

## Module 13 Enzymes and Vitamins

### Lecture 34 Enzymes

#### 13.1 Introduction

Enzymes are proteins that catalyze body's chemical reactions. They can increase the rate of reaction as much  $10^6$  by reducing the activation energy without affecting the equilibrium. Since the enzymes are proteins, they are made up of amino acids linked together by peptide bonds. The reactant of an enzyme-catalyzed reaction is called as substrate (Figure 1). The specificity of the enzyme for its substrate is known as molecular recognition.

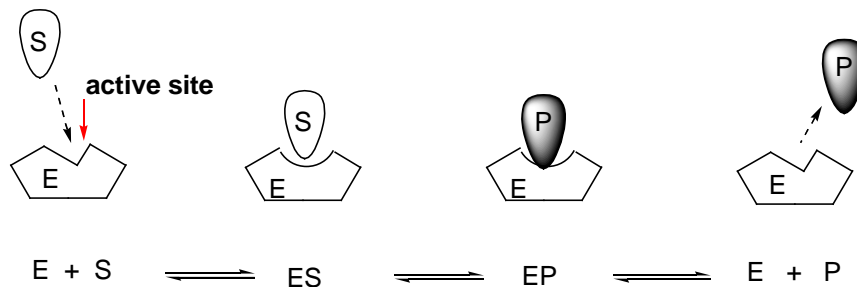


Figure 1. Enzyme-catalyzed reactions.

#### 13.2 Active Site

Active site of an enzyme is generally a hollow on the protein surface where the substrate can bind (Figure 2). The substrate is generally bound to amino acids present in the binding site by

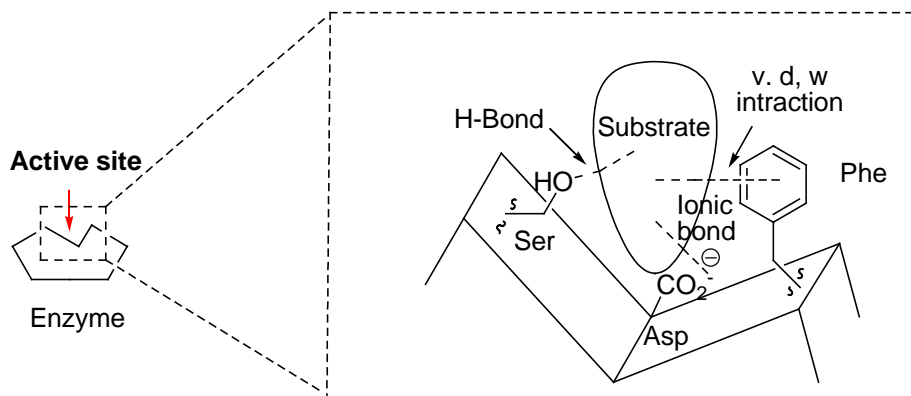


Figure 2

variety of interactions such as van der Waals interaction, H-bonding and ionic bonding. These interactions are strong enough to hold the substrate for the reaction to take place, but weak enough to allow the product to depart once it is produced.

The amino acids present in the active site also assist in the reaction mechanism. For example, nucleophilic amino acid such as serine is commonly involved in enzyme-catalyzed reaction mechanisms and will form covalent bond with the substrate as part of the reaction mechanism (Figure 3).

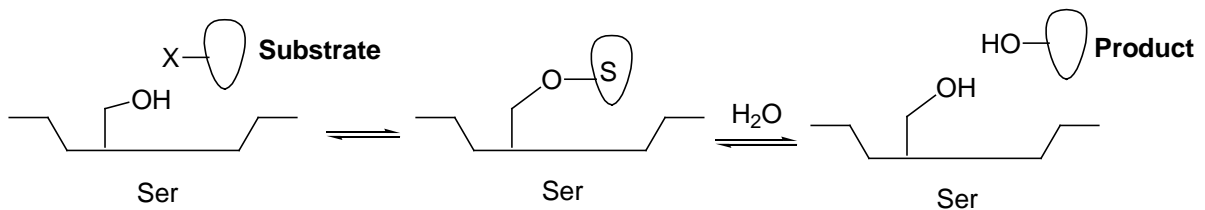


Figure 3

### 13.3 Acetylcholine Hydrolysis

There are many reasons why enzymes catalyze the reactions. In figure 3 we have seen an example where amino acid in the active site can assist the enzyme mechanism acting as a nucleophile. Another reason why enzyme acts as catalyst is the binding process itself. The active site is not ideal shape for the substrate, but when the binding takes place the shape changes to accommodate the substrate and maximizes the possible interactions of the substrate with amino acids of the active site. This is called as an **induced fit** (Figure 4).

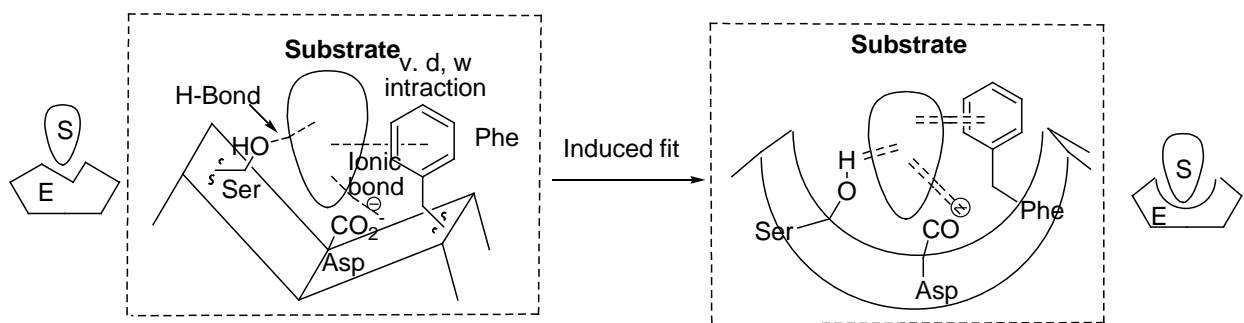
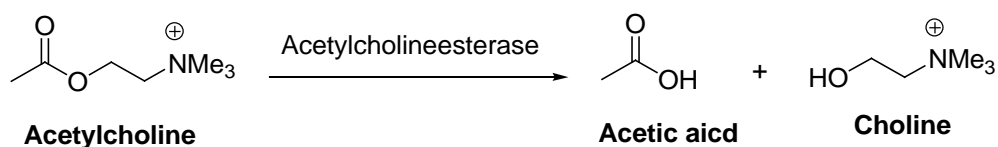
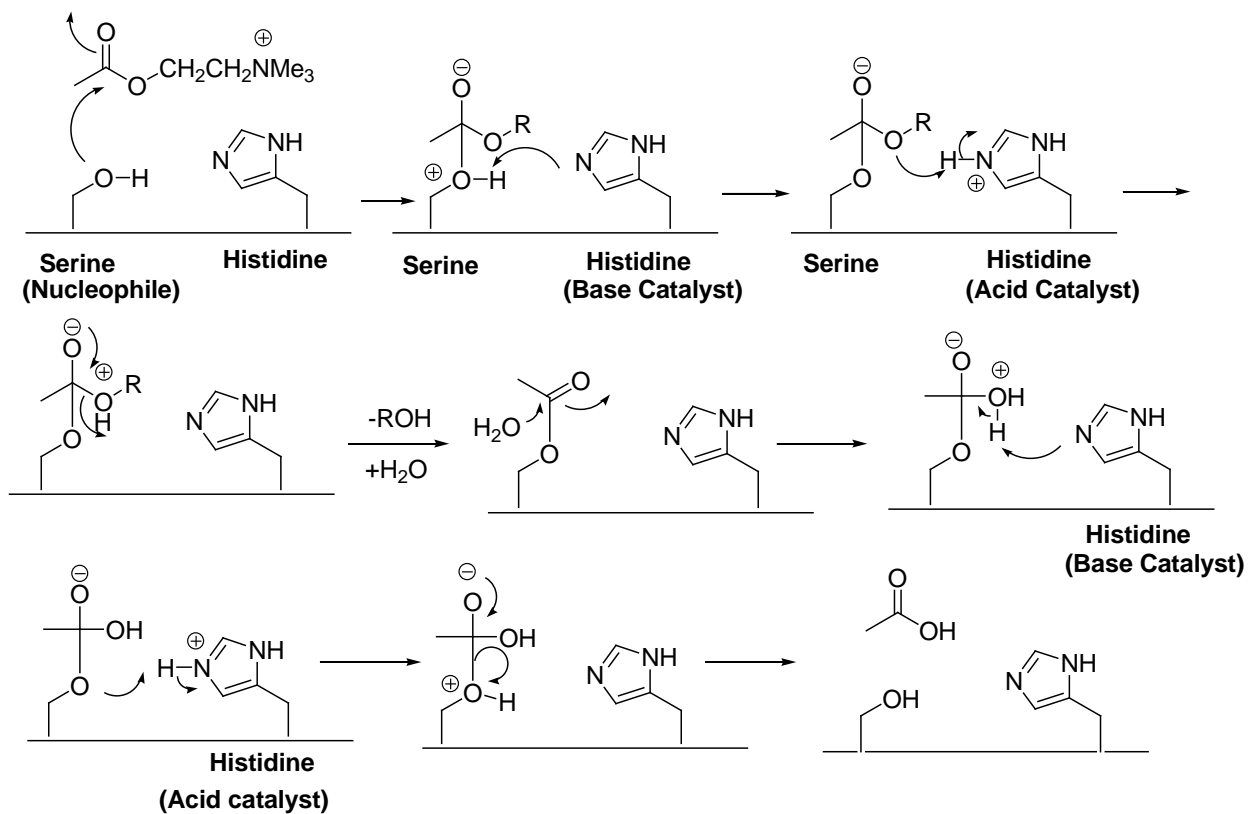


Figure 4. Induced fit

These binding process also force the substrate to adopt a specific conformation which is ideal for the reaction with nucleophile and catalytic amino acids. Furthermore, the binding between the substrate and enzyme is that important bonds in the substrate may be strained and weakened, allowing the reaction takes place more easily. For an example, neurotransmitter acetylcholine is hydrolyzed by the enzyme acetylcholinesterase (Scheme 1). In this process, acetylcholine is bound in the active site such that it is held in position to undergo reaction with nucleophilic serine OH group. A histidine residue is also position such a way to act as an acid/base catalyst. H-Bonding between the ester group of the substrate with a tyrosine residue in the active site also aids to weaken the ester linkage, allowing it to be cleaved more easily.



### Mechanism



Scheme 1. Acetylcholine hydrolysis

### 13.4 Enzyme Inhibitors

Enzyme inhibitors are drugs which inhibit the catalytic activity of enzyme. They can be classified as competitive and noncompetitive inhibitors. **Competitive inhibitors** compete with the substrate for the active site. The greater the concentration of the inhibitor, there is a less probability for the substrate to enter the active site (Figure 5). On the other hand, the greater the concentration of the natural substrate, the less efficient is the inhibition.

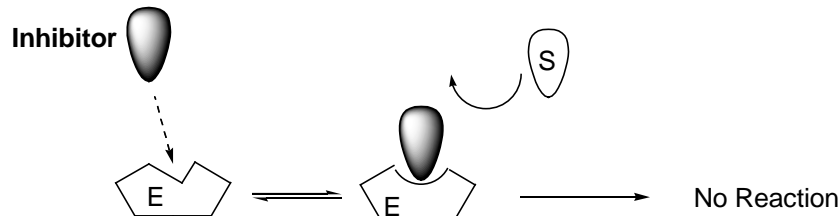


Figure 5. Competitive Inhibition

**Noncompetitive inhibitors** do not compete with the substrate to enter the active site. Instead, they bind at different region of the enzyme and produces an induced fit, which changes the shape of the enzyme such as the active site no longer available to the substrate (Figure 6). Increasing the concentration of the substrate will have no effect on the level of inhibition.

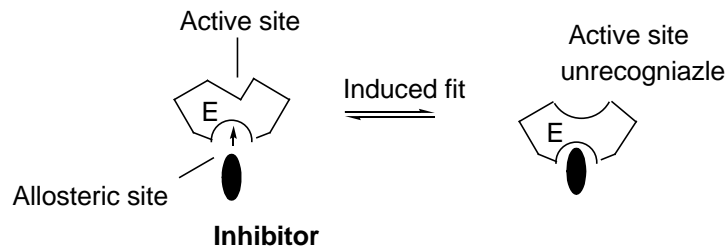


Figure 6. Noncompetitive Inhibition

The binding site of the noncompetitive inhibitors is known as **allosteric site**. It is often present in the enzyme at the start of the biosynthesis. The allosteric site binds the final product of the biosynthesis and provides a feedback control for the biosynthesis pathway. When the concentration of the biosynthesis product is high, the allosteric site is occupied, the activity of the enzyme is off. The final biosynthetic product is thus used as a lead compound for the design of inhibitors that will bind to the allosteric site (Figure 7).

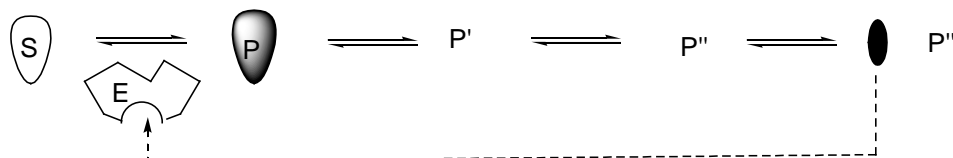


Figure 7. Allosteric Inhibition

The inhibitors can be reversible or nonreversible. In case of reversible inhibitor, the inhibitor binds with the enzyme through intermolecular interactions such as H-bond, ionic bonding and van der Waals interactions. There will be equilibrium between the enzyme-inhibitor complex and free enzyme and unbound inhibitor. The position of the equilibrium will depend on the strength of the intermolecular interactions between the enzyme and inhibitor. On the other hand, in case of **non-reversible inhibitors**, the inhibitor may react with enzyme generating a covalent bond. Such bonds are strong and the enzyme remains permanently blocked making the inhibition irreversible (Figure 8).

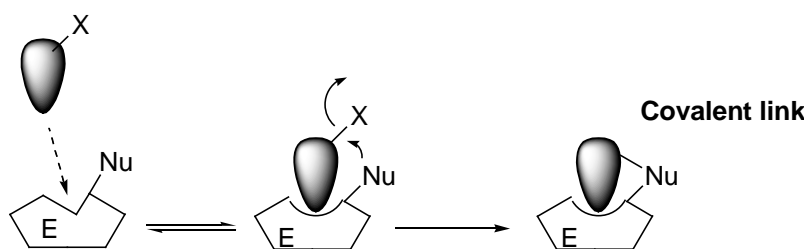


Figure 8. Irreversible Inhibition

### 13.5 Enzyme Selectivity

The inhibitor should be selective for the target enzyme in order to avoid the side effects. There are instances where a particular enzyme may exist in different forms having different amino acid composition, but identical catalytic reaction. These enzymes are called isozymes. If the variation is in the binding site, it is possible to design drugs that will be the isozyme selective.

## Module 13 Enzymes and Vitamins

### Lecture 35 Coenzymes and Vitamins

#### 13.6 Introduction

Coenzymes are organic molecules commonly derived from vitamins. Vitamin is a substance that body can not normally synthesize, but they are required in small amount for normal body function. In this lecture we will cover niacin, vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, vitamin H and vitamin K. For the details of the vitamin A, D and E, please see the lecture 28.

#### 13.7 Niacin

Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) is a coenzyme that is used by enzyme as oxidizing agent for biological oxidations. Since *niacin* is the portion of coenzyme NAD<sup>+</sup>, it is to be added in the diet so that the body can synthesize NAD<sup>+</sup> (Figure 1). Figure 2 illustrates an example for the enzyme catalyzed oxidation/reduction reactions using NAD<sup>+</sup>/NADH.

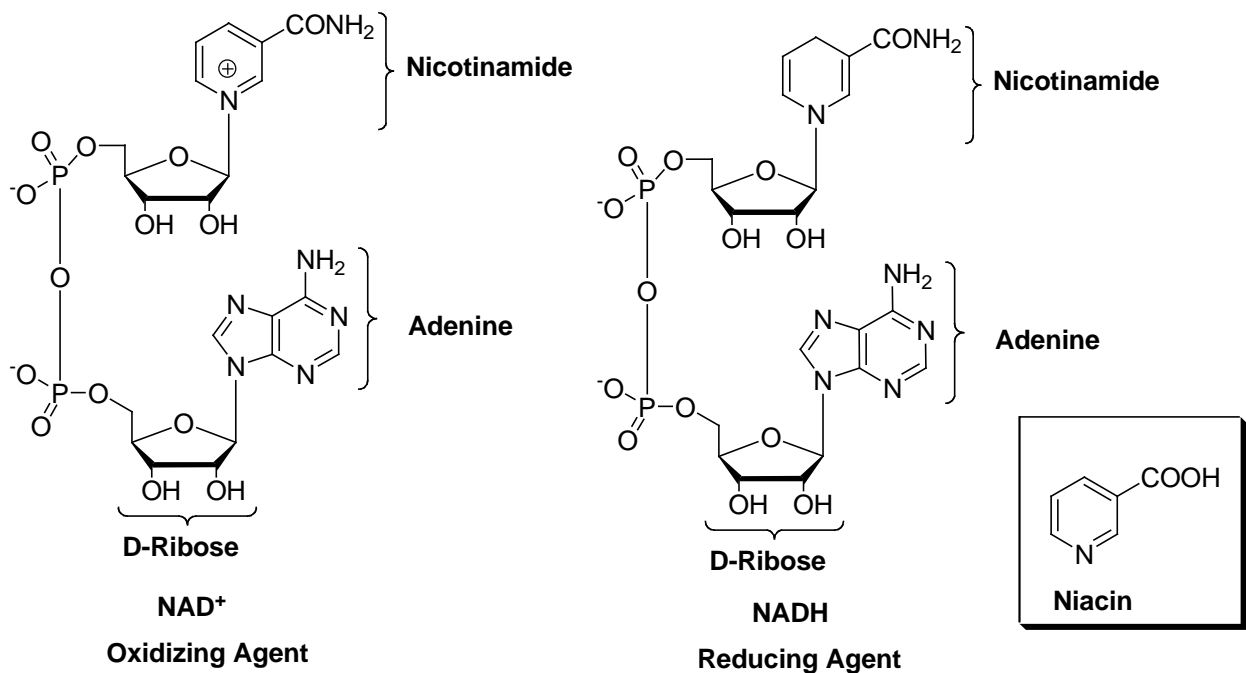


Figure 1

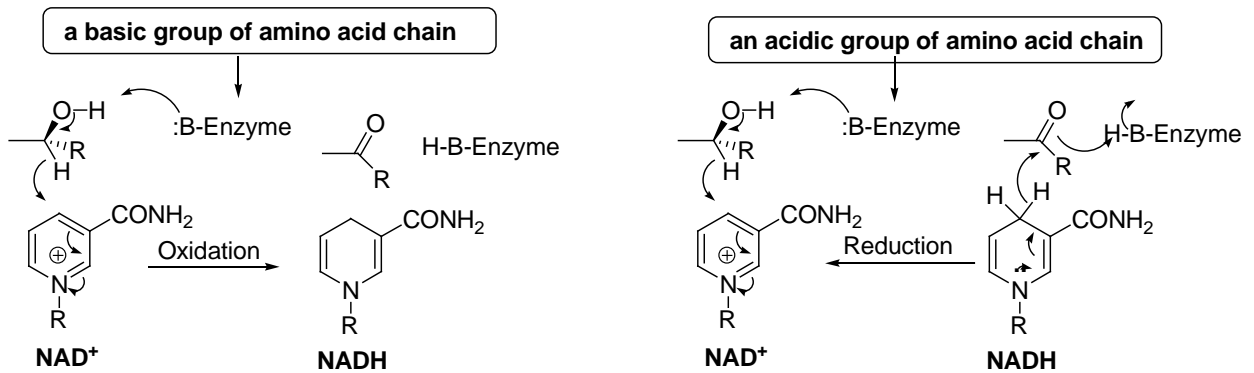
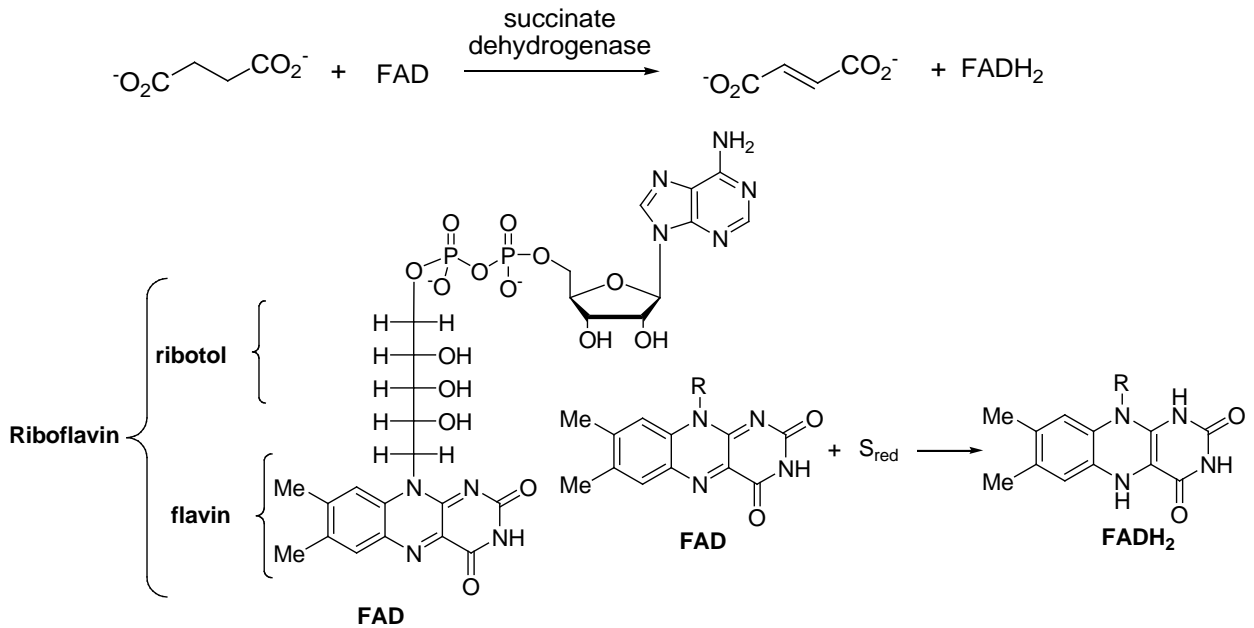


Figure 2

### 13.8 Vitamin B<sub>2</sub>

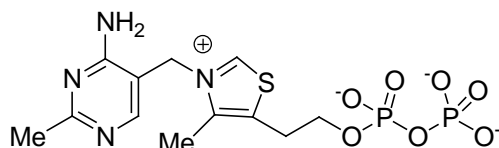
Riboflavin (flavin plus ribitol) is known as vitamin B<sub>2</sub>. It is required to synthesize coenzyme *flavin adenine dinucleotide* (FAD) which is used as oxidizing agent in enzyme catalyzed oxidation reactions. For example, FAD is used by succinate dehydrogenase to oxidize succinate to fumarate (Scheme 1).



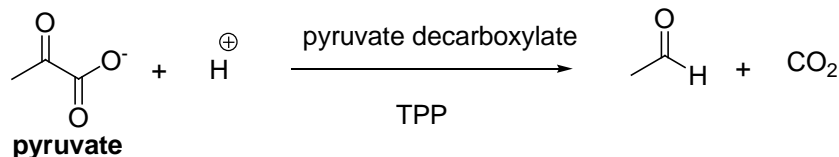
Scheme 1

### 13.9 Vitamin B<sub>1</sub>

Thiamine is known as vitamin B<sub>1</sub>. It is required to form the coenzyme thiamine pyrophosphate (TPP). TPP is required by enzymes that catalyze the transfer of a two carbon fragment from one species to another. For example, pyruvate decarboxylase enzyme requires TPP for the decarboxylation of pyruvate and transfer the remaining two-carbon fragment to a proton to afford acetaldehyde (Scheme 2).



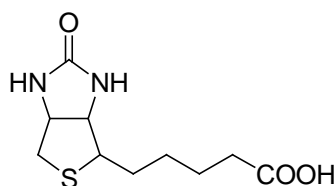
**Thiamine pyrophosphate (TPP)**



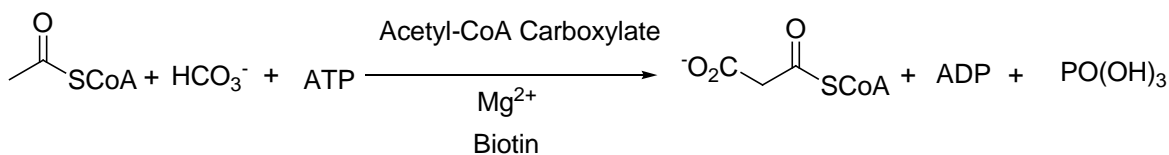
**Scheme 2**

### 13.10 Vitamin H

Biotin is known as vitamin H, which is synthesized by bacteria that live in intestines. Thus, biotin does not have to be incorporated in diet and the deficiencies are generally rare. Biotin is the coenzyme required by enzymes that catalyze carboxylation of a carbon adjacent to a carbonyl group. For example, acetyl-CoA carboxylase converts acetyl-CoA into malonyl-CoA. HCO<sub>3</sub><sup>-</sup> is used as the source of carboxyl group which is attached to the substrate (Scheme 3). The reaction is also required ATP and Mg<sup>2+</sup>.



**Biotin**

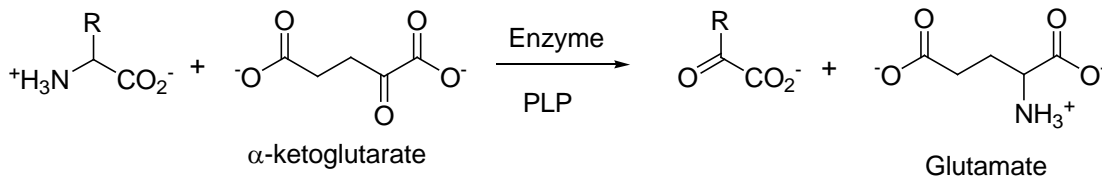
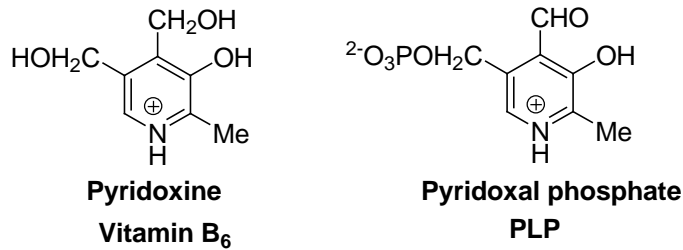


**Scheme 3**



### 13.11 Vitamin B<sub>6</sub>

The coenzyme pyridoxal phosphate (PLP) is derived vitamin B<sub>6</sub>. The deficiency of vitamin B<sub>6</sub> causes anemia. Enzymes that catalyze the reactions of amino acids require PLP. One of the common examples is the transamination using enzyme aminotransferases (Scheme 4).

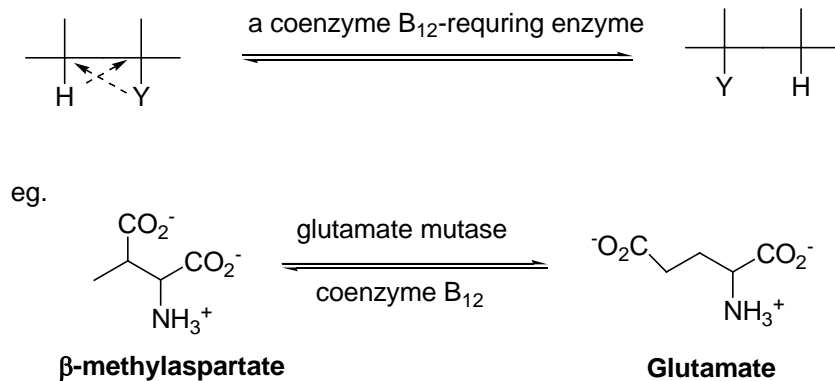


Scheme 4

### 13.2 Vitamin B<sub>12</sub>

Enzymes that catalyze certain rearrangement reactions require coenzyme B<sub>12</sub> which is derived from vitamin B<sub>12</sub>. In vitamin B<sub>12</sub>, cyano group is coordinated to cobalt. In coenzyme B<sub>12</sub>, this group is replaced by a 5'-deoxyadenosyl group.

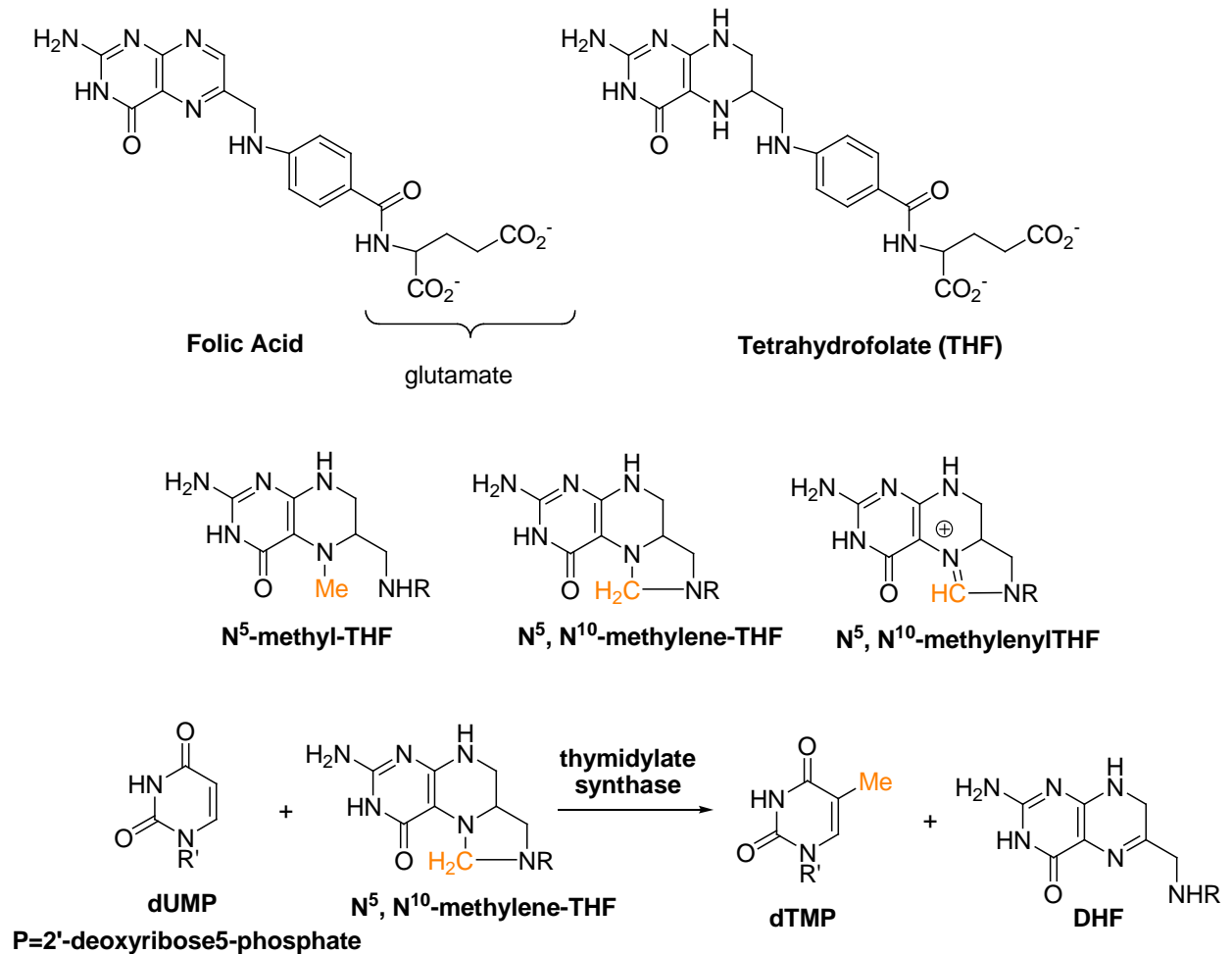
Coenzyme B<sub>12</sub> is used by enzymes which catalyze reactions in that a group (Y) bonded to one carbon changes to hydrogen bonded an adjacent carbon (Scheme 5). For example,  $\square$  methylaspartate is converted into glutamate where CO<sub>2</sub><sup>-</sup> has migrated to the adjacent carbon.



Scheme 5

### 13.13 Folic Acid

Tetrahydrofolate (THF) is the coenzyme required by enzymes which catalyze the transfer of a group having a single carbon to their substrates. The one carbon may be methyl ( $\text{CH}_3$ ), methylene ( $\text{CH}_2$ ) or formyl ( $\text{CH}$ ) group. The coenzyme THF is required for the synthesis of bases in RNA and DNA and synthesis of some aromatic amino acids. Folic acid is the vitamin used for the synthesis of the coenzyme THF. For example, thymidylate synthase is the enzyme that catalyzes the synthesis of T's from U's. This transformation requires  $\text{N}^5, \text{N}^{10}$ -methylene-THF as a coenzyme (Scheme 6).



Scheme 6

### 13.14 Vitamin K

- Vitamin K is synthesized by intestinal bacteria.
- Vitamin K is required for proper clotting of blood. In order for blood to clot, blood-clotting proteins must bind to  $\text{Ca}^{2+}$ .
- Vitamin K is synthesized by intestinal bacteria. Thus, deficiency of vitamin K is rare.
- Vitamin  $\text{KH}_2$  is the coenzyme that is derived from vitamin K.
- The coenzyme vitamin  $\text{KH}_2$  is required by enzyme that catalyzes the carboxylation of the  $\gamma$ -carbon of glutamate side chains in proteins, forming  $\gamma$ -carboxyglutamates (Scheme 7).
- The process uses  $\text{CO}_2$  for the carboxyl group that is introduced in to the glutamate chains.
- The protein involved in the blood clotting contains several glutamates near the N-terminal ends.
- $\gamma$ -Carboxyglutamate makes stronger complex with  $\text{Ca}^{2+}$  compared to that of glutamate.

