How Enzymes Work

Pratt & Cornely Ch 6

Enzymes

- Biocatalyst—active site
- Proteins
- Substrate
- Reaction specificity
- Stereospecificity
- Coupled reactions
- Regulation
Rate Enhancement

### TABLE 6-1 Rate Enhancements of Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Half-Life (uncatalyzed)*</th>
<th>Uncatalyzed Rate (s⁻¹)</th>
<th>Catalyzed Rate (s⁻¹)</th>
<th>Rate Enhancement (catalyzed rate/uncatalyzed rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orotidine 5'-monophosphate decarboxylase</td>
<td>78,000,000 years</td>
<td>2.8 x 10⁻⁶</td>
<td>39</td>
<td>1.4 x 10⁷</td>
</tr>
<tr>
<td>Staphylococcal nuclease</td>
<td>130,000 years</td>
<td>1.7 x 10⁻⁵</td>
<td>95</td>
<td>3.6 x 10⁴</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>120 years</td>
<td>1.8 x 10⁻⁴</td>
<td>370</td>
<td>2.1 x 10⁴</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>20 years</td>
<td>1.0 x 10⁻²</td>
<td>190</td>
<td>1.7 x 10⁻¹</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>1.9 years</td>
<td>4.2 x 10⁻⁵</td>
<td>4,300</td>
<td>1.0 x 10⁴</td>
</tr>
<tr>
<td>Chorismate mutase</td>
<td>2.4 hours</td>
<td>2.6 x 10⁻⁵</td>
<td>50</td>
<td>1.9 x 10⁴</td>
</tr>
<tr>
<td>Carbonyl anhydrase</td>
<td>5 seconds</td>
<td>1.3 x 10⁻⁷</td>
<td>1,000,000</td>
<td>7.7 x 10⁴</td>
</tr>
</tbody>
</table>

*The half-times of very slow reactions were estimated by extrapolating from measurements made at very high temperatures.


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Orotidine Decarboxylase

- Key enzyme in production of nucleotides for DNA
- $T_{1/2} = 14$ ms
- But what makes it a great enzyme?
The Speed of the Uncatalyzed Rxn

![Temperature dependence of the rate of decarboxylation of l-methylorotic acid in 0.1 M potassium phosphate buffer, pH 6.8 (6).]

Mechanism and RDS

![Reactions and structures related to the mechanism and RDS]
EC Nomenclature

**Enzyme Classification**

<table>
<thead>
<tr>
<th>Class of Enzyme</th>
<th>Type of Reaction Catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Oxidoreductases</td>
<td>Oxidation–reduction reactions</td>
</tr>
<tr>
<td>2. Transferases</td>
<td>Transfer of functional groups</td>
</tr>
<tr>
<td>3. Hydrolases</td>
<td>Hydrolysis reactions</td>
</tr>
<tr>
<td>4. Lyases</td>
<td>Group elimination to form double bonds</td>
</tr>
<tr>
<td>5. Isomerases</td>
<td>Isomerization reactions</td>
</tr>
<tr>
<td>6. Ligases</td>
<td>Bond formation coupled with ATP hydrolysis</td>
</tr>
</tbody>
</table>

Enzyme Classes

1. Oxidoreductase

- Recognize Redox reactions
- Redox cofactors: \( \text{NAD}^+ / \text{NADH} \), \( \text{FAD} / \text{FADH}_2 \), \( \text{Q} / \text{QH}_2 \)
- Dehydrogenases, oxidases, peroxidases, reductase
Enzyme Classes

2. Transferase

- 2 substrates
- Coenzymes—PLP
- Transferase, kinase

3. Hydrolase

- Water nucleophile
- Phosphatase, nuclease, protease, peptidase
Enzyme Classes

4. Lyase

- Hardest to recognize—not redox, hydrolysis
- Elimination of a group to give double bond
- Reversible
- Hydratase, decarboxylase, (formerly synthases)

5. Isomerase

- Rearrangement without loss/add
- Racemase, isomerase, mutase (phosphate)
6. Ligase

- Joining together with ATP input
- Irreversible
- Synthetase

Problem 10

- To which class do the enzymes that catalyze the following reactions belong?
Problems 11-12

• Draw the structures of the products

\[
\begin{align*}
\text{Succinate} & \quad \text{succinate dehydrogenase} \\
\text{Malate} & \quad \text{malate dehydrogenase}
\end{align*}
\]

Problem 14

• Propose a name for each enzyme.
Catalysis

- Thermodynamics
- Kinetics
- Rxn coordinate
- Transition state
- 5.7 kJ ~ 10x change

Mechanisms

- Two major mechanisms—any or all may be used in a given enzyme
  - Chemical Mechanisms
    - Acid-base catalysis
    - Covalent catalysis
    - Metal ion catalysis
  - Binding Mechanisms
    - Proximity/orientation effect
    - Transition State Stabilization
    - Electrostatic catalysis
1. Acid/Base Catalysis

- Sidechains affect most proton transfers

<table>
<thead>
<tr>
<th>Group</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal α-carboxyl</td>
<td>3-4</td>
</tr>
<tr>
<td>Side-chain carboxyl</td>
<td>6-5</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6-7</td>
</tr>
<tr>
<td>Terminal α-amino</td>
<td>7.5-9</td>
</tr>
<tr>
<td>Thiol</td>
<td>8-9.5</td>
</tr>
<tr>
<td>Phenol</td>
<td>9.5-10</td>
</tr>
<tr>
<td>α-Amino</td>
<td>−10</td>
</tr>
<tr>
<td>Guanidine</td>
<td>−12</td>
</tr>
<tr>
<td>Hydroxyethyl</td>
<td>−16</td>
</tr>
</tbody>
</table>

- General Acid-Base Catalysis
  - $H^+$ and $HO^-$ are “specific acid/base” and depend on pH
  - Amino acid sidechains are general acid-base, and can conduct reactions inside active site pocket that aren’t possible in solution
What’s Wrong with This?

\[
\begin{align*}
\text{Ketone} & \quad \begin{array}{c}
R \\
C=O \\
\text{CH}_2 \\
\text{H}
\end{array} & \quad \begin{array}{c}
R \\
\text{C} \cdots \text{O}^- \\
\text{CH}_2 \\
\text{H}^+
\end{array} & \quad \begin{array}{c}
R \\
\text{C} \cdots \text{O}^{-} \\
\text{CH}_2
\end{array} & \quad \begin{array}{c}
R \\
\text{C} \cdots \text{O}^{-} \\
\text{CH}_2
\end{array} & \quad \begin{array}{c}
R \\
\text{C} \cdots \text{O}^{-} \\
\text{CH}_2
\end{array} & \quad \begin{array}{c}
R \\
\text{C} \cdots \text{O}^{-} \\
\text{CH}_2
\end{array}
\end{align*}
\]

Ketone \quad Transition state \quad Enol

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Triose Phosphate Isomerase

\[
\begin{align*}
{}^1\text{CH}_2\text{OH} & \quad \begin{array}{c}
{}^1\text{CH}_2\text{OH}
\end{array} & \quad \begin{array}{c}
\text{C} \cdots \text{O}
\end{array} & \quad \begin{array}{c}
{}^1\text{CH}_2\text{OPO}_3^{\text{+}}
\end{array} & \quad \begin{array}{c}
{}^1\text{CH}_2\text{OH}
\end{array} \quad \text{Triose phosphate isomerase} & \quad \begin{array}{c}
\text{H}
\end{array} & \quad \begin{array}{c}
\text{C} \cdots \text{O}
\end{array} & \quad \begin{array}{c}
\text{H} \cdots \text{OH}
\end{array} & \quad \begin{array}{c}
{}^1\text{CH}_2\text{OPO}_3^{\text{+}}
\end{array} \quad \text{\(\Delta\)-Glyceraldehyde 3-phosphate (G3P)}
\end{align*}
\]

Dihydroxyacetone phosphate (DHAP) \quad \text{Triose phosphate isomerase} \quad \alpha\text{-Glyceraldehyde 3-phosphate (G3P)}
Mechanism

Be able to explain catalytic function of AA in each step of a mechanism

2. Covalent Catalysis

- Can act as active site nucleophile
- Can produce a more reactive electrophile
- Example:

\[
\begin{align*}
\text{Acetoacetate} & \rightarrow \text{Enolate} \rightarrow \text{Acetone} \\
& \text{H}_3\text{C} - \text{C} = \text{CH}_2 - \text{CO} & \text{H}_3\text{C} - \text{C} = \text{CH}_2 & \text{H}_3\text{C} - \text{C} = \text{CH}_2
\end{align*}
\]
Covalent Intermediate

\[
\begin{align*}
\text{Acetoacetate} & \quad \text{Schiff base (imine)} \\
H_3C-C\text{-CH}_2-C\text{O}^+ & + \text{RNH}_2 \rightarrow H_3C-C\text{-CH}_2-C\text{O}^- + \text{OH}^-
\end{align*}
\]

3. Metal Ion Catalysis

- Redox reactions
- Stabilization of charges

\[
\begin{align*}
\text{Acetaldehyde} & \quad \text{Ethanol} \\
H_3C-C\text{CHO} & \quad H_3C-C\text{CH}_2\text{OH} \\
\text{Zn}^{2+} & \quad 2\text{H}
\end{align*}
\]
Metalloprotease

- Problem 47: Propose a mechanism for this protease:

\[ \text{Metalloprotease} \]

\[ \text{ pH affects Enzyme Catalysis} \]

Propose possible explanations of pH profile
Binding Energy

- Binding based on intermolecular forces
- “Lock and Key”
- Selectivity
- Rate Enhancement
  - Effective concentration
  - Entropy trap

Productive orientation of two molecules in the active site
Effective Molarity

- May be higher than actual molarity possibility
- Entropic help

Induced Fit

- “Lock and Key” too simplistic
- Enzymes are actually somewhat flexible
- Substrate specificity comes at catalytic price
- $k_{\text{cat}} = 10^3$ per second, but worth cost
Lowering Activation Energy

- Transition state stabilization is half the story

Weak Binding of Substrate

- Substrate binding: too much of a good thing
- Thermodynamic pit
- $K_M \sim 10^{-4}$ M
- Can be $10^{-6}$ M for cofactors
Case Study: Chymotrypsin

- Serine protease
- Catalytic triad
- Oxyanion hole
Substrate Specificity

- Specificity pocket
- Binding affinity
- Promiscuity