How Enzymes Work

Pratt & Cornely Ch 6

Enzymes

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

\[
\begin{align*}
R - NH - CH - C\text{H} & \xrightarrow{\text{enzyme}} R - NH - C\text{H} - COO^- \\
+ H_2O & \rightarrow R - NH - CH - C\text{H} + H_3O^+
\end{align*}
\]
Rate Enhancement

Orotidine Decarboxylase

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

### TABLE 6-1: Rate Enhancements of Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Half-Time (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>$2.8 \times 10^{-5}$</td>
<td>39</td>
<td>$1.4 \times 10^{9}$</td>
</tr>
<tr>
<td>Staphylococcal nuclease</td>
<td>130,000 years</td>
<td>$1.7 \times 10^{-5}$</td>
<td>95</td>
<td>$5.6 \times 10^{5}$</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>120 years</td>
<td>$1.8 \times 10^{-9}$</td>
<td>370</td>
<td>$2.1 \times 10^{4}$</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>20 years</td>
<td>$1.0 \times 10^{-6}$</td>
<td>190</td>
<td>$1.7 \times 10^{3}$</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>1.9 years</td>
<td>$4.3 \times 10^{-4}$</td>
<td>4,300</td>
<td>$1.0 \times 10^{3}$</td>
</tr>
<tr>
<td>Chorismate mutase</td>
<td>2.4 hours</td>
<td>$2.6 \times 10^{-3}$</td>
<td>50</td>
<td>$1.9 \times 10^{4}$</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>5 seconds</td>
<td>$1.3 \times 10^{-1}$</td>
<td>1,000,000</td>
<td>$7.7 \times 10^{4}$</td>
</tr>
</tbody>
</table>

*The half times of very slow reactions were estimated by extrapolating from measurements made at very high temperatures. (Data mostly from Radicka, R., and Wolfenden, R., Science 267, 99–93 (1995)).

© John Wiley & Sons, Inc. All rights reserved.
The Speed of the Uncatalyzed Rxn

Figure 4  Temperature dependence of the rate of decarboxylation of 1-methylorotate in 0.1 M potassium phosphate buffer, pH 6.8 (6).

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**

   
   ![Oxidoreductase Reaction Equation](image)

2. **Transferase**

   
   ![Transferase Reaction Equation](image)
Enzyme Classes

3. Hydrolase

\[ \text{Pyrophosphate} \rightarrow \text{Phosphate} + \text{H}_2\text{O} \]

Pyrophosphatase

4. Lyase

\[ \text{Pyruvate} \rightarrow \text{Acetaldehyde} + \text{Carbon dioxide} \]

Pyrurate decarboxylase

5. Isomerase

\[ \text{L-Alanine} \leftrightarrow \text{D-Alanine} \]

Alanine racemase

6. Ligase

\[ \text{L-Glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{Glutamine} + \text{ADP} + \text{P}_i \]
Problem 10

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

![Chemical structures]

Problems 11-12

- Draw the structures of the products

![Chemical reactions]

succinate dehydrogenase

malate dehydrogenase
Problem 14

- Propose a name for each enzyme.

![Enzyme structures](image)

- Catalysis
  - Thermodynamics
  - Kinetics
  - Rxn coordinate
  - Transition state
  - $5.7 \text{ kJ} \sim 10x$ 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 6.2 Typical $pK_a$ values of reactive groups of amino acids in proteins

<table>
<thead>
<tr>
<th>Group</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal $\alpha$-carboxyl</td>
<td>3-4</td>
</tr>
<tr>
<td>Sidechain $\alpha$-carboxyl</td>
<td>4-5</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6-7</td>
</tr>
<tr>
<td>Terminal $\alpha$-amino</td>
<td>7.5-9</td>
</tr>
<tr>
<td>Thiol</td>
<td>8-9.5</td>
</tr>
<tr>
<td>Phenol</td>
<td>9.3-10</td>
</tr>
<tr>
<td>$\epsilon$-Amino</td>
<td>$\sim$10</td>
</tr>
<tr>
<td>Guanidine</td>
<td>$\sim$12</td>
</tr>
<tr>
<td>Hydroxyethyl</td>
<td>$\sim$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?

\[
\begin{align*}
\text{Ketone} & \quad \text{Transition state} \quad \text{Enol} \\
\text{CH}_2 & \quad \vdash \quad \vdash \\
\text{H} & \quad \vdash \\
\end{align*}
\]
Triose Phosphate Isomerase

Dihydroxyacetone phosphate (DHAP) $\xrightarrow{\text{Triose phosphate isomerase}}$ \( \beta \)-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:

\[
\begin{align*}
\text{Acetoacetate} & \xrightarrow{\text{CO}_2} \text{Enolate} \xrightarrow{\text{H}^+} \text{Acetone} \\
H_2C-C^=CH_2-C^=O & \rightarrow [H_2C-C^{=}CH_2]^{-} \rightarrow H_2C-C^{=}CH_3 \\
\text{Acetoacetate} & \rightarrow \text{Schiff base (imine)}
\end{align*}
\]
3. Metal Ion Catalysis

- Redox reactions
- Stabilization of charges

\[
\text{Acetaldehyde} \quad \rightleftharpoons \quad \left[ \text{H}_3\text{C} - \text{C} - \text{O} \right]^{\text{Zn}^{2+}} \quad 2 \text{H} \quad \rightleftharpoons \quad \text{H}_3\text{C} - \text{C} - \text{H}^{\text{OH}} \quad \text{Ethanol}
\]

Metalloprotease

- Problem 47: Propose a mechanism for this protease:
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
- $K_M \approx 10^{-4}$ M
- Can be $10^{-6}$ M for cofactors

Case Study: Chymotrypsin

- Serine protease
- Catalytic triad
- Oxyanion hole
Substrate Specificity

- Specificity pocket
- Binding affinity
- Promiscuity