Chapter 13. Introduction to Enzymes
Enzyme reaction

- Much higher reaction rates – $10^6$ to $10^{14}$ times faster than uncatalyzed reactions
- Milder reaction conditions – Low temperature, 1 atm, near neutral pH
- Greater reaction specificity – Specific for substrates, no side products
- Capacity for regulation – Enzymatic activity varies in response to the substrate concentration
Turnover

- Number of catalytic event per unit time (s\(^{-1}\))
- Typical value: 1 – 10\(^6\) s\(^{-1}\)

\[ \text{e.g. Urease} \]

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{H}_2\text{N} \\
\text{O} & \quad \text{O}
\end{align*}
\]

Urea

Nonenzymatic reaction rate: 3 x 10\(^{-10}\) s\(^{-1}\) (t\(_{1/2}\)=7.3 yrs)
Enzymatic reaction rate: 3 x 10\(^4\) s\(^{-1}\) (t\(_{1/2}\) = 20 \(\mu\)sec)
Rate acceleration = 10\(^{14}\)

Carbonic anhydrase

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \quad \leftrightarrow \quad \text{HCO}_3^- + \text{H}^+
\end{align*}
\]

Nonenzymatic reaction rate: 3.8 x 10\(^{-2}\) s\(^{-1}\) (t\(_{1/2}\)=18 sec)
Enzymatic reaction rate: 1 x 10\(^6\) s\(^{-1}\) (t\(_{1/2}\) = 0.1 \(\mu\)sec)
Rate acceleration = 10\(^8\)
### Turnover numbers, $k_3$, for some enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$k_3$ (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>$\text{H}_2\text{O}_2$</td>
<td>40,000,000</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>$\text{HCO}_3^-$</td>
<td>400,000</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
<td>25,000</td>
</tr>
<tr>
<td>Penicillinase</td>
<td>Benzylpenicillin</td>
<td>2,000</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Lactate</td>
<td>1,000</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Glycyltyrosinylglycine</td>
<td>100</td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>DNA</td>
<td>15</td>
</tr>
<tr>
<td>Ribulose-1,5-bisphosphate carboxylase</td>
<td>Ribulose-1,5-bisphosphate + CO$_2$</td>
<td>3.3</td>
</tr>
<tr>
<td>Ribulose-1,5-bisphosphate oxygenase</td>
<td>Ribulose-1,5-bisphosphate + O$_2$</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Cofactors

• Metal ions - Mn$^{2+}$, Mg$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Fe$^{2+}$
• Coenzymes
  – Cosubstrates (Transient binding)
  – Prosthetic groups (Tight covalent binding)
• Holoenzyme vs. apoenzyme

Apoenzyme (inactive) + cofactor $\rightleftharpoons$ holoenzyme (active)
# Common coenzymes

<table>
<thead>
<tr>
<th>Coenzyme</th>
<th>Reaction Mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>Carboxylation</td>
</tr>
<tr>
<td>Cobalamin (B\textsubscript{12}) coenzymes</td>
<td>Alkylation</td>
</tr>
<tr>
<td>Coenzyme A</td>
<td>Acyl transfer</td>
</tr>
<tr>
<td>Flavin coenzymes</td>
<td>Oxidation–reduction</td>
</tr>
<tr>
<td>Lipoic acid</td>
<td>Acyl transfer</td>
</tr>
<tr>
<td>Nicotinamide coenzymes</td>
<td>Oxidation–reduction</td>
</tr>
<tr>
<td>Pyridoxal phosphate</td>
<td>Amino group transfer</td>
</tr>
<tr>
<td>Tetrahydrofolate</td>
<td>One-carbon group transfer</td>
</tr>
<tr>
<td>Thiamine pyrophosphate</td>
<td>Aldehyde transfer</td>
</tr>
</tbody>
</table>
Water-soluble vitamins are coenzyme precursors

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Coenzyme</th>
<th>Human Deficiency Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>Biocytin</td>
<td>a</td>
</tr>
<tr>
<td>Cobalamin (B₁₂)</td>
<td>Cobalamin (B₁₂) coenzymes</td>
<td>Pernicious anemia</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Tetrahydrofolate</td>
<td>Megaloblastic anemia</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>Nicotinamide coenzymes</td>
<td>Pellagra</td>
</tr>
<tr>
<td>Pantothenate</td>
<td>Coenzyme A</td>
<td>a</td>
</tr>
<tr>
<td>Pyridoxine (B₆)</td>
<td>Pyridoxal phosphate</td>
<td>a</td>
</tr>
<tr>
<td>Riboflavin (B₂)</td>
<td>Flavin coenzymes</td>
<td>a</td>
</tr>
<tr>
<td>Thiamine (B₁)</td>
<td>Thiamine pyrophosphate</td>
<td>Beriberi</td>
</tr>
</tbody>
</table>

*aNo specific name; deficiency in humans is rare or unobserved.*
Biotin (B₇)  Thiamine (B₁)  Cobalamine (B₁₂)

Nicotinamide (B₃)  Riboflavin (B₂)  Pyridoxine (B₆)

Pantothenic acid  Folic acid (B₉)
Substrate specificity

- Lock-and-key model
- Induced-fit model

Van der Waals interactions
Electrostatic interactions
Hydrogen bondings
Hydrophobic interactions
Stereospecificity

- Enzymes are highly specific both in binding chiral substrate and in catalyzing their reactions.
- Enzymes provide asymmetric active sites due to their inherent chirality.

Enzyme

\[
\begin{align*}
\text{Citrate} & \quad \text{Isocitrate} \\
\text{Prochiral} & \quad \text{(prochiral)}
\end{align*}
\]

\[
\begin{align*}
\text{aconitase}
\end{align*}
\]
The structure and reaction of NAD$^+$

Nicotinamide

$X = H$  \text{Nicotinamide adenine dinucleotide (NAD$^+$)}

$X = PO_3^{2-}$  \text{Nicotinamide adenine dinucleotide phosphate (NADP$^+$)}
Alcohol dehydrogenase (ADH)

\[
\text{Ethanol} \quad \text{ADH} \quad \text{Acetaldehyde}
\]

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ & \quad \overset{\text{ADH}}{\rightleftharpoons} \quad \text{CH}_3\text{CH} + \text{NADH} + \text{H}^+ \\
\end{align*}
\]
Stereochemistry of the ADH reaction

\[
\text{ADH} \quad \text{+} \quad \text{DODH} \quad \leftrightarrow \quad \text{ADH} \quad \text{+} \quad \text{DODH} + \text{H}^+ \]

Westheimer & Vennesland (1953)
Stereochemistry of the ADH reaction

Westheimer & Vennesland (1953)
Substrate specificity

- Some enzymes are extremely specific
  - e.g. DNA and RNA polymerases
- Other enzymes recognize related molecules
  - e.g. ADH
- Some enzymes have very broad specificity
  - e.g. Digestive enzymes (peptidases, lipases etc)
The Arrhenius equation is given by:

\[ k = A e^{-\frac{E_a}{RT}} \]

where:
- \( k \) is the rate constant,
- \( A \) is the pre-exponential factor or the frequency factor,
- \( E_a \) is the activation energy,
- \( R \) is the gas constant, and
- \( T \) is the temperature.

The diagram illustrates a free energy profile with:
- \( A + B \) as the initial reactants,
- \( P + Q \) as the products,
- \( X^\dagger \) as the intermediate state,
- \( \Delta G^\dagger \) as the activation energy, and
- \( \Delta G_{reaction} \) as the reaction free energy change.

The activation energy \( E_a \) is the energy barrier that needs to be overcome for the reaction to proceed.
Multistep reaction

Rate-determining step: the slowest step (= the highest activation energy)
Principles of catalysis

3% H₂O₂
37°C
+ Fe³⁺ + Catalase

(d) Higher temperature

- Lower barrier height (activation energy)
- Stabilization of transition state
- Destabilization of the ground state
Catalysts reduce the activation energy

Rate enhancement = \( \frac{k_{\text{cat}}}{k_{\text{uncat}}} = e^{\frac{\Delta E_a}{RT}} \)

e.g. When \( \Delta E_a = 5.7 \text{ kJ/mol} \), the reaction rate increases 10 fold
When \( \Delta E_a = 34 \text{ kJ/mol} \), the reaction rate increases \( 10^6 \) fold
Catalytic mechanisms

- Proximity and orientation effects
- Covalent catalysis – intermediates
- Acid/Base catalysis – removal and addition of H⁺
- Metal ion catalysis – Stabilization of electrophiles or generation of nucleophiles etc.
- Stabilization of transition state
- Strain and/or distortion of substrates
Regulation of enzymatic activity

- Enzyme availability
  - Rate of synthesis
  - Rate of degradation

- Enzyme’s substrate binding affinity
  - Feedback inhibition
  - Allosteric control
Allosteric effectors of aspartate transcarboxylase (ATCase)

Graph showing the relative reaction rate against [Aspartate] (mM) with different effectors:
- ATP (activator)
- CTP (inhibitor)
- No allosteric effectors
Feedback inhibition

Carbamoyl phosphate + Aspartate $\xrightarrow{\text{ATCase}}$ N-Carbamoyl aspartate

INHIBITION

6 enzymatic reaction steps

NH$_2$

Cytidine triphosphate (CTP)
Structural basis of allosterism in ATCase

T state (inactive, CTP binding)  R state (active, ATP binding)
IUBMB nomenclature of enzymes

Enzyme Classification According to Reaction Type

<table>
<thead>
<tr>
<th>Classification</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</td>
</tr>
<tr>
<td>6. Ligases</td>
<td>Bond formation coupled with ATP hydrolysis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Common name</th>
<th>Systematic name</th>
<th>Classification number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol dehydrogenase</td>
<td>Alcohol:NAD⁺ oxidoreductase</td>
<td>EC 1.1.1.1</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>Peptidyl-L-aminoacid hydrolase</td>
<td>EC 3.4.17.1</td>
</tr>
</tbody>
</table>

Enzyme Nomenclature Database (http://www.chem.qmw.ac.uk/iubmb/enzyme)