Chapter 7 - Coenzymes and Vitamins

- Some enzymes require **cofactors** for activity
  
  (1) **Essential ions** (mostly metal ions)
  (2) **Coenzymes** (organic compounds)

\[
\text{Apoenzyme} + \text{Cofactor} \rightarrow \text{Holoenzyme}
\]

(protein only)   (active)

(inactive)
Coenzymes

- Coenzymes act as group-transfer reagents
- Hydrogen, electrons, or other groups can be transferred
- Larger mobile metabolic groups can be attached at the reactive center of the coenzyme
- Coenzyme reactions can be organized by their types of substrates and mechanisms
Types of cofactors

- Essential ions
  - Activator ions (loosely bound)
  - Metal ions of metalloenzymes (tightly bound)
- Coenzymes
  - Cosubstrates (loosely bound)
  - Prosthetic groups (tightly bound)
Many Enzymes Require Inorganic Cations

- Enzymes requiring metal ions for full activity:
  1. **Metal-activated enzymes** have an absolute requirement or are stimulated by metal ions (examples: $K^+$, $Ca^{2+}$, $Mg^{2+}$)
  2. **Metalloenzymes** contain firmly bound metal ions at the enzyme active sites (examples: iron, zinc, copper, cobalt)
Mechanism of carbonic anhydrase

• Action of carbonic anhydrase, a metalloenzyme

• Zinc ion promotes the ionization of bound $\text{H}_2\text{O}$. Resulting nucleophilic $\text{OH}^-$ attacks carbon of $\text{CO}_2$

Iron in metalloenzymes

• Iron undergoes reversible oxidation and reduction:

\[ \text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+} \]

• Enzyme heme groups and cytochromes contain iron

• Nonheme iron exists in iron-sulfur clusters (iron is bound by sulfide ions and S\(^-\) groups from cysteines)

• Iron-sulfur clusters can accept only one e\(^-\) in a reaction
Iron-sulfur clusters

- Iron atoms are complexed with an equal number of sulfide ions ($S^{2-}$) and with thiolate groups of Cys side chains
Coenzyme Classification

• There are two classes of coenzymes

1) Cosubstrates are altered during the reaction and regenerated by another enzyme

2) Prosthetic groups remain bound to the enzyme during the reaction, and may be covalently or tightly bound to enzyme
Classification of coenzymes in mammals

(1) **Metabolite coenzymes** - synthesized from common metabolites

(2) **Vitamin-derived coenzymes** - derivatives of vitamins (vitamins cannot be synthesized by mammals, but must be obtained as nutrients)
Metabolite Coenzymes

• Nucleoside triphosphates are examples
Reactions of ATP

• ATP is a versatile reactant that can donate its:
  (1) Phosphoryl group (γ-phosphate)
  (2) Pyrophosphoryl group (γ,β phosphates)
  (3) Adenylyl group (AMP)
  (4) Adenosyl group
SAM synthesis

- ATP is also a source of other metabolite coenzymes such as S-adenosylmethionine (SAM)
- SAM donates methyl groups in many biosynthesis reactions

\[
\text{Methionine} + \text{ATP} \rightarrow \text{S-Adenosylmethionine} + P_i + PP_i
\]
S-Adenosylmethionine
Vitamin-Derived Coenzymes and Nutrition

- Vitamins are required for coenzyme synthesis and must be obtained from nutrients.
- Animals rely on plants and microorganisms for vitamin sources (meat supplies vitamins also).
- Most vitamins must be enzymatically transformed to the coenzyme.
<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Disease</th>
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<tbody>
<tr>
<td>Ascorbate (C)</td>
<td>Scurvy</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>Pellagra</td>
</tr>
<tr>
<td>Riboflavin (B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>Growth retardation</td>
</tr>
<tr>
<td>Pantothenate (B&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>Dermatitis in chickens</td>
</tr>
<tr>
<td>Thiamine (B&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>Beriberi</td>
</tr>
<tr>
<td>Pyridoxal (B&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>Dermatitis in rats</td>
</tr>
<tr>
<td>Biotin</td>
<td>Dermatitis in humans</td>
</tr>
<tr>
<td>Folate</td>
<td>Anemia</td>
</tr>
<tr>
<td>Cobalamin (B&lt;sub&gt;12&lt;/sub&gt;)</td>
<td>Pernicious anemia</td>
</tr>
</tbody>
</table>
Vitamin C: a vitamin but not a coenzyme

- A reducing reagent for hydroxylation of collagen
- Deficiency leads to the disease scurvy
- Most animals (not primates) can synthesize Vit C
NAD$^+$ and NADP$^+$

- Nicotinic acid (niacin) is precursor of NAD and NADP
- Lack of niacin causes the disease pellagra
- Humans obtain niacin from cereals, meat, legumes
Oxidized, reduced forms of NAD$^+$ (NADP$^+$)
NAD and NADP are cosubstrates for dehydrogenases

- Oxidation by pyridine nucleotides always occurs two electrons at a time.
- Dehydrogenases transfer a hydride ion (H:\-\) from a substrate to pyridine ring C-4 of NAD\(^+\) or NADP\(^+\).
- The net reaction is:

\[
\text{NAD(P)}^+ + 2e^- + 2H^+ \rightarrow \text{NAD(P)H} + H^+
\]
Ordered mechanism for lactate dehydrogenase

- Reaction of lactate dehydrogenase

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
H_3C-CH-COO^- & \quad \text{H}_3C-C-COO^- \\
\text{Lactate} & \quad \text{Pyruvate} \\
\text{NAD}^+ & \quad \text{NADH} + H^+ \\
\end{align*}
\]  

- NAD\(^+\) is bound first and NADH released last

\[
\begin{array}{cccc}
\text{NAD}^+ & \quad \text{Lactate} & \quad \text{Pyruvate} & \quad \text{NADH} \\
\text{Apoenzyme} & \quad \text{Holoenzyme} & \quad \text{E-NAD}^+ & \quad \text{E-NADH} \\
\text{E-Lactate} & \quad \text{E-Pyruvate} & \quad \text{E-NADH} & \quad \text{E} \\
\end{array}
\]
Mechanism of lactate dehydrogenase

- Hydride ion (H⁻) is transferred from C-2 of L-lactate to the C-4 of NAD⁺
FAD and FMN
(Riboflavin and its coenzymes)
Reduction, reoxidation of FMN or FAD
Coenzyme A (CoA or HS-CoA)

- Derived from the vitamin pantothenate (Vit B₃)
- Participates in acyl-group transfer reactions with carboxylic acids and fatty acids
- CoA-dependent reactions include oxidation of fuel molecules and biosynthesis of carboxylic acids and fatty acids
- Acyl groups are covalently attached to the -SH of CoA to form thioesters
Coenzyme A

[Chemical structure diagram of Coenzyme A]
Acyl carrier protein

(b)

Phosphopantetheine prosthetic group

Protein

Serine
Thiamine Pyrophosphate (TPP)

- TPP is a derivative of thiamine (Vit B$_1$)
- Reactive center is the thiazolium ring (with a very acidic hydrogen atom at C-2 position)
- TPP participates in reactions of:
  1. Decarboxylation
  2. Oxidative decarboxylation
  3. Transketolase enzyme reactions
Thiamine (Vitamin $B_1$) and TPP
Mechanism of pyruvate dehydrogenase
Hydroxyethylthiamine pyrophosphate (HETPP)
Acetaldehyde → Ylid → TPP

Enz → B: → H
Pyridoxal Phosphate (PLP)

• PLP is derived from Vit B₆ family of vitamins (deficiencies lead to dermatitis and disorders of protein metabolism)

• Vitamin B₆ is phosphorylated to form PLP

• PLP is a prosthetic group for enzymes catalyzing reactions involving amino acid metabolism (isomerizations, decarboxylations, side chain eliminations or replacements)
B₆ Vitamins and Pyridoxal Phosphate (PLP)
Mechanism of transaminases
Binding of substrate to a PLP-dependent enzyme

- Internal aldimine (PLP-enzyme)
- Geminal diamine (a tetrahedral intermediate)
External aldimine
(Schiff base with substrate)
Quinonoid intermediate
(resonance-stabilized carbanion)

(3)
\[ \alpha \text{-Keto acid} \quad R \quad C \quad COO^- \]

Pyridoxamine phosphate (PMP)
Isomerization
Isomerization
Isomerization
Biotin

- Biotin is required in very small amounts because it is available from intestinal bacteria.
- Avidin (raw egg protein) binds biotin very tightly and may lead to a biotin deficiency (cooking eggs denatures avidin so it does not bind biotin).
- Biotin (a prosthetic group) enzymes catalyze:
  1. Carboxyl-group transfer reactions
  2. ATP-dependent carboxylation reactions
Enzyme-bound biotin

- Biotin is linked by an amide bond to the ε-amino group of a lysine residue of the enzyme.
- The reactive center of biotin is the N-1 (red)
Reaction catalyzed by pyruvate carboxylase

Two step mechanism

Step 1: Formation of carboxybiotin-enzyme complex (requires ATP)

Step 2: Enolate form of pyruvate attacks the carboxyl group of carboxybiotin forming oxaloacetate and regenerating biotin
Tetrahydrofolate (THF)

• Vitamin folate is found in green leaves, liver, yeast

• The coenzyme THF is a folate derivative where positions 5,6,7,8 of the pterin ring are reduced

• THF contains 5-6 glutamate residues which facilitate binding of the coenzyme to enzymes

• THF participates in transfers of one carbon units at the oxidation levels of methanol (CH$_3$OH), formaldehyde (HCHO), formic acid (HCOOH)
Pterin, folate and tetrahydrofolate (THF)
Formation of tetrahydrofolate (THF) from folate
• One-carbon derivatives of THF
Cobalamin (Vitamin B₁₂)

- Coenzymes: methylcobalamin, adenosylcobalamin
- Cobalamin contains a corrin ring system and a cobalt (it is synthesized by only a few microorganisms)
- Humans obtain cobalamin from foods of animal origin (deficiency leads to pernicious anemia)
- Coenzymes participate in enzyme-catalyzed molecular rearrangements in which an H atom and a second group on the substrate exchange places
Cobalamin (Vit B$_{12}$) and its coenzymes
(b) Abbreviated structure of cobalamin coenzymes
Intramolecular rearrangements catalyzed by adenosylcobalamin enzymes

(a) Rearrangement of an H and substituent X on an adjacent carbon
(b) Rearrangement of methylmalonyl CoA
Methylcobalamin participates in the transfer of methyl groups.

Chemical diagram showing the conversion of Homocysteine to Methionine through the involvement of 5-Methyltetrahydrofolate, Tetrahydrofolate, and Methylcobalamin.
**Lipoamide**

- Coenzyme lipoamide is the protein-bound form of lipoic acid
- Animals can synthesize lipoic acid, it is not a vitamin
- Lipoic acid is an 8-carbon carboxylic acid with sulfhydryl groups on C-6 and C-8
- Lipoamide functions as a “swinging arm” that carries acyl groups between active sites in multienzyme complexes
Lipoamide

- Lipoic acid is bound via an amide linkage to the ε-amino group of an enzyme lysine
- Reactive center of the coenzyme shown in red
Transfer of an acyl group between active sites

- Acetyl groups attached to the C-8 of lipoamide can be transferred to acceptor molecules.

- In the pyruvate dehydrogenase reaction, the acetyl group is transferred to coenzyme A to form acetylSCoA.
Lipid Vitamins

- Four lipid vitamins: A, D, E, K
- All contain rings and long, aliphatic side chains
- All are highly hydrophobic
- The lipid vitamins differ widely in their functions
Vitamin A (Retinol)

- Vit A is obtained from liver, egg yolks, milk products or β-carotene from yellow vegetables
- Vit A exists in 3 forms: alcohol (retinol), aldehyde and retinoic acid
- Retinol and retinoic acid have roles as protein receptors
- Rentinal (aldehyde) is a light-sensitive compound with a role in vision
Formation of vitamin A from $\beta$-carotene
Vitamin D

• A group of related lipids involved in control of Ca$^{2+}$ utilization in humans

• Vitamin D$_3$ and 1,25-dihydroxycholecalciferol
Vitamin E (α-tocopherol)

• A reducing reagent that scavenges oxygen and free radicals
• May prevent damage to fatty acids in membranes
Vitamin K (phylloquinone)

- Required for synthesis of blood coagulation proteins
- A coenzyme for mammalian carboxylases that convert glutamate to \( \gamma \)-carboxyglutamate residues
- Calcium binds to the \( \gamma \)-carboxyGlu residues of these coagulation proteins which adhere to platelet surfaces
- Vitamin K analogs (used as competitive inhibitors to prevent regeneration of dihydrovitamin K) are given to individuals who suffer excessive blood clotting
(a)

Vitamin K (Phylloquinone)

(b)

Glutamate residue

\[ \text{Vitamin K–dependent carboxylase} \]

\[ \text{CO}_2 \quad \text{H}^+ \]

\[ \gamma\text{-Carboxyglutamate residue} \]
Ubiquinone (Coenzyme Q)

- Found in respiring organisms and photosynthetic bacteria
- Transports electrons between membrane-embedded complexes
- Plastoquinone (ubiquinone analog) functions in photosynthetic electron transport
(a) Ubiquinone,  
(b) Plastoquinone

- Hydrophobic tail of each is composed of 6 to 10 five-carbon isoprenoid units
- The isoprenoid chain allows these quinones to dissolve in lipid membranes
• Three oxidation states of ubiquinone
• Ubiquinone is reduced in two one-electron steps via a semiquinone free radical intermediate. Reactive center is shown in red.
Protein Coenzymes

- **Protein coenzymes** (group-transfer proteins) contain a functional group as part of a protein or as a prosthetic group.

- Participate in:
  1. **Group-transfer reactions**
  2. **Oxidation-reduction reactions** where transferred group is a hydrogen or an electron.

- Metal ions, iron-sulfur clusters and heme groups are commonly found in these proteins.
Cytochromes

- Heme-containing coenzymes whose Fe(III) undergoes reversible one-electron reduction
- Cytochromes $a, b$ and $c$ have different visible absorption spectra and heme prosthetic groups
- Electron transfer potential varies among different cytochromes due to the different protein environment of each prosthetic group
Heme group of cyt a
(c) Heme group of cyt c

Cytochrome c heme group
Absorption spectra of oxidized and reduced cytochrome c

- **Reduced cyt c** (blue) has 3 absorbance peaks: \(\alpha, \beta, \gamma\)
- **Oxidized cyt c** (red) has only a \(\gamma\) (Soret) band