Chapter 6: Outline

Outline

Properties of Enzymes
Classification of Enzymes
Enzyme Kinetics
  Michaelis-Menten Kinetics
  Lineweaver-Burke Plots
  Enzyme Inhibition
Catalysis
  Cofactors
    Temperature and pH
Enzyme Regulation
Enzymes are catalysts which increase the rate of reactions by up to $10^{12}$. Enzyme lower the activation energy of the reaction by changing the reaction pathway. Enzymes do not change the standard free energy change ($\Delta G^\circ$) for the reaction nor the Keq. Enzymes are temperature and pH sensitive and will not work outside their normal range.

**Classification of Enzymes**

The International Union of Biochemistry (IUB) classifies and names enzymes according to the type of chemical reaction it catalyzes. Enzymes are assigned an E.C. number and a systematic name to characterize the reaction they catalyze (also a shorten common name).

**Ethanol + NAD+ $\rightarrow$ Acetaldehyde + NADH**

*alcohol dehydrogenase* (common name)
alcohol:NAD$^+$ oxidoreductase (systematic name)
E.C. 1.1.1.1 number
**Enzyme Classes**

**Six Major Classes of Enzymes**

1. **Oxidoreductases** catalyze redox reactions. (dehydrogenases, reductases or peroxidases)
2. **Transferases** transfer a group from one molecule to another (transaminases or transcarboxylases)
3. **Hydrolases** cleave bonds by adding water. (phosphatases or peptidases)
4. **Lyases** catalyze removal of groups to form double bonds or the reverse (decarboxylases or synthases)
5. **Isomerases** catalyze intramolecular rearrangements (epimerases or mutases)
6. **Ligases** bond two molecules together. Many are called synthetases (carboxylases).

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**Examples of Reactions Mediated by Each Enzyme Class**

<table>
<thead>
<tr>
<th>Enzyme Class</th>
<th>Example</th>
<th>Reaction Catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidoreductase</td>
<td>Alcohol dehydrogenase</td>
<td>$\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ \rightarrow \text{CH}_3\text{CH}_2\text{OH} + \text{NADH} + \text{H}^+$</td>
</tr>
<tr>
<td>Transferase</td>
<td>Hexokinase</td>
<td>$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose-6-phosphate} + \text{ADP}$</td>
</tr>
<tr>
<td>Hydrolase</td>
<td>Chymotrypsin</td>
<td>$\text{Polypeptide} + \text{H}_2\text{O} \rightarrow \text{Peptides}$</td>
</tr>
<tr>
<td>Lyase</td>
<td>Pyruvate decarboxylase</td>
<td>$\text{Pyruvate} \rightarrow \text{HCO}_3^- + \text{H}_2\text{O} + \text{CO}_2$</td>
</tr>
<tr>
<td>Isomerase</td>
<td>Alanine racemase</td>
<td>$\text{D-Alanine} \leftrightarrow \text{L-Alanine}$</td>
</tr>
<tr>
<td>Ligase</td>
<td>Pyruvate carboxylase</td>
<td>$\text{Pyruvate} + \text{ATP} \rightarrow \text{Oxaloacetate}$</td>
</tr>
</tbody>
</table>
General Reaction Rates

**A → P**

Initial velocity = \( v_0 = -\frac{\Delta[A]}{\Delta t} \) or \( \frac{\Delta[P]}{\Delta t} \)

First order: \( v_0 = -\frac{\Delta[A]}{\Delta t} = k[A]^1 \)

General order: \( v_0 = k[A]^x \); where \( k \) is the rate constant and \( x \) is the order of the reaction (both experimentally determined).

When \( x = 0 \) then \( v_0 \) is not dependent on \([A]\).

When \( x = 2 \) then \( v_0 \) increases by four when the \([A]\) is doubled.

Bisubstrate Reactions

**A + B → P (E + S → P)**

\( v_0 = -\frac{\Delta[A]}{\Delta t} \) or \( -\frac{\Delta[B]}{\Delta t} \) or \( \frac{\Delta[P]}{\Delta t} \)

\( v_0 = -k[A]^1[B]^1 \)

(overall second order)

If B (or S) is not in excess, then the increase in \( v_0 \) is linear.

If B (or S) is in large excess then there is no increase in \( v_0 \) (pseudo first order).

Velocity = \( k[A]^1[B]^0 = k'[A]^1 \)
Example of Hydrolysis of Gly-Gly

Gly-Gly + H₂O → 2 Gly

\[ v_0 = k[Gly-Gly]^x[H₂O]^y \]

<table>
<thead>
<tr>
<th>[Gly-Gly]</th>
<th>[H₂O]</th>
<th>Velocity, Ms⁻¹ x 10⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) 0.1</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>b) 0.2</td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>c) 0.1</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>d) 0.2</td>
<td>0.2</td>
<td>?</td>
</tr>
</tbody>
</table>

When the [Gly-Gly] is doubled, velocity doubles (x=1)
When the [H₂O] is doubled, velocity doubles (y = 1)

Michaelis-Menten Enzyme Kinetics

\[ v_0 = \frac{V_{max}[S]}{K_m + [S]} \]

At low substrate concentrations the reaction is first order in substrate (\( v_0 \rightarrow V_{max}[S]/K_m \)).
At high substrate concentrations the reaction approaches zero order (\( v_0 \rightarrow V_{max} \)).
Lineweaver-Burke Plot

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

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

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

Y-intercept = \( \frac{1}{V_{\text{max}}} \)

X-intercept = \(-\frac{1}{K_m}\)

Meaning of \( V_{\text{max}} \) and \( K_m \)

\[ V_{\text{max}} = k_3E_t \]

\[ k_{\text{cat}} = \frac{V_{\text{max}}}{E_t} \text{ (s}^{-1}\text{)} \]

Turnover

number = \( \frac{1}{k_{\text{cat}}} \text{ (s)} \)

\[ K_m = \frac{k_2 + k_3}{k_1} = \frac{k_2}{k_1} \text{ (M)} \]

\[ K_m = \text{dissociation constant of ES} \]
### Enzyme Inhibition

**Competitive Inhibitors**
- I binds to only E
- $K_m^{\text{app}}$ increases
- $V_{\text{max}}^{\text{app}}$ does not change

**Uncompetitive Inhibitors**
- I binds to only ES
- $K_m^{\text{app}}$ decreases
- $V_{\text{max}}^{\text{app}}$ decreases

**Noncompetitive Inhibitors**
- I binds to both E and ES
- $K_m^{\text{app}}$ does not change
- $V_{\text{max}}^{\text{app}}$ decreases

### Allosteric Proteins

Exhibits sigmoidal velocity curve rather than hyperbolic curve.

Are often regulatory enzymes since they switch between **efficient** and **less efficient** forms with increasing $[S]$.

**T-state $\rightarrow$ R-state**

Analogous to Hemoglobin binding $O_2$. 
Enzymes are pH and Temperature Sensitive

**pH**: Different amino acid R-groups contribute to the catalytic mechanism of the enzyme. Titration of the weak acid residues often lead to lowering of the catalytic efficiency.

\[ \text{R-CO}_2\text{H} \rightarrow \text{R-CO}_2^- \]

Proton donor  Proton acceptor

**Temperature**: Higher temperatures increase velocity until the protein unfolds.

Catalytic Mechanisms to Lower the Activation Energy of a Biochemical Rx

**Proximity Effects**
- Substrates are oriented in the catalytic site of the enzyme with the proper orientation (decrease in entropy).

**Strain Effects**
- Enzyme-substrate adopts a conformation that stabilizes the transition state of the reaction

**Electrostatic Effects**
- Charged (or hydrogen bonding) groups on the protein stabilizes ionic species formed in the reaction.

**Acid-Base Catalysis**
- Proton donors and acceptors provide high acid or base strength in the catalytic site of the reaction.

**Covalent Catalysis**
- Covalent bonds between E and S stabilize intermediates
Cofactors are Essential in for the Mechanism of many Biological Enzyme Reactions

Metal Cofactors
Fe, Zn, Cu, Co are permanently bound to metalloenzymes
Ca, Mg, K, and to a lesser extent Na are needed in metal-activated enzymes

Coenzyme Cofactors
coenzymes like ATP or NAD⁺ bind to enzymes like substrates and are released like products
prosthetic groups like many vitamin-derived coenzymes are permanently bound to the enzyme

NAD⁺ is a cosubstrate in oxidation-reduction reactions

\[
\text{Nicotinamide} \quad \text{NAD}^+ \quad \text{NADH}
\]

\[
\text{(a)} \quad \text{NAD}^+ + \text{H}^+ + 2e^- \rightleftharpoons \text{NADH} + \text{H}^+
\]

\[
\text{(b)} \quad \text{NAD}^+ \quad \text{NADH}
\]
FAD is a prosthetic group of other oxidation-reduction reactions.

Metal Activated Enzyme

Zn²⁺ is Permanently Bound to Alcohol Dehydrogenase
Many Water Soluble Vitamins are Precursors to Coenzymes

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Coenzyme Form</th>
<th>Reaction or Process Promoted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-Soluble Vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamine (B₁)</td>
<td>Thiamine pyrophosphate</td>
<td>Decarboxylation, aldehyde group transfer</td>
</tr>
<tr>
<td>Riboflavin (B₂)</td>
<td>FAD and FMN</td>
<td>Redox</td>
</tr>
<tr>
<td>Pyridoxine (B₆)</td>
<td>Pyridoxal phosphate</td>
<td>Amino group transfer</td>
</tr>
<tr>
<td>Nicotinic acid (niacin)</td>
<td>NAD and NADP</td>
<td>Redox</td>
</tr>
<tr>
<td>Pantothetic acid</td>
<td>Coenzyme A</td>
<td>Acyl transfer</td>
</tr>
<tr>
<td>Biotin</td>
<td>Biocytin</td>
<td>Carboxylation</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Tetrahydrofolic acid</td>
<td>One-carbon group transfer</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>Deoxyadenosylcobalamin, methylcobalamin</td>
<td>Intramolecular rearrangements</td>
</tr>
<tr>
<td>Ascorbic acid (vitamin C)</td>
<td>Unknown</td>
<td>Hydroxylation</td>
</tr>
<tr>
<td>Lipid-Soluble Vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Retinal</td>
<td>Vision, growth, and reproduction</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>1,25-Dihydroxycholecalciferol</td>
<td>Calcium and phosphate metabolism</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Unknown</td>
<td>Lipid antioxidant</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>Unknown</td>
<td>Blood clotting</td>
</tr>
</tbody>
</table>
Enzyme Regulation

Regulation is need to control different metabolic fluxes through anabolic and catabolic pathways

**Genetic Control** – biosynthesis of enzymes

**Covalent Modification** – phosphorylation-dephosphorylation switch activity of enzymes on an off. Hydrolysis of *zymogens* used to turn enzymes on.

**Compartmentation** – separate catabolic and anabolic metabolism

**Allosteric Regulation** – allosteric activators and inhibitors switch shift sigmoidal velocities