_{\text{cat}} \) and \( k_{\text{cat}}/K_m \)

Turnover number

Specificity constant

\[
\begin{align*}
\text{Region A:} & \quad ES \xrightarrow{k_{\text{cat}}} E + P \\
\text{Region B:} & \quad E + S \xrightarrow{K_m} E + P \\
& \quad (E + S \rightarrow ES \rightarrow E + P)
\end{align*}
\]

See pages 110-111

Examples of catalytic constants

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>( k_{\text{cat}}(\text{s}^{-1}) )</th>
<th>See also Table 7.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papain</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>(10^2)</td>
<td></td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>(10^2)</td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>(10^2) (to (10^3))</td>
<td></td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>(10^3)</td>
<td></td>
</tr>
<tr>
<td>Kinases</td>
<td>(10^3)</td>
<td></td>
</tr>
<tr>
<td>Dehydrogenases</td>
<td>(10^3)</td>
<td></td>
</tr>
<tr>
<td>Transaminases</td>
<td>(10^3)</td>
<td></td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>(10^6)</td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>(10^6)</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>(10^7)</td>
<td></td>
</tr>
</tbody>
</table>

Also note: (Km usually \(10^{-1}\) to \(10^{-7}\)M) (100 mM to 0.1 µM)
Values of $k_{\text{cat}}/K_m$

- $k_{\text{cat}}/K_m$ can approach rate of encounter of two uncharged molecules in solution ($10^8$ to $10^9$M$^{-1}$s$^{-1}$)
- $k_{\text{cat}}/K_m$ is also a measure of enzyme specificity for different substrates (specificity constant)
- rate acceleration = $k_{\text{cat}}/k_n$
  \[
  (k_n = \text{rate constant in the absence of enzyme})
  \]

$k_{\text{cat}}/K_m$ - This ratio is often thought of as a measure of enzyme efficiency. Either a large value of $k_{\text{cat}}$ (rapid turnover) or a small value of $K_m$ (high affinity for substrate) makes $k_{\text{cat}}/K_m$ large.

Comparison of $k_{\text{cat}}/K_m$ allows direct comparison of the effectiveness of an enzyme toward different substrates (or of different enzymes for the same substrate)

The larger the $k_{\text{cat}}/K_m$ the more efficient the enzyme is for a reaction.

<table>
<thead>
<tr>
<th>Table 7.3 Substrate preferences of chymotrypsin</th>
<th>Amino acid in ester</th>
<th>Amino acid side chain</th>
<th>$k_{\text{cat}}/K_m$ (s$^{-1}$ M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>H</td>
<td>$\text{-}\text{CH}_2$</td>
<td>$1.3 \times 10^3$</td>
</tr>
<tr>
<td>Valine</td>
<td>$\text{-CH}$</td>
<td>$\text{-CH}_3$</td>
<td>2.0</td>
</tr>
<tr>
<td>Norvaline</td>
<td>$\text{-CH}_2\text{CH}_3$</td>
<td>$\text{-CH}_2\text{CH}_3$</td>
<td>$3.6 \times 10^3$</td>
</tr>
<tr>
<td>Norleucine</td>
<td>$\text{-CH}_2\text{CH}_2\text{CH}_3$</td>
<td>$\text{-CH}_2\text{CH}_2\text{CH}_3$</td>
<td>$3.0 \times 10^3$</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>$\text{-CH}_2$</td>
<td>$\text{Ph}$</td>
<td>$1.0 \times 10^5$</td>
</tr>
</tbody>
</table>

Source: After A. Forsht, Structure and Mechanism in Protein Science; A Guide to Enzyme Catalysis and Protein Folding (W. H. Freeman and Company, 1999), Table 6.3.
rate acceleration = \( k_{\text{cat}}/k_n \)

\( k_n = \text{rate constant in the absence of enzyme} \)

### Table 6.1 Rate enhancement by selected enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Nonenzymatic half-life</th>
<th>Uncatalyzed rate ( (k_{\text{cat}} \text{ s}^{-1}) )</th>
<th>Catalyzed rate ( (k_{\text{cat}} \text{ s}^{-1}) )</th>
<th>Rate enhancement ( (k_{\text{cat}} \text{ s}^{-1})/k_n \text{ s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMP decarboxylase</td>
<td>78,000,000 years</td>
<td>( 2.8 \times 10^{-16} )</td>
<td>39</td>
<td>( 1.4 \times 10^{17} )</td>
</tr>
<tr>
<td>Staphylococcal nuclease</td>
<td>130,000 years</td>
<td>( 1.7 \times 10^{-13} )</td>
<td>95</td>
<td>( 5.6 \times 10^{14} )</td>
</tr>
<tr>
<td>AMP nucleosidase</td>
<td>69,000 years</td>
<td>( 1.0 \times 10^{-11} )</td>
<td>60</td>
<td>( 6.0 \times 10^{13} )</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>7.3 years</td>
<td>( 3.0 \times 10^{-6} )</td>
<td>578</td>
<td>( 1.9 \times 10^{13} )</td>
</tr>
<tr>
<td>Ketosteroid isomerase</td>
<td>7 weeks</td>
<td>( 1.7 \times 10^{-7} )</td>
<td>66,000</td>
<td>( 3.9 \times 10^{14} )</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>1.0 days</td>
<td>( 4.3 \times 10^{-4} )</td>
<td>4,300</td>
<td>( 1.0 \times 10^{4} )</td>
</tr>
<tr>
<td>Chorismate mutase</td>
<td>7.4 hours</td>
<td>( 2.6 \times 10^{-5} )</td>
<td>50</td>
<td>( 1.9 \times 10^{5} )</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>5 seconds</td>
<td>( 1.3 \times 10^{-1} )</td>
<td>( 1 \times 10^{1} )</td>
<td>( 7.7 \times 10^{1} )</td>
</tr>
</tbody>
</table>

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Vmax and Km can be determined by measuring the rate of the reaction at different \([S]\) if an enzyme operates my Michaelis-Menten kinetics

Transformation of the Michaelis-Menten equation (i.e. taking the reciprocal of both sides)

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

Recall \(y = a + bx\) \(b = \text{slope}, a = \text{y intercept}\)

A plot of \(1/V\) versus \(1/[S]\) is a Lineweaver-Burk plot (a straight line plot)
Measurement of $K_m$ and $V_{max}$

**Fig 7.5** The double-reciprocal Lineweaver-Burk plot is a linear transformation of the Michaelis-Menten plot ($1/v_o$ versus $1/[S]$)

$K_m$ and $V_{max}$ can be determined from the Lineweaver-Burk plot

$\frac{1}{v_o} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$

Useful information regarding enzyme kinetics

$K_m$ and $V_{max}$ can be determined from the Lineweaver-Burk plot

$K_m$ depends on enzyme, pH, temperature and ionic strength of environment

The fraction of enzyme sites filled $= f_{ES}$

$f_{ES} = \frac{V}{V_{max}}$
BCMB 3100 - Chapter 5 Lecture

- Enzymes
- Six Classes (IUBMB)
- Kinetics
- Michaelis-Menten Equation
- Vo, Km, Vmax, Kcat
- Lineweaver-Burk Plot
- Kinetics of Multisubstrate Reactions

Kinetics of Multisubstrate Reactions
Cleland notation for bi-substrate reactions

Kinetic Mechanisms

Fig 7.6 Notations for bisubstrate reactions
(a) Sequential (ordered or random)

A + B → P + Q
Fig. 7.6 (b) Bisubstrate reactions

\[ A + B \rightarrow P + Q \]

Ping-pong reaction
(Double-displacement reaction)

(b) Ping-pong

BCMB 3100 - Chapter 5 Lecture

- Kinetics
- Michaelis-Menten Equation
- Vo, Km, Vmax, Kcat
- Lineweaver-Burk Plot
- Kinetics of Multisubstrate Reactions
- Reversible Inhibition
Reversible Enzyme Inhibition

- Three types of reversible inhibition: **Competitive**, **Uncompetitive** and **Noncompetitive**

**Fig 8.10** Reversible enzyme inhibitors

(a) __________. S and I bind to same site on E

(b) **Nonclassical competitive**. Binding of S at active site prevents binding of I at separate site. Binding of I at separate site prevents S binding at active site.
See Fig 8.10 **Competitive inhibition.** (a) Kinetic scheme. (b) Lineweaver–Burk plot

Apparent ______ is increased  
________ stays the same.

Most common type of inhibitors

---

**Benzamidline competes with arginine for binding to trypsin**

Example of a competitive inhibitor

![Diagram of benzamidline and arginine structures]
(c) ______________.
I binds only to ES (inactivates E)

(d) ______________.
I binds to either E or ES to inactivate the enzyme

See Figs 8.11 & 8.12

See Fig 8.11 Uncompetitive inhibition

(a) $E + S \rightleftharpoons ES \rightarrow E + P$

$K_i$

ESI

Apparent ______ is decreased
_______ is decreased
See Fig 8.12 Noncompetitive inhibition

(a)

\[ E + S \xleftrightarrow{K_i} ES \xrightarrow{K_i} E + P \]

\[ EI + S \xleftrightarrow{} ESI \]

Apparent ______ stays the same

_______ is decreased

(b)

\[ \frac{1}{V_0} \]

\[ \frac{1}{[S]} \]

Control

\[ [II] \]

BCMB 3100 - Chapter 5 Lecture

- Michaelis-Menten Equation
- \( V_0, K_m, V_{max}, K_{cat} \)
- Lineweaver-Burk Plot
- Kinetics of Multisubstrate Reactions
- Reversible Inhibition
- Irreversible Inhibition
Irreversible Enzyme Inhibition

- Irreversible inhibitors form stable covalent bonds with the enzyme
- Irreversible inhibitors can be used to identify the amino acid residues at enzyme active sites
- Incubation of inhibitor with enzyme results in loss of activity

Covalent complex with lysine residues

- Reduction of a Schiff base forms a stable substituted enzyme
Example of irreversible inhibition

Inhibition of acetylcholine esterase (enzyme that hydrolyses the neurotransmitter acetylcholine & restores polarization of a nerve).

Nerve gases are organic phosphate compounds (e.g. DFP) that form stable phosphoryl-enzymes complexes by reacting with the OH of the active-site Ser. They kill by causing respiratory paralysis.

• Reaction of DFP with Ser-195 of chymotrypsin

DFP = diisopropyl fluorophosphate
Affinity labels for studying enzyme active sites

• ___________ are active-site directed reagents
• They are irreversible inhibitors
• Affinity labels resemble substrates, but contain reactive groups to interact covalently with the enzyme

Site-Directed Mutagenesis Modifies Enzymes

• _________________ (SDM) can be used to test the functions of individual amino acid side chains
• One amino acid is replaced by another using molecular biology techniques
• Example: Subtilisin protease (used in detergents) was made more resistant to chemical oxidation by replacing Met-222 with Ala-222
BCMB 3100 - Chapter 5 Lecture

- Vo, Km, Vmax, Kcat
- Lineweaver-Burk Plot
- Kinetics of Multisubstrate Reactions
- Reversible Inhibition
- Irreversible Inhibition
- Regulatory Enzymes

General Features of Regulatory Enzymes (1)

1. Regulatory compounds (__________________) bind regulatory sites separate from catalytic sites. They can be activators or inhibitors.

2. Allosteric modulators bind noncovalently, may alter Km or Vmax, and are NOT chemically altered by the enzyme they regulate

3. Catalytic and regulatory sites are different
General Features of Regulatory Enzymes (2)

4. \( v_0 \) vs \([S]\) curve for regulatory enzymes is often sigmoidal, suggesting cooperativity of \( S \) binding

5. Most regulatory enzymes have 4\(^o\) structure. Subunits may be identical or different

Levels of enzyme regulation

1. allosteric \( R \rightarrow T \) transition (fastest)
2. covalent modification of enzyme (e.g. phosphorylation)
3. regulation of enzyme synthesis or degradation (slowest)

Some enzymes do NOT obey Michael-Menten kinetics

Example: Allosteric enzymes often have a sigmoidal rather than hyperbolic \( v_0 \) vs \([S]\) plot

For enzymes that do not obey Michaelis-Menten kinetics, measurement of kinetics at different \([S]\) can distinguish competitive from noncompetitive inhibition.

\[ \text{_________________ : Vmax is NOT altered, but apparent Km is increased} \]
\[ \text{_________________ : Vmax is decreased but Km is not affected} \]
\[ \text{_________________ : Vmax and Km are decreased} \]
Plots of initial velocity versus F6P for PFK-1 (phosphofructokinase-1)

- ADP is an allosteric activator of PFK-1 and lowers the apparent $K_m$ without affecting $V_{max}$
- For a given F6P concentration the $v_o$ is larger in the presence of ADP

See Fig. 16.12

Regulatory enzymes usually occur at the first committed step of a pathway

Regulatory enzymes are bound by allosteric effectors at the regulatory site

Role of cooperativity of binding in regulation

- Addition of modulators alters enzyme activity
- Activators can lower $K_m$, inhibitors can raise $K_m$
Example of regulation of enzyme activity by covalent modification of enzyme (e.g. phosphorylation)

**Pyruvate dehydrogenase regulation**

- **Phosphorylation** stabilizes the inactive state (red)
- **Dephosphorylation** stabilizes the active state (green)