CHEM 529
Enzyme and Coenzyme Mechanisms

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Map of the major metabolic pathways in a typical cell
ATP and NADPH are sources of free energy for biosynthetic reactions.

Diagram:
- Complex metabolites
  - ADP + HPO$_4^{2-}$
  - NADP$^+$
  - ATP
  - NADPH
- Degradation
- Biosynthesis
- Simple products
Overview of human catabolism
Review of biologically-important functional groups and their properties

blackboard discussion
Basic reaction of an amine: \[ R\text{-}\text{NH}_2 + H^+ \rightarrow R\text{-}N^+\text{-}H \]

Nucleophilic reaction of an amine: \[ R\text{-}\text{NH}_2 + \begin{array}{c} R' \text{C} = O \rightarrow R\text{-}N\text{-}C\text{-}OH \end{array} \]

Imine formation: \[ \begin{array}{c} R\text{-}\text{NH}_2 + \text{C} = O \rightarrow R\text{-}N\text{-}C\text{-}OH \end{array} \]
Proposed mechanism of rabbit muscle aldolase: Imine (Schiff base) intermediate
The bovine pancreatic RNase A catalyzed hydrolysis of RNA is a two-step process with the intermediate formation of a 2',3'-cyclic nucleotide.

two histidines serve as proton donor and acceptor; an acid-base catalytic mechanism
Catalytic mechanism of serine proteases

1. Nucleophilic attack
   Substrate polypeptide
   Enzyme–substrate complex

2. Water displacement
   Tetrahedral intermediate
   New N-terminus of cleaved polypeptide chain
   Acyl–enzyme intermediate

3. Deacylation
   Tetrahedral intermediate

4. Hydrolysis
   New C-terminus of cleaved polypeptide chain
   Active enzyme
Free energies of hydrolysis of some biologically-important compounds
Standard free energies of hydrolysis of common functional groups in biochemistry

- **Enol phosphate bond**
  - Reaction: \( \text{H}_2\text{C}=\text{C}-\text{O} \rightarrow \text{HO}_2^+ \)
  - Standard free energy change (\( \Delta G^\circ \)): -14.8 (-61.9)

- **Anhydride bond to carbon**
  - Reaction: \( \text{C}-\text{O} \rightarrow \text{CO} \)
  - Example: 1,3-bisphosphoglycerate
    - Reaction: \( \text{HO}_2^+ \rightarrow \text{HO}_2^- \)
    - Standard free energy change (\( \Delta G^\circ \)): -11.7 (-49.0)

- **Phosphate bond in creatine phosphate**
  - Reaction: \( \text{N}^+ \text{H}_2 \text{C}-\text{C}-\text{N}^- \text{H}_2 \text{C}-\text{N}^- \text{H}_2 \text{C} \rightarrow \text{N}^+ \text{H}_2 \text{C}-\text{C}-\text{N}^- \text{H}_2 \text{C}-\text{N}^- \text{H}_2 \text{C} \)
  - Example: Creatine phosphate
    - Reaction: \( \text{N}^+ \text{H}_2 \text{C}-\text{C}-\text{N}^- \text{H}_2 \text{C}-\text{N}^- \text{H}_2 \text{C} \rightarrow \text{N}^+ \text{H}_2 \text{C}-\text{C}-\text{N}^- \text{H}_2 \text{C}-\text{N}^- \text{H}_2 \text{C} \)
    - Standard free energy change (\( \Delta G^\circ \)): -10.3 (-43.0)

- **Anhydride bond to phosphate** (phospho-anhydride bond)
  - Reaction: \( \text{O}^+ \text{H}_2 \text{O} \rightarrow \text{O}^- \text{H}_2 \text{O} \)
  - Example: ATP when hydrolyzed to ADP
    - Reaction: \( \text{O}^+ \text{H}_2 \text{O} \rightarrow \text{O}^- \text{H}_2 \text{O} \)
    - Standard free energy change (\( \Delta G^\circ \)): -7.3 (-30.6)

- **Phosphoester bond**
  - Reaction: \( \text{C}-\text{O} \rightarrow \text{O}^-\text{H}_2 \text{O} \)
  - Example: Glucose 6-phosphate
    - Reaction: \( \text{C}-\text{O} \rightarrow \text{O}^-\text{H}_2 \text{O} \)
    - Standard free energy change (\( \Delta G^\circ \)): -3.3 (-17.5)

**Type of phosphate bond**

**Specific examples showing the standard free-energy change (\( \Delta G^\circ \)) for hydrolysis of phosphate bond**

*Figure 2-74 Molecular Biology of the Cell S/e © Garland Science 2008*
Standard free energies of hydrolysis of some phosphate-containing compounds of biological interest

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\Delta G$ (kJ⋅mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoenolpyruvate</td>
<td>$-61.9$</td>
</tr>
<tr>
<td>1,3-Bisphosphoglycerate</td>
<td>$-49.4$</td>
</tr>
<tr>
<td>Acetyl phosphate</td>
<td>$-43.1$</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>$-43.1$</td>
</tr>
<tr>
<td>PP$_i$</td>
<td>$-33.5$</td>
</tr>
<tr>
<td>ATP ($\rightarrow$ AMP + PP$_i$)</td>
<td>$-32.2$</td>
</tr>
<tr>
<td>ATP ($\rightarrow$ ADP + P$_i$)</td>
<td>$-30.5$</td>
</tr>
<tr>
<td>Glucose-1-phosphate</td>
<td>$-20.9$</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>$-13.8$</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>$-13.8$</td>
</tr>
<tr>
<td>Glycerol-3-phosphate</td>
<td>$-9.2$</td>
</tr>
</tbody>
</table>

The flow of phosphoryl groups from high-energy phosphate donors, via the ATP–ADP system, to low-energy phosphate acceptors (note the central role of ATP as energy currency).
A covalent bond is formed between glyceraldehyde 3-phosphate (the substrate) and the –SH group of a cysteine side chain of the enzyme glyceraldehyde 3-phosphate dehydrogenase, which also binds noncovalently to NAD$^+$. 

Oxidation of glyceraldehyde 3-phosphate occurs, as two electrons plus a proton (a hydride ion, see Figure 2–60) are transferred from glyceraldehyde 3-phosphate to the bound NAD$^+$, forming NADH. Part of the energy released by the oxidation of the aldehyde is thus stored in NADH, and part goes into converting the bond between the enzyme and its substrate glyceraldehyde 3-phosphate into a high-energy thioester bond.

A molecule of inorganic phosphate displaces the high-energy bond to the enzyme to create 1,3-bisphosphoglycerate which contains a high-energy acyl-anhydride bond.

**Generation of a high-energy phosphate in glycolysis**
Explanation of the very negative change in standard free energy associated with the pyruvate kinase reaction

Phosphoenol-pyruvate (PEP) + Mg$^{2+}$ + K$^+$ → ADP + Mg$^{2+}$ + K$^+$

1 ATP formation

ATP + Mg$^{2+}$ + H$^+$ → Enolpyruvate + Mg$^{2+}$

2 Tautomerization

Enolpyruvate → Pyruvate

Hydrolysis

$G^0 = -16 \text{ kJ} \cdot \text{mol}^{-1}$

$\text{Phosphoenol-pyruvate} \rightarrow \text{Pyruvate (enol form)} + \text{H}_2\text{O}$

Tautomerization

$G^0 = -46 \text{ kJ} \cdot \text{mol}^{-1}$

$\text{Pyruvate (enol form)} \leftrightarrow \text{Pyruvate (keto form)}$

Overall reaction

$G^0 = -61.9 \text{ kJ} \cdot \text{mol}^{-1}$

$\text{Phosphoenol-pyruvate} \rightarrow \text{Pyruvate} + \text{H}_2\text{O} + \text{HPO}_4^{2-}$

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**Coupled reactions involving ATP synthesis:** The pyruvate kinase-catalyzed phosphorylation of ADP by phosphoenolpyruvate (PEP) to form ATP and pyruvate (the second substrate-level phosphorylation reaction of glycolysis)

Exergonic half-reaction 1

\[
\text{CH}_2=\text{C}^{\text{COO}^-} + \text{H}_2\text{O} \rightleftharpoons \text{CH}_3=\text{C}^{\text{COO}^-} + \text{P}_i
\]

\[\Delta G^{\circ} (\text{kJ} \cdot \text{mol}^{-1}) = -61.9\]

<table>
<thead>
<tr>
<th><strong>Phosphoenolpyruvate</strong></th>
<th><strong>Pyruvate</strong></th>
</tr>
</thead>
</table>

Endergonic half-reaction 2

\[
\text{ADP} + \text{P}_i \rightleftharpoons \text{ATP} + \text{H}_2\text{O}
\]

\[\Delta G^{\circ} (\text{kJ} \cdot \text{mol}^{-1}) = +30.5\]

Overall coupled reaction

\[
\text{CH}_2=\text{C}^{\text{COO}^-} + \text{ADP} \rightleftharpoons \text{CH}_3=\text{C}^{\text{COO}^-} + \text{ATP}
\]

\[\Delta G^{\circ} (\text{kJ} \cdot \text{mol}^{-1}) = -31.4\]
Acetyl CoA: A biological thioester

Acetyl-coenzyme A

Coenzyme A = CoASH
Acetyl-CoA hydrolysis

\[
\text{Acetyl-CoA} + \text{H}_2\text{O} \rightarrow \text{acetate}^- + \text{CoA} + \text{H}^+ \\
\Delta G^{\circ} = -31.4 \text{ kJ/mol}
\]
Explanation of the energetics of acetyl CoA hydrolysis

Thioester

\[ \text{CH}_3\text{C} = \text{O} \quad \text{S} \rightarrow \text{R} \]

\[ \Delta G \text{ for thioester hydrolysis} \]

\[ \text{CH}_3\text{C} = \text{C} + \text{R-SH} \]

Oxygen ester

Extra stabilization of oxygen ester by resonance

\[ \text{O} \rightarrow \text{R} \]

\[ \text{CH}_3\text{C} = \text{C} \quad \delta^- \rightarrow \text{O} \]

\[ \Delta G \text{ for oxygen ester hydrolysis} \]

\[ \text{CH}_3\text{C} = \text{C} + \text{R-OH} \]
Some fundamental chemical mechanisms
Modes of $\text{C—H}$ bond breaking

**Homolytic:**

\[
\text{C} - \text{H} \xrightarrow{\text{homolytic cleavage}} \text{C}^\cdot + \text{H}^\cdot
\]

**Radicals**

**Heterolytic:**

(i) \[
\text{C} \xrightarrow{} \text{C}^- + \text{H}^+
\]

Carbanion Proton

(ii) \[
\text{C}^+ + \text{H}^-
\]

Carbocation Hydride ion
Biologically-important nucleophilic groups

(a) Nucleophiles

\[
\begin{align*}
R\overset{\cdot}{\cdot}H & \iff \overset{\cdot}{\cdot}RO^{-} \quad \text{+ } H^+ \quad \text{Hydroxyl group} \\
R\overset{\cdot}{\cdot}SH & \iff \overset{\cdot}{\cdot}RS^{-} \quad \text{+ } H^+ \quad \text{Sulfhydryl group} \\
RNH_3^+ & \iff RNH_2 \quad \text{+ } H^+ \quad \text{Amino group} \\
\end{align*}
\]
Biologically-important electrophilic groups

(b) Electrophiles

\[ \text{H}^+ \quad \text{Protons} \]

\[ M^{n+} \quad \text{Metal ions} \]

R
\[
\text{C}==\text{O} \quad \text{Carbonyl carbon atom}
\]
R'

R
\[
\text{C}==\text{NH}^+ \quad \text{Cationic imine (Schiff base)}
\]
R'
Metabolic group-transfer reactions:  
**Acyl group transfer**

\[
\begin{align*}
R-\overset{\text{O}}{C}X + Y^- & \rightarrow R-\overset{\text{O}^-}{C}X \rightarrow R-\overset{\text{O}}{C}Y + X^- \\
& \text{Tetrahedral intermediate}
\end{align*}
\]
Metabolic group-transfer reactions: 

Phosphoryl group transfer

\[
\begin{align*}
Y^- + \overset{\text{X}}{\overset{\text{P}}{\text{O}}} & \rightarrow \left[ \overset{\text{X}}{\overset{\text{P}}{\text{O}}} \right] \\
& \rightarrow \overset{\text{Y}}{\overset{\text{P}}{\text{O}}} O^- \\
& + X^-
\end{align*}
\]

Trigonal bipyramidal intermediate
A phosphoryl group transfer reaction: Hexokinase

Glucose + ATP → Glucose-6-phosphate + ADP

Trigonal bipyramid intermediate
Metabolic group-transfer reactions: 
**Glycosyl group transfer**
Elimination reaction mechanisms using dehydration as an example

**Concerted**

\[
\begin{align*}
R - C - C - R' & \rightarrow C = C + H^+ + OH^- \\
H & \quad \quad \quad \quad \quad \quad H \\
& \quad \quad \quad \quad \quad \quad \text{OH} \\
& \quad \quad \quad \quad \quad \quad \text{H} \\
& \quad \quad \quad \quad \quad \quad \text{R'}
\end{align*}
\]

**Stepwise via a carbocation**

\[
\begin{align*}
R - C - C - R' & \rightarrow R - C - C - R' + H^+ + OH^- \\
H & \quad \quad \quad \quad \quad \quad H \\
& \quad \quad \quad \quad \quad \quad \text{OH} \\
& \quad \quad \quad \quad \quad \quad \text{OH} \\
& \quad \quad \quad \quad \quad \quad \text{H} \\
& \quad \quad \quad \quad \quad \quad \text{R'} \\
& \quad \quad \quad \quad \quad \quad \text{H} \\
& \quad \quad \quad \quad \quad \quad \text{R'}
\end{align*}
\]

**Stepwise via a carbanion**

\[
\begin{align*}
R - C - C - R' & \rightarrow R - C - C - R' + H^+ + OH^- \\
H & \quad \quad \quad \quad \quad \quad H \\
& \quad \quad \quad \quad \quad \quad \text{OH} \\
& \quad \quad \quad \quad \quad \quad \text{H} \\
& \quad \quad \quad \quad \quad \quad \text{H} \\
& \quad \quad \quad \quad \quad \quad \text{R'} \\
& \quad \quad \quad \quad \quad \quad \text{H} \\
& \quad \quad \quad \quad \quad \quad \text{R'}
\end{align*}
\]
SCHEME 10.2 Three general mechanisms for dehydration.
Elimination reaction mechanisms using dehydration as an example

Reaction stereochemistry

\[ \begin{align*}
R - C - C - R' \quad &\rightarrow \quad \text{trans (anti)} \\
\text{H} &\quad \text{H} \\
\text{H} &\quad \text{OH} \\
\text{H} &\quad \text{OH} \\
\text{H} &\quad \text{H} \\
\end{align*} \]

\[ \begin{align*}
\text{H}^+ + \text{OH}^- \\
\text{H}^+ + \text{OH}^- \\
\end{align*} \]
Example of an enzyme-catalyzed elimination reaction

SCHEME 10.1  Reactions catalyzed by dehydratases and hydratases.
Mechanism of aldose-ketose isomerization

Aldose

B: \[ \overset{\text{H}}{\text{C}} \overset{\text{O}}{\text{H}} \overset{\text{R}}{\text{H}} \]

\[ \overset{\text{B}}{\text{H}} \overset{\text{C}}{\text{O}} \overset{\text{H}}{\text{H}} \]

Ketose

B: \[ \overset{\text{H}}{\text{C}} \overset{\text{O}}{\text{H}} \overset{\text{R}}{\text{R}} \]

\[ \overset{\text{B}}{\text{H}} \overset{\text{C}}{\text{O}} \overset{\text{O}}{\text{H}} \]

\[ \overset{\text{BH}^+}{\text{C}} \overset{\text{O}}{\text{H}} \overset{\text{R}}{\text{R}} \]

\[ \overset{\text{BH}^+}{\text{C}} \overset{\text{O}}{\text{O}} \overset{\text{H}}{\text{H}} \]

\[ \overset{\text{cis-Enediolate intermediates}}{\text{B}} \]
C—C bond formation and cleavage reactions: 
\textit{Aldol condensation}

(a) \textit{Aldol condensation}

\begin{align*}
\text{Ketone} & \quad \text{Resonance-stabilized carbanion (enolate)}
\end{align*}

Second ketone (electrophilic center)
C—C bond formation and cleavage reactions: Claisen condensation (ester)

(b) Claisen ester condensation

\[ \text{Acetyl-CoA} \quad \rightleftharpoons \quad \text{Addition to electrophilic center [as in (a)]} \]

Resonance-stabilized enolate
C–C bond formation and cleavage reactions: Decarboxylation of a β-keto acid

\[ \overset{\text{R–C–CH}_2}{\text{O}} \overset{\text{O}}{\text{C–O}} \iff \text{CO}_2 + \begin{cases} \text{O} \\
\text{R–C–CH}_2 \\
\text{O}^- \\
\text{R–C–CH}_2 
\end{cases} \rightarrow \text{Addition to electrophilic center [as in (a)]} \]

\( \beta \)-Keto acid

Resonance-stabilized enolate