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Mechanisms of actions of coenzymes

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ABSTRACT

Each living species uses coenzymes in numerous important reactions catalyzed by enzymes. There are two types of coenzymes depending on the interaction with apoenzymes: coenzymes frequently called co-substrates and coenzymes known as prosthetic groups. Main metabolic roles of co-substrates (adenosine triphosphate (ATP), *S*-adenosyl methionine, uridine diphosphate glucose, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$), coenzyme A (CoA), tetrahydrofolate and ubiquinone (Q)) and prosthetic groups (flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), thiamine pyrophosphate (TPP), pyridoxal phosphate (PLP), biotin, adenosylcobalamin, methylcobalamin, lipoamide, retinal, and vitamin K) are described in the review.

Keywords: *Coenzyme, Co-substrates, Prosthetic groups, Mechanisms.*

Introduction

Coenzymes can be classified into two groups depending on the interaction with apoenzyme. The coenzymes of the first type-often called co-substrates are substrates in the reactions catalyzed by enzymes. Co-substrate is changing during the reaction and dissociating from the active center. The original structure of co-substrate is regenerating in the next reaction catalyzed by other enzymes. Therefore, co-substrates cover mobile metabolic group between different reactions catalyzed by enzymes (http://www.uwyo.edu/molecbio/courses/molb-3610/files/chapter%207%20coenzymes%20and%20vitamines.pdf).

The second type of the coenzymes is called the prosthetic groups. The prosthetic group remains bonded for the enzyme during the reaction. In some cases, the prosthetic group is covalently bound for its apoenzyme, while in other cases it is weakly bound to the active center by numerous weak interactions. Similarly to ionic amino acid residues of the active site, the prosthetic group must return to its original form during the whole catalytic event or holoenzyme will not remain catalytically active (http://www.uwyo.edu/molecbio/courses/molb-3610/files/chapter%207%20coenzymes%20and%20vitamines.pdf).

Every living species uses coenzymes in a different number of the important reactions catalyzed by enzymes. Numerous species can synthesize their coenzymes from simple precursors. This ability is particularly important in four out of five kingdoms: prokaryotes, protozoa, fungi, and plants. Animals, generally, lost their ability to synthesize some coenzymes. Mammals (including humans) have the ability for the source coenzymes, or their direct precursors in order to survive. Final vitamin sources are usually plants and microorganisms, although carnivores can get vitamins from meat. Majority of vitamins must be transformed enzymatically into their corresponding coenzymes (Arsic et al., 2016).

The illnesses emerged due to the deficiency, *i.e.*, when there is a lack of vitamins, or it is absent in the food nutrition (Arsic et al., 2016). Majority of vitamins are converted into coenzymes, most often after the reaction with ATP (Huennekens et al., 1974). A portion of ATP molecule which is transferred to the vitamin is the group which binds the coenzyme for the enzyme active centers. Vitamins soluble in water are necessary in small quantities because they are excreted by urine, and cell depots of their coenzymes are unstable (Schellack et al., 2015).

On the other side, lipid vitamins, like vitamins A, D, E, I, K are stored in animals, and increased intake can cause toxic states known as hypervitaminoses (Engelking, 2015).

The most important enzymes are listed in Table 1 together with their roles in metabolism and their vitamin sources.

Table 1. The most important coenzymes (Horton et al., 2006)

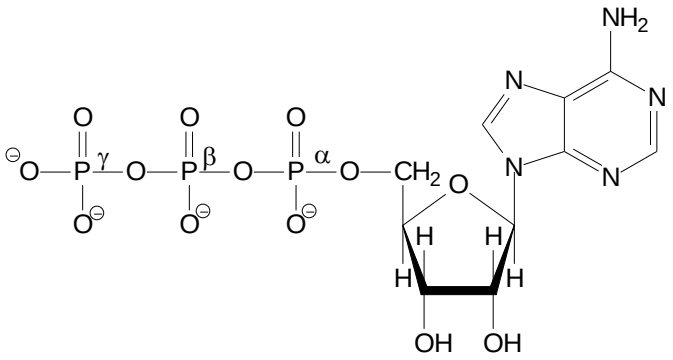
Coenzyme	**Vitamin**	**Main metabolic role**	**Mechanistic role**
Adenosine triphosphate (ATP)	-	Transfer of phosphoryl or nucleotidyl groups	Co-substrate
S-Adenosyl methionine	-	Transfer of metal groups	Co-substrate
Uridine diphosphate glucose	-	Transfer of glycosyl groups	Co-substrate
Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$)	Niacin	Oxidation-reduction reactions involving 2-electron transfers	Co-substrate
Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)	Riboflavin (B_2)	Oxidation-reduction reactions including one and two electron transfers	Prosthetic group
Coenzyme A (CoA)	Pantothenate (B_3)	Transfer of acyl groups	Co-substrate
Thiamine pyrophosphate (TPP)	Thiamine (B_1)	Transfer of fragments from two carbons containing carbonyl group	Prosthetic group
Pyridoxal phosphate (PLP)	Pyridoxine (B_6)	Transfer of groups from and to amino	Prosthetic group

		acids	
Biotin	Biotin	ATP dependent carboxylation of substrate or transfer of carboxylic groups between substrates	Prosthetic group
Tetrahydrofolate	Folate	Transfer of substituents with one carbon, particularly formyl and hydroxymethyl groups; giving methyl group for thiamine in DNA	Co-substrate
Adenosylcobalamin	Cobalamine (B_{12})	Intramolecular rearrangement	Prosthetic group
Methylcobalamin	Cobalamine (B_{12})	Transfer of methyl groups	Prosthetic group
Lipoamide	-	Oxidation of hydroxyalkyl group from TPP and the next transfer as an acyl group	Prosthetic group
Retinal	Vitamin A	Eyesight	Prosthetic group
Vitamin K	Vitamin K	Carboxylation of some glutamic residues	Prosthetic group
Ubiquinone (Q)	-	Electron carrier soluble in fats	Co-substrate

ATP and other nucleotide co-substrates

There are many nucleoside triphosphates which behave as coenzymes. Among them, adenosine triphosphate (ATP) is the most abundant. Other frequent examples are GTP, *S*-adenosyl methionine and nucleotide sugars such as uridine diphosphate glucose (UDP-glucose). ATP (Figure 1a) can donate phosphoryl, pyrophosphoryl, adenylyl (AMP), or adenosyl groups in reactions of group transfers.

a)



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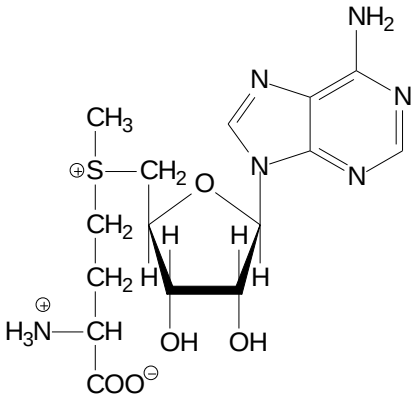


Figure 1. a) Nitrogen base adenine is connected to the ribose which carries three phosphoryl groups. Transfer of the phosphoryl group gives ADP, and the transfer of nucleotidyl group (AMP) gives pyrophosphate; b) *S*-adenosyl methionine

The most usual reaction involving ATP is the transfer of the phosphoryl group. In the reactions catalyzed by enzymes, for example, γ-phosphoryl group ATP is transferred to the

nucleophile, leaving ADP. The second most usual reaction is a transfer of the nucleotidyl group (transfer of AMP part), leaving pyrophosphate (PP_i).

S-adenosyl methionine (Figure 1b) is synthesized in the reaction of methionine with ATP. Different from a thiomethyl group of methionine, positively charged sulfonium *S*-adenosyl methionine is highly reactive, so it reacts readily with nucleophilic acceptors, and practically it is a donor of all methyl groups used in biosynthetic reactions (*e.g.*, conversion of hormone norepinephrine into epinephrine). Methylation reactions which require *S*-adenosyl methionine involve methylation of phospholipids, proteins, DNA, and RNA. In plants, *S*-adenosyl methionine is involved in the regulation of fruit ripening as a precursor of plant hormone ethylene (Chiang et al., 1996).

Nucleotide-sugar coenzymes are involved in the metabolism of carbohydrates. The most common nucleotide sugar, uridine diphosphate glucose (UDP-glucose) is formed in the reaction of glucose 1-phosphate with uridine triphosphates (UTP) (Figure 2). UDP-glucose can donate its glycosyl group to the corresponding acceptor releasing UDP. UDP-glucose is regenerated when UDP accepted phosphoryl group from ATP and obtained UTP reacts with another molecule of glucose-1-phosphate.

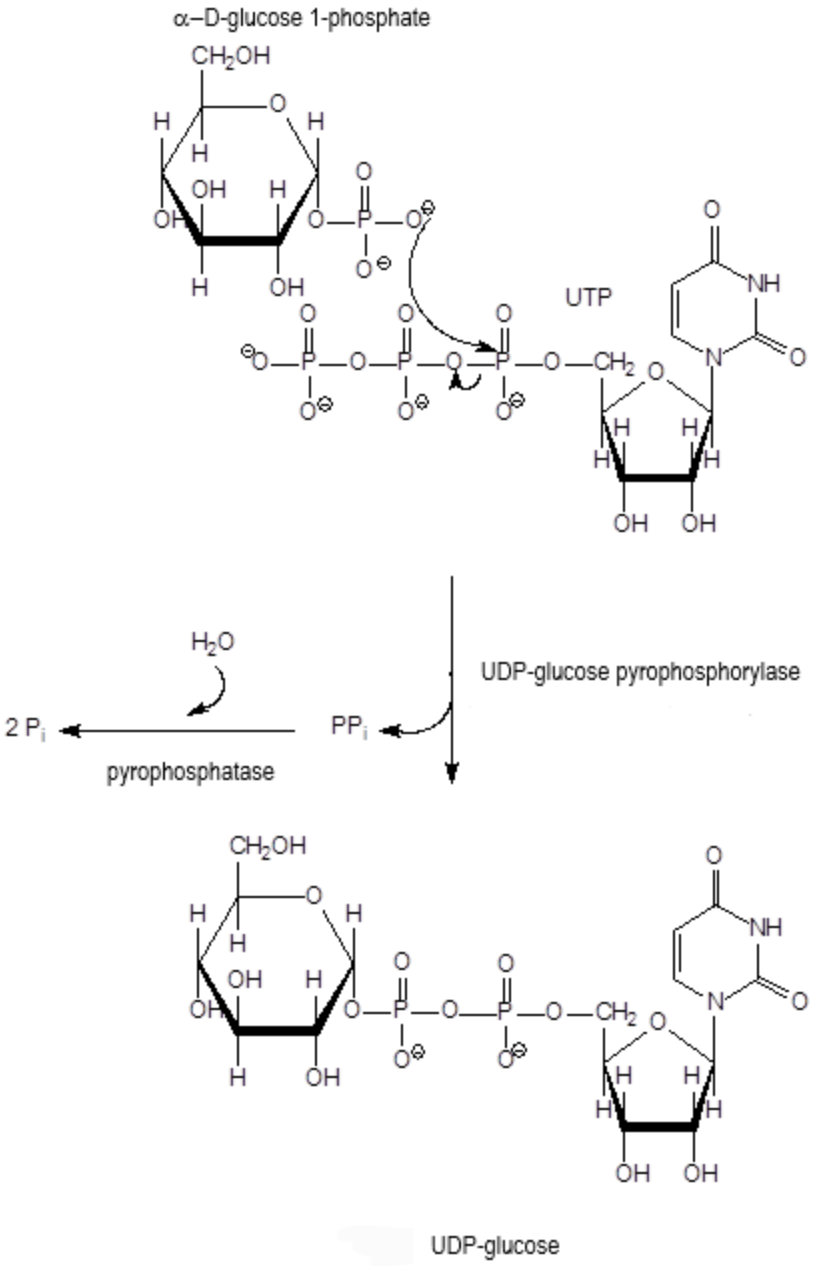


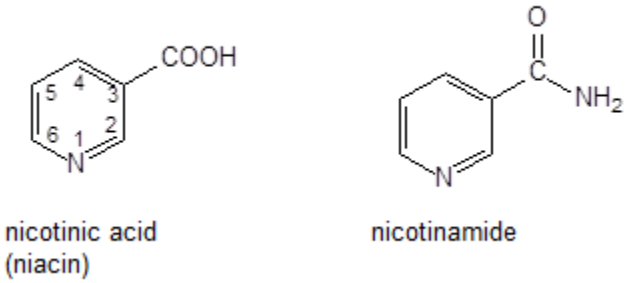
Figure 2. The formation of UDP glucose catalyzed with UDP-glucose phosphorylase (Horton et al., 2006)

In the mechanism of the formation of UDP glucose, the oxygen of the phosphoric group of α-D-glucose 1-phosphate attacks α-phosphorus of UTP. The released PP_i is hydrolyzed fast to 2 P_i by the action of pyrophosphatase. This hydrolysis enables the occurring of the reaction catalyzed by pyrophosphorylase.

NAD^+ and $NADP^+$

Nicotinamide coenzymes are nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). The both contain nicotinamide (Figure 3a).

a)



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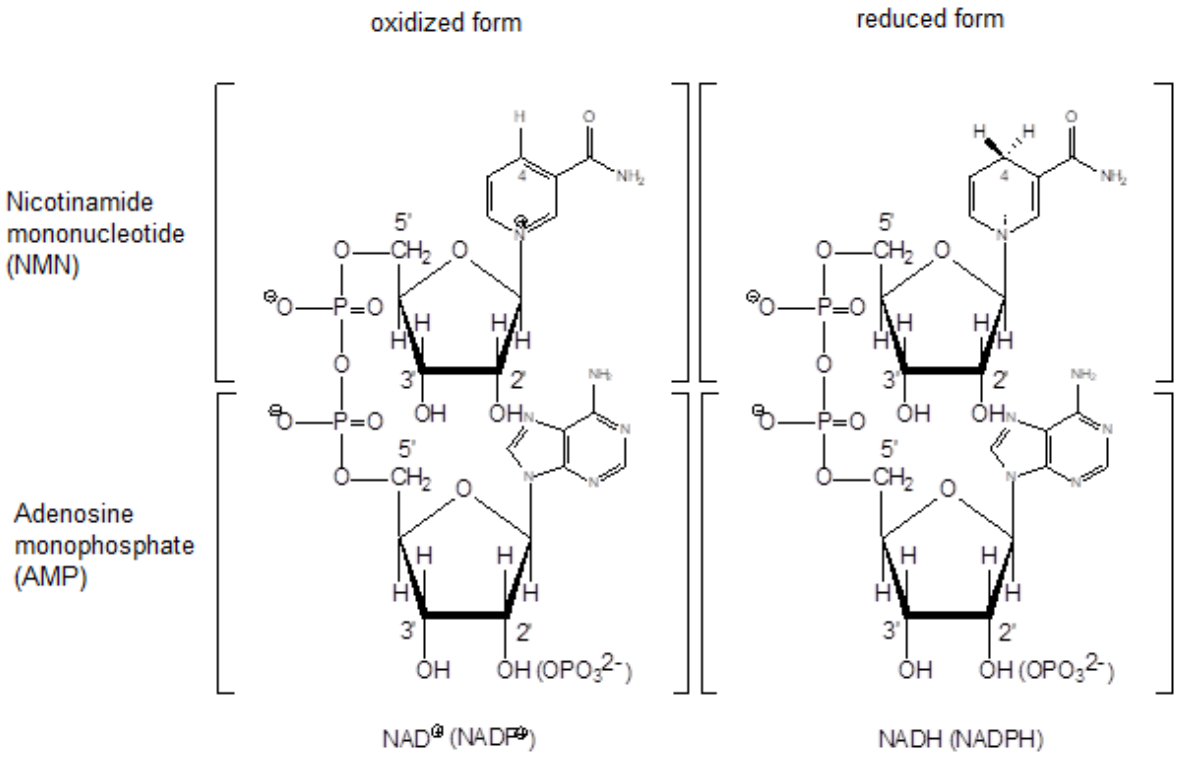


Figure 3. a) Nicotinic acid (niacin) and nicotinamide; b) Oxidation and reduction forms of NAD (and NADP)

The deficiency of nicotinic acid (niacin) causes the pellagra disease. Nicotinic acid or nicotinamide is essential as a precursor of NAD^+ and $NADP^+$ (pyridine nucleotide coenzymes) (Figure 3b) (Sorci et al., 2010). Nicotinamide coenzymes play a role in numerous oxidation-reduction reactions in the form of electron transfers from and to the metabolite. Pyridine ring NAD^+ is reduced by the addition of hydride ion onto C-4 when NAD^+ is transformed into NADH (and when $NADP^+$ is transformed into NADPH) (Sorci et al., 2010).

NAD^+ and $NADP^+$ almost always behave as dehydrogenase substrates (Bellamacina, 1996). Dehydrogenase catalyzes the oxidation of the substrate by transferring two electrons and proton in the form of hydride ion (H^-) onto C-4 of nicotinamide group NAD^+ and $NADP^+$. In this way, the reduced forms are formed (NADH and NADPH), where new C-H bond is created on C-4 (Bellamacina, 1996).

NADH and NADPH (stable in solutions containing oxygen) possess reductive power (Kukielka and Cederbaum, 1990). The stability of reduced pyridine nucleotides allows them to carry their reduction potential from one enzyme to another; the characteristics not owned by flavin coenzymes. The majority of reactions in which NADH and NADPH are formed are catabolic reactions. The oxidation of NADH in mitochondria is coupled with ATP synthesis. The most significant part of NADPH is used as a reduction agent in biosynthetic reactions (Kukielka and Cederbaum, 1990).

NADH and NADPH show a maximum in ultraviolet region at 340 nm caused by dihydropyridine ring, while NAD^+ and $NADP^+$ do not absorb the light at this wavelength. The appearance and disappearance of the absorbance at 340 nm are useful for the measurement of the rate of the oxidation (McComb et al., 1976).

In the mechanism of the oxidation of lactate to pyruvate catalyzed by lactate dehydrogenase (Figure 4), the coenzyme accepts the hydride ion on C-4 in nicotinamide group. This phenomenon leads to the bond rearrangement in the ring when electrons are moving to the positively charged nitrogen atom. The enzyme represents acid-base catalyst and the suitable place for binding coenzyme, and the substrate as well. Two hydrogens are moving from the lactate to produce pyruvate. One of these hydrogens are moving to NAD^+ as a hydride ion bearing two electrons, and the another is transferring to His-195 as a proton. The second hydrogen is then releasing as H^+ to regenerate the base catalyst (His-195) (Kane, 2014; Speers and Reguera, 2012).

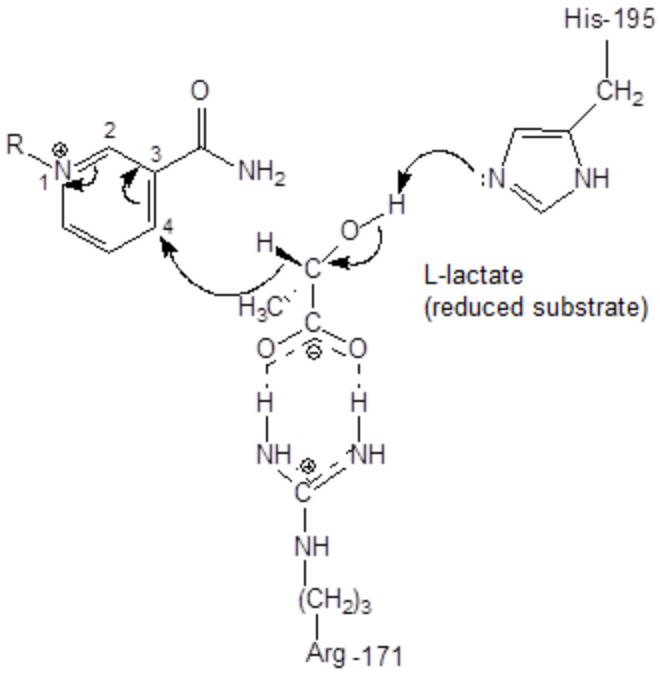


Figure 4. The mechanism of lactate dehydrogenase (Speers and Reguera, 2012)

FAD and FMN

Coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are derived from riboflavin or vitamin B_2. Riboflavin is synthesized by bacteria, protozoa, fungi, plants and some animals. Mammals are getting riboflavin from food. Riboflavin consists from ribitol connected to N-10 atom of heterocyclic ring system called isoalloxazine (Figure 5a). Similarly to NAD^+ and $NADP^+$, FAD contains AMP and pyrophosphate bond (Figure 5b).

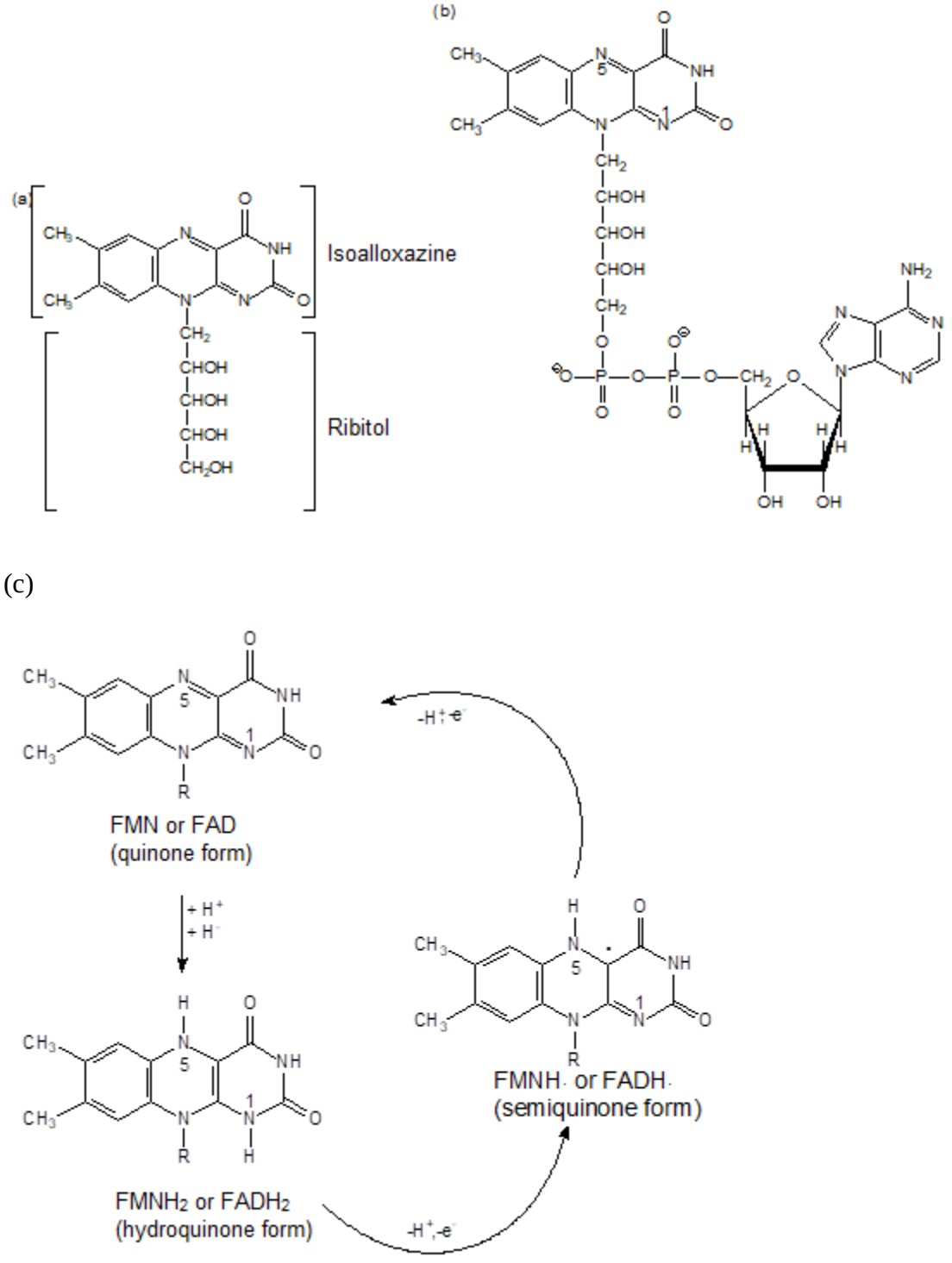


Figure 5. Riboflavin and its coenzymes. (a) Riboflavin; (b) Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD); (c) Reduction and reoxidation of FMN or FAD

FAD or FMN are necessary as prosthetic groups for many oxidoreductases. Reduced flavin coenzymes can be easily oxidized in the presence of oxygen. FAD and FMN are reduced to $FADH_2$ and $FMNH_2$ by taking proton and two electrons in the form of hydride ion (Figure 5c). Oxidized enzymes are light yellow as a result of the system of conjugated double bonds of the isoalloxazine cyclic system. Different from NADH and NADPH, which exclusively participate in two-electron systems, $FMNH_2$ and $FADH_2$ are donated electrons (one ($FADH\cdot$ or $FMNH\cdot$ are formed) or two). The intermediates are relatively stable free radicals called semiquinones. Oxidation of $FADH_2$ and $FAMNH_2$ is often coupled with the reduction of metalloproteins containing Fe^{3+} (in [Fe-S] cluster). Since iron-sulfur cluster can accept only one electron, reduced flavin must be oxidized into two one-electron steps *via* semiquinone intermediate (Ghisla and Massey, 1989).

Coenzyme A

Numerous metabolic processes depend on coenzyme A (CoA, or HS-CoA), including the oxidation of fuel molecule and biosynthesis of some carbohydrates and fats. The coenzyme is involved in the reactions of acyl group transfers (Leonardi et al., 2005). It has three main components: 2-mercaptoethylamine unit with free -SH group, pantothenate vitamin (vitamin B_3), and ADP part (Figure 6a). Acetyl CoA is energetically rich compound because of the high energy of thioester bond.

Phosphopantetheine, phosphate ester which contains 2-mercaptoethylamine and pantothenate parts of coenzyme A, is a prosthetic group of a small protein (77 amino acid residues), known as an acyl carrier protein (ACP). The prosthetic group is esterified to ACP *via* oxygen in the side chain of a serine residue (Figure 6b). The intermediates acetylate SH of the prosthetic group ACP in the fatty acids' biosynthesis (Leonardi et al., 2005).

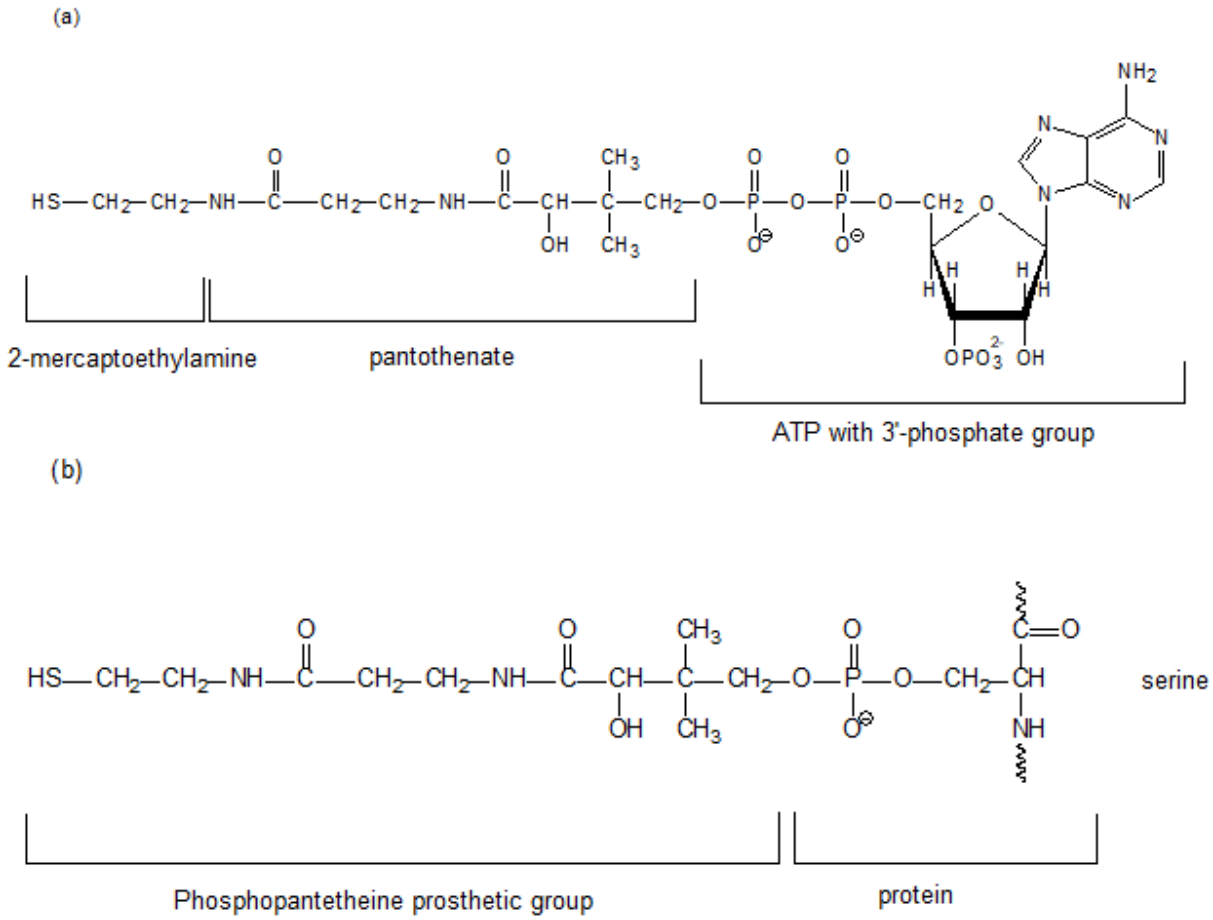


Figure 6. (a) Coenzyme A; (b) Acyl carrier protein (ACP)

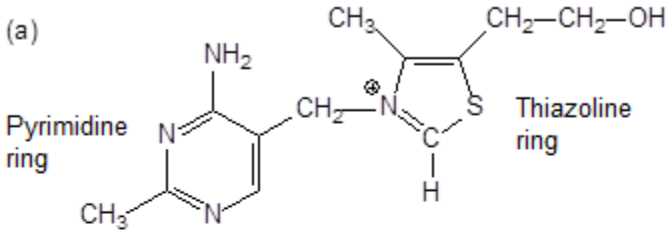
Thiamine pyrophosphate

Thiamine (or vitamin B_1) contains pyrimidine ring and positively charged thiazoline ring (Figure 7a). In mammals, thiamine is the essential vitamin, wide-spread in the rice peel and other wheat. Its deficiency causes beriberi. Coenzyme is the thiamine pyrophosphate (TPP) (Figure 7b). TPP is synthesized from thiamine by the enzymatic transfer of pyrophosphoryl group from ATP (Shepard and Broderick, 2010).

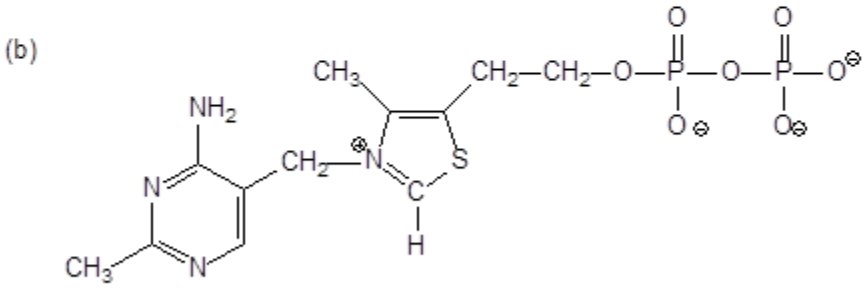
Numerous decarboxylases (carboxylases) require TPP as a coenzyme (*e.g.*, pyruvate decarboxylase of the yeast) (Figure 7c).

TPP is also the coenzyme involved in oxidative decarboxylation of α-keto acids, except for pyruvates. The first steps in these reactions are occurring according to the mechanism shown in

Figure 7c. Besides, TPP is a prosthetic group for the enzymes known as transketolases, which catalyze the transfer between the sugar molecules of two carbon groups containing keto group.



Thiamine (vitamin B_1)



Thiamine pyrophosphate (TPP)

(c)

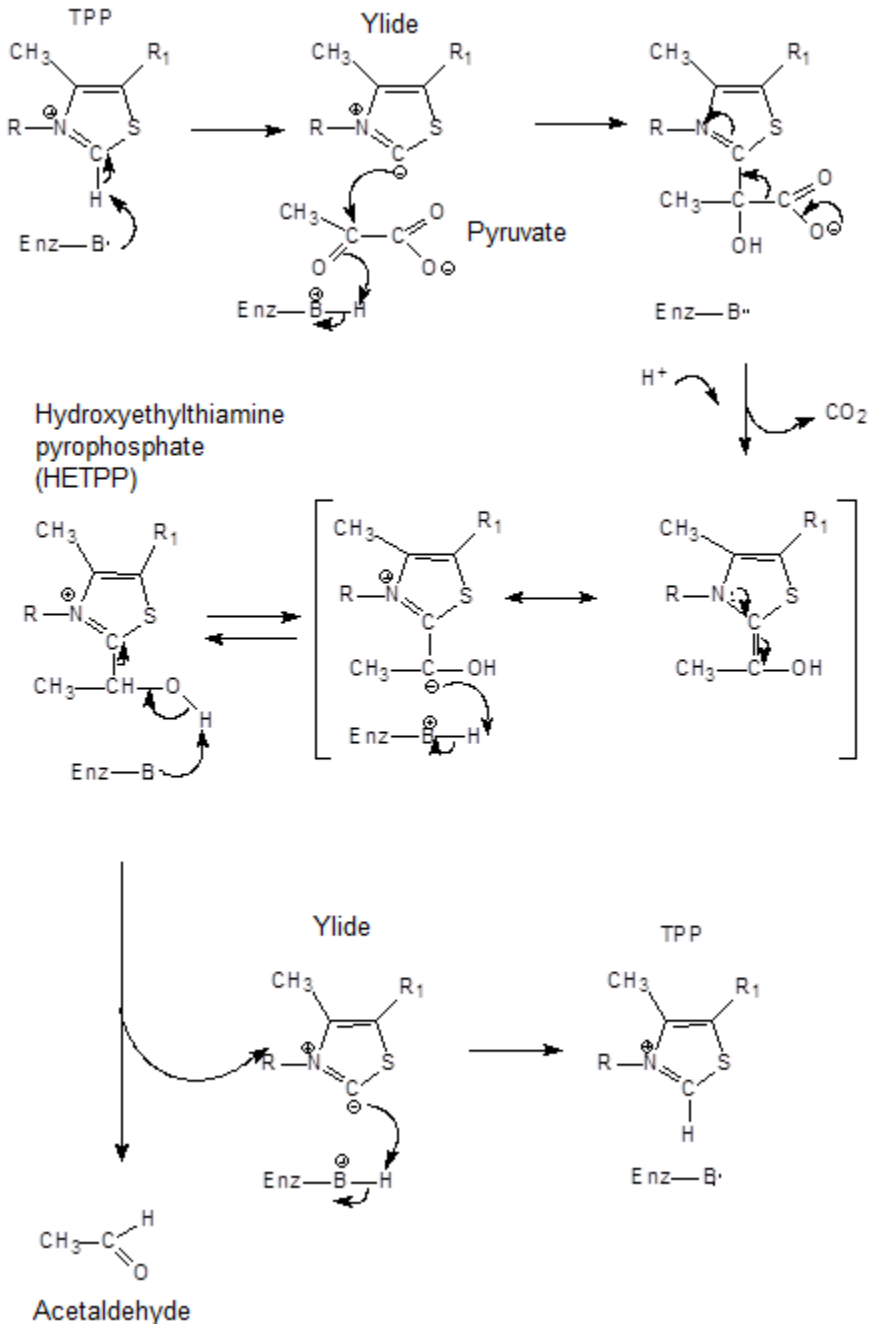


Figure 7. (a) Thiamine (vitamin B_1); (b) Thiamine pyrophosphate (TPP); (c) The mechanism of pyruvate decarboxylase from the yeast

Thiazoline ring of the coenzyme contains the reactive center. C-2 of TPP has unusual activity; it is acidic despite high pK_a in aqueous solution. Experiments show that pK_a value for the ionization of hydroxyethylamine pyrophosphate (HETPP) (*i.e.*, forming of dipolar carbanion)

changes from 15 in water to 6 on the active center of pyruvate decarboxylase. This increased acidity is ascribed to low polarity of active center, which is also responsible for the increased reactivity of TPP itself. The positive charge of thiazoline ring of TPP attracts electrons, weakening the bond between C-2 and hydrogen. The proton is mostly removing by base part of the enzyme. Ionization gives resonantly stable dipolar carbanion known as ylide. Negatively charged C-2 attacks electron-deficit carbonyl carbon of pyruvate substrate, and the first product (CO_2) is releasing. Two carbons of pyruvate are now attached to thiazoline ring as a part of resonance-stabilized carbanion. In the next step, the protonation of carbanion gives hydroxyethylamine pyrophosphate (HETPP). HETPP is separating, releasing acetaldehyde (the second product). TPP is forming again when ylide is protonating from the side of the enzyme (Figure 7c) (Shepard and Broderick, 2010).

Pyridoxal phosphate

The family of B_6 vitamins soluble in water consists of three closely connected molecules differing only in the state of the oxidation or amination of the carbon bound to the position 4 of the pyridine ring (Figure 8a). Induced deficiency of vitamin B_6 in mice causes dermatitis and various disorders connected to the metabolism of proteins; deficiencies of vitamin B_6 in humans are rare. Once B_6 enters the cell, enzymatic transfer of γ-phosphoryl group from ATP forms coenzyme pyridoxal 5'-phosphate (PLP) (Figure 8b) (Hayashi et al., 1990).

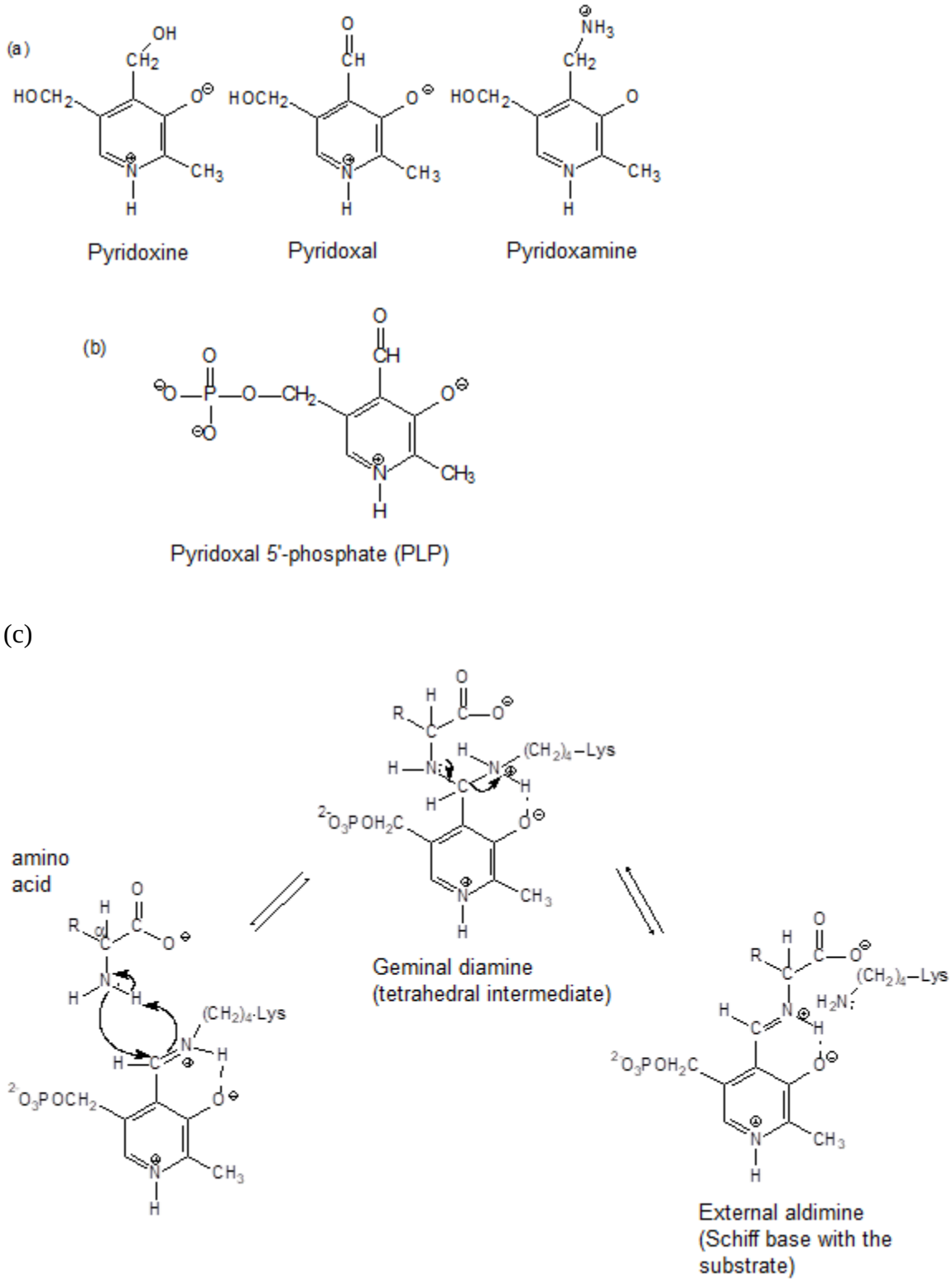


Figure 8. (a) Vitamins of B_6 family: pyridoxine, pyridoxal, and pyridoxamine; (b) Pyridoxal 5'-phosphate (PLP); (c) The binding of the substrate to the PLP-dependent enzyme

Pyridoxal phosphate is a prosthetic group for many enzymes which catalyze different reactions, including isomerizations, decarboxylations, and eliminations in side chain or substitutions. In enzymes dependent on PLP, the carbonyl group of the prosthetic group is binding as Schiff base (imine) for ε-amino group of a lysine residue in the active center (Figure 8c) (Hayashi et al., 1990; Horton et al., 2006).

Transamination is most frequently dependent reaction on PLP, and the mechanism of this type of the reaction is presented in Figure 9.

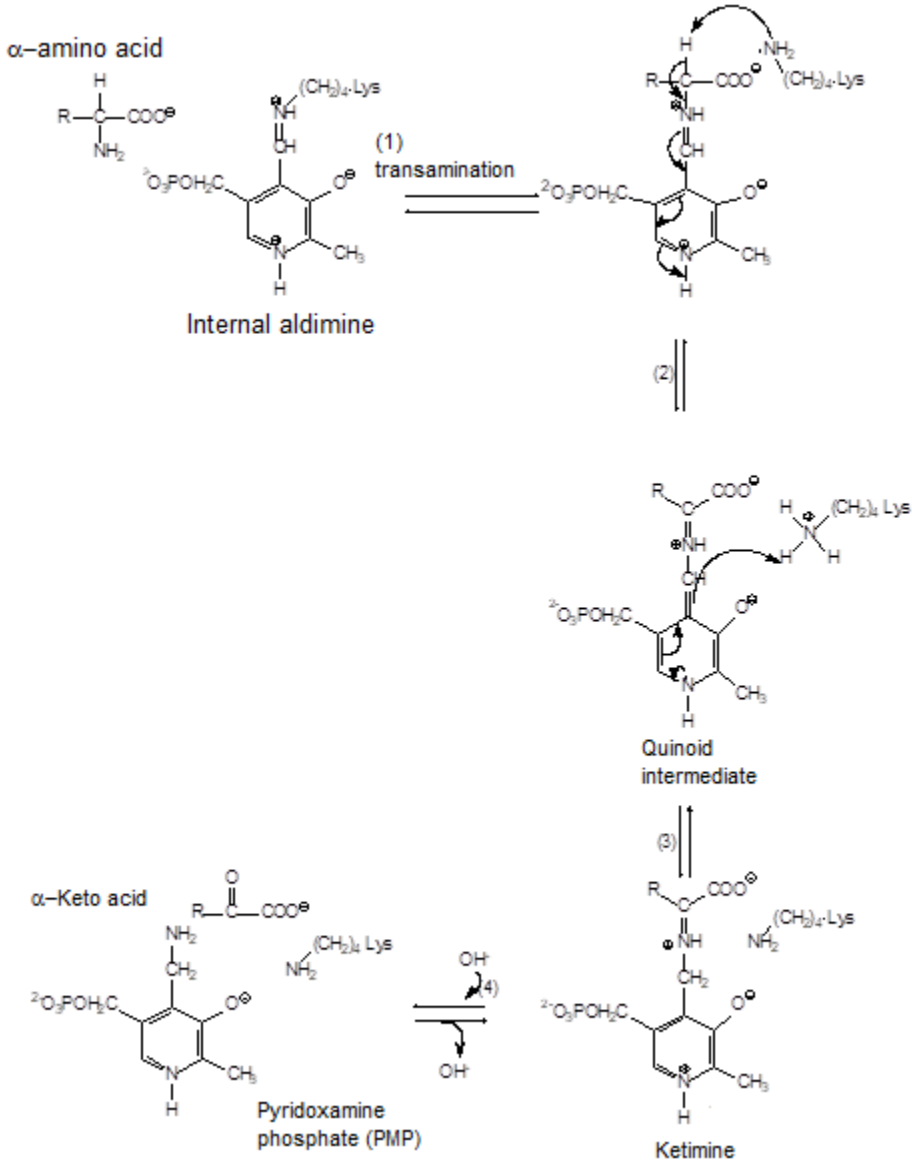


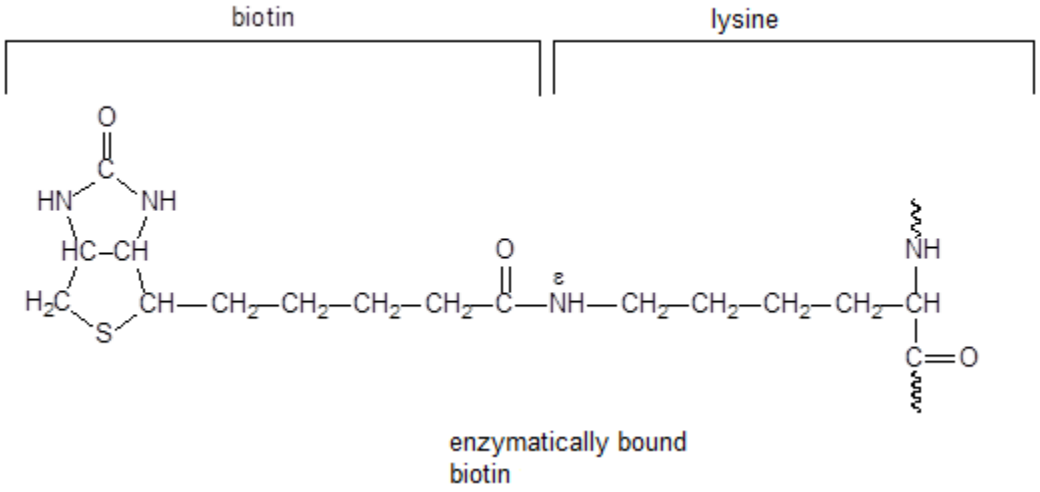
Figure 9. The mechanism of transaminase (Horton et al., 2006)

In the first step of the mechanism, amino acid replaces lysine from internal aldimine which binds PLP for the enzyme forming the external aldimine. In the second step, α-hydrogen of the amino acid is taken with the base catalyst by the same lysine residue. Electronic rearrangement leads to the quinoid intermediate. In the third step, the protonation of the intermediate with the lysine residue gives ketoimine. In the fourth step, hydrolysis of the ketoimine gives α-keto acid, which dissociates, and PMP remains bound to the enzyme. If another α-keto acid enters, each step goes in reverse. The amino acid is transferred to α-keto acid, giving new amino acid and regenerates the original PLP form of the enzyme (Figure 9) (Hayashi et al., 1990; Horton et al., 2006).

Biotin

Biotin is a prosthetic group for enzymes which catalyze the reactions of the transfer of carboxyl group and the reaction of carboxylation dependent on ATP. It is covalently bound to the active center of its enzyme host by amide bond for ε-amino group of a lysine residue (Figure 10a).

(a)



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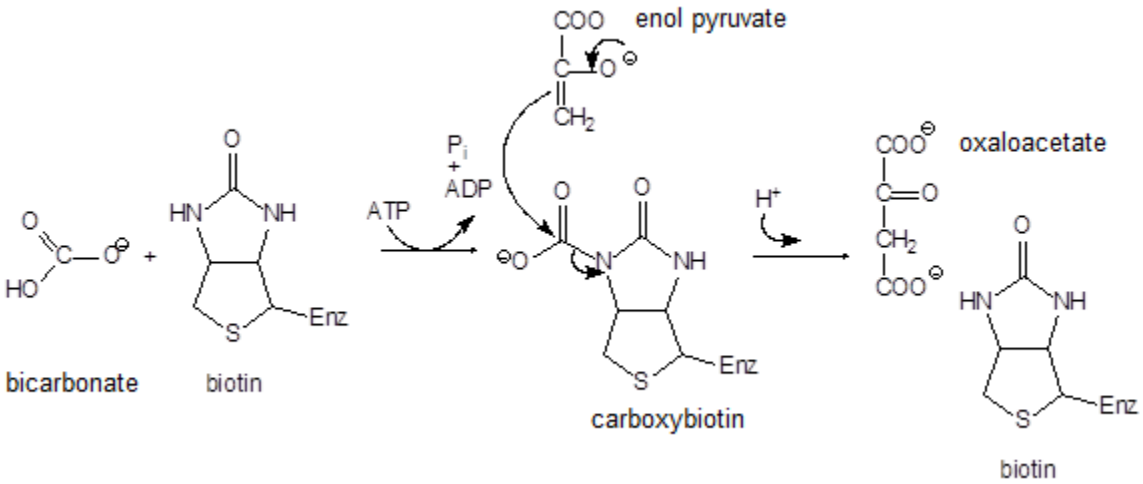


Figure 10. (a) Enzymatically bound biotin. (b) The reaction catalyzed by pyruvate carboxylase (Knowles, 1989)

The reaction of pyruvate carboxylase demonstrates the role of biotin as a carrier of carbon dioxide (Figure 10b). Firstly, bicarbonate and ATP react forming carboxybiotin. Carboxybiotinyl-enzyme complex gives stable activated form CO_2 which can be transferred to the pyruvate. Then the enolate form of the pyruvate attacks carboxyl group of carboxybiotin, forming oxaloacetate and regenerated biotin (Figure 10b).

The biotin is synthesized in intestine bacteria, and it is necessary in micrograms daily, so the deficiency of biotin is rare in humans or animals with regular feeding.

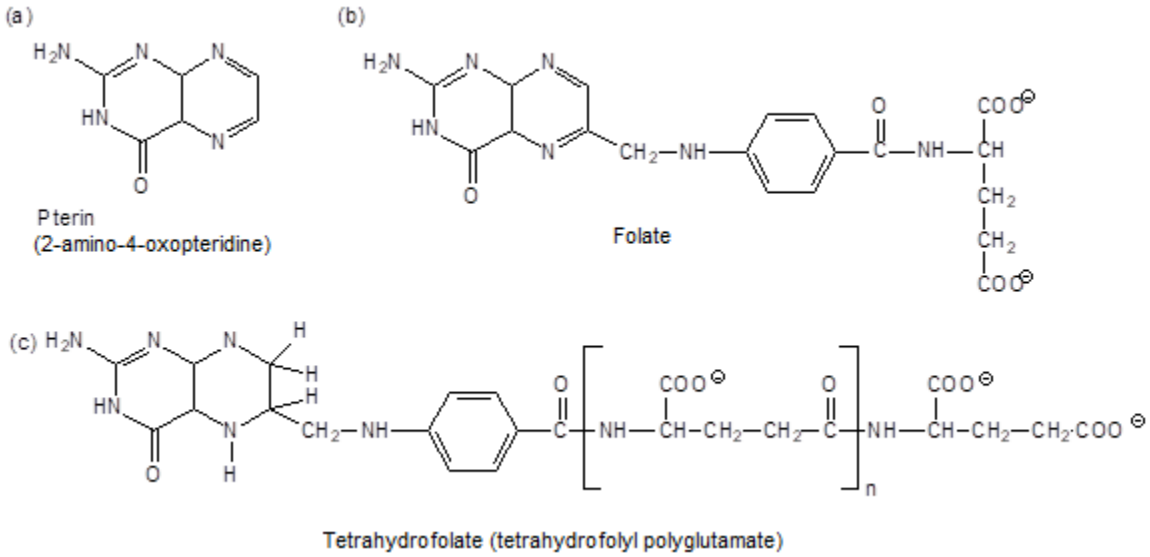
Different laboratory techniques use high affinity of avidin for biotin. For example, the substance for which biotin is covalently bound can be extracted from the complex mixture by affinity chromatography on the column immobilized with avidin (Hsu, 1985).

Tetrahydrofolate

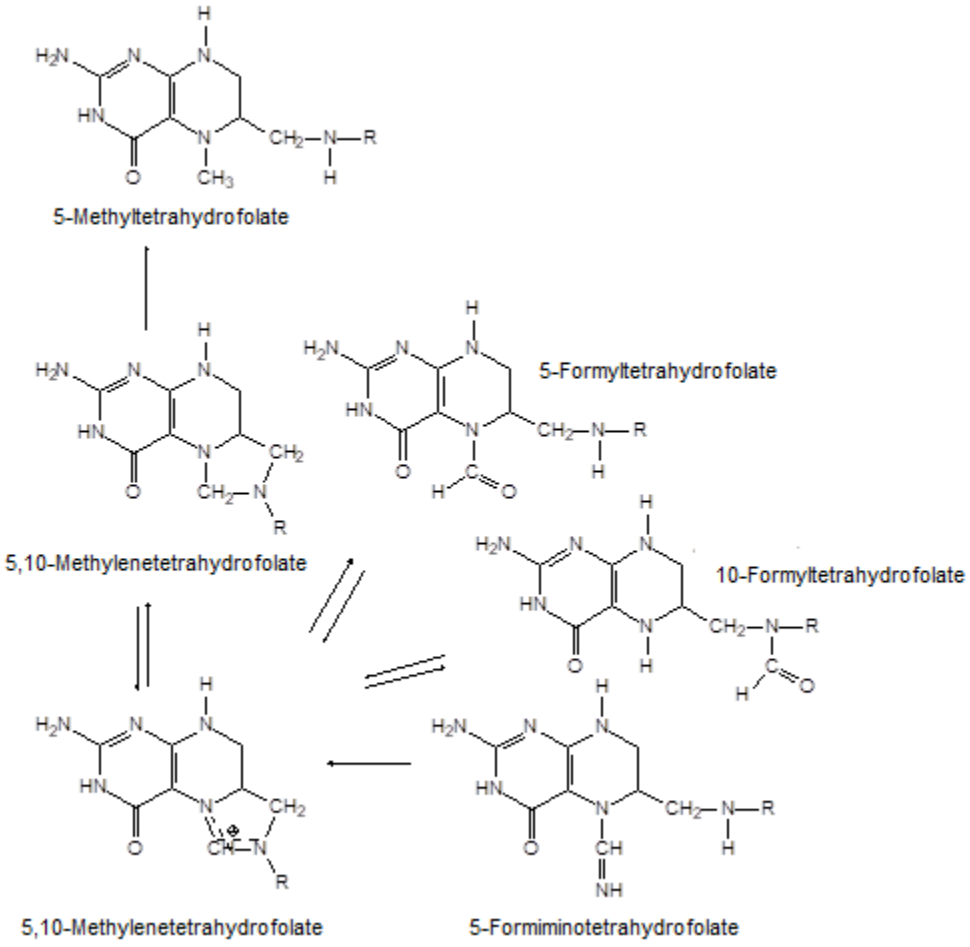
Vitamin folate is isolated for the first time in the early 1940s from green leaves, liver, and yeast. Folate has three main components: pterin (2-amino-4-oxopteridine) (Figure 11a), the

residue of *p*-aminobenzoic acid, and residue of the glutamate. Humans need folate in nutrition because they are not in the position to synthesize pterin-*p*-aminobenzoic acid intermediate (PABA) (Wallig and Keenan, 2013).

Coenzyme formed from the folate (Figure 11b) is known as tetrahydrofolate (Figure 11c) (Wallig and Keenan, 2013).



(d)



(e)

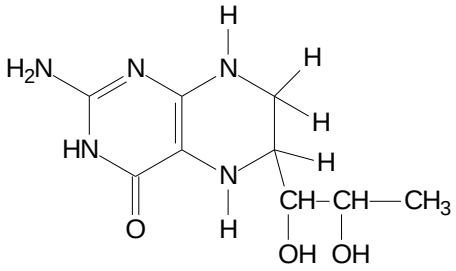


Figure 11. (a) Pterin; (b) Folate; (c) Tetrahydrofolate; (d) Monocarbonic derivatives of tetrahydrofolates; (e) 5,6,7,8-tetrahydrobiopterin

The anionic polyglutamic residue, usually five to six residues long, takes part in the binding of coenzymes for enzymes (Wallig and Keenan, 2013).

Tetrahydrofolate is formed by the addition of hydrogens into positions 5, 6, 7 and 8 of pterin cyclic system.

The reduction of dihydrofolate obtained during the formation of the methyl group of thymidylates (dTMP) is the primary metabolic function of dihydrofolate reductase. This reaction which uses derivative of tetrahydrofolate is the essential step in the synthesis of DNA. Because the cell division cannot be achieved when DNA synthesis is stopped, dihydrofolate reductase is intensively studied as an aim in chemotherapy for cancer treatment (Horton et al., 2006).

5,6,7,8-tetrahydrofolate is necessary to enzymes which catalyze biochemical transfers of several monocarbon units. The groups bound to tetrahydrofolate are methylene, methyl, and formyl. Figure 11d shows the structure of several monocarbon derivatives of tetrahydrofolate and enzymatic interconversion happens between them (Horton et al., 2006).

Monocarbonic metabolic groups are covalently bound for the secondary amine N-5 or N-10 of tetrahydrofolates, or both in cyclic form. 10-Formyltetrahydrofolate is a donor of formyl groups, and 5,10-methylenetetrahydrofolate is the donor of hydroxymethyl groups (Figure 11d).

The second pterin coenzyme, 5,6,7,8-tetrahydrobiopterin has a side chain with three carbons on C-6 pterin part instead of long side chain which is situated in tetrahydrofolate (Figure 11e) (Wallig and Keenan, 2013). This coenzyme is not derived from vitamin; it is synthesized by animals alone and by other organisms. Tetrahydrobiopterin is a cofactor for several hydroxylases, and it is a reducing agent in the conversion of phenylalanine to tyrosine. Also, it is necessary to the enzyme which catalyzes the synthesis of nitrogen oxide from arginine.

Cobalamin

Cobalamin (vitamin B_{12}) is the biggest B vitamin, and it is last isolated. The structure of cobalamin includes corrin ring system which is similar to the porphyrinic cyclic system of heme. Cobalamin contains cobalt instead of iron which is situated in heme. In coenzyme form of cobalamin, R group is either methyl group (in methyl-cobalamin) or 5'-deoxyadenosyl group (in adenosylcobalamin) (Banerjee and Ragsdale, 2003).

Cobalamin is necessary as a micro substance to all animals and some bacteria and algae, but it is not necessary for plants, and they are not synthesizing them. Thus, humans will generally get vitamin B_{12} from food of animal origin. Vegetarians are getting appropriate quantity from microorganisms. The cobalamin deficiency can cause anemia, potentially fatal diseases in which

there is decreasing in the production of blood cells by bone marrow. This type of anemia can lead to neurological disorders. Majority of victims of this anemia do not excrete necessary glycoprotein (called internal factor) (Arsic et al., 2016).

The role of adenosylcobalamin reflects the reactivity of C-Co bond. The coenzyme takes part in several intramolecular rearrangements catalyzed by enzymes where hydrogen atom and another group bound to the neighboring carbon atoms inside the substrate, by exchanging places (Figure 12a). The example is methyl malonyl-CoA mutase reaction (Figure 12b), important in the fatty acids' metabolism containing an odd number of carbon atoms, which leads to the formation of succinyl CoA, intermediate in the chain of citric acid (Banerjee and Ragsdale, 2003).

Methylcobalamin takes part in the transfer of methyl groups and the regeneration of methionine from homocysteine in mammals. In this reaction, the methyl group of 5-methyltetrahydrofolate transits into reactive, reduced form of cobalamin creating methylcobalamin, which can transfer a methyl group to thiol chain of homocysteine.

The third group of cobalamin enzymes is reduced dehalogenase dependent on vitamin B_{12}. They are bacterial enzymes making detoxication of chlorinated organic molecules including PCB (Banerjee and Ragsdale, 2003; Horton et al., 2006).

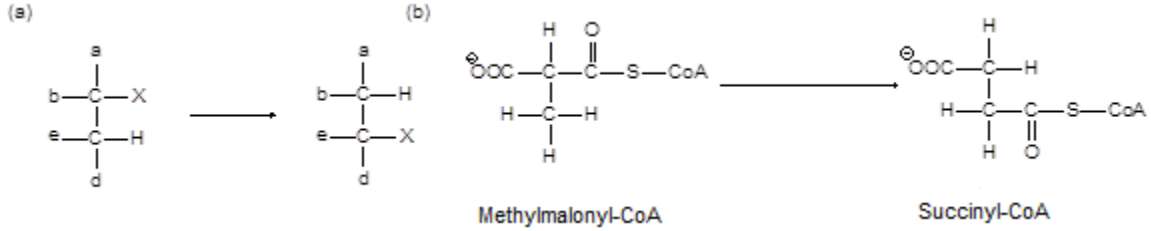


Figure 12. (a) The rearrangement in which hydrogen atom and the substituent on a neighboring carbon atom are exchanging places; (b) The rearrangement of methyl malonyl CoA in succinyl CoA, catalyzed with methyl malonyl-CoA mutase (Horton et al., 2006)

Lipoamide

Coenzyme lipoamide is a protein binding form of lipoic acid. Although lipoic acid is often described as vitamin B, it seems that the animals are capable of synthesizing it. It is necessary for particular bacteria and protozoa to grow (Figure 13) (Shen et al., 2011).

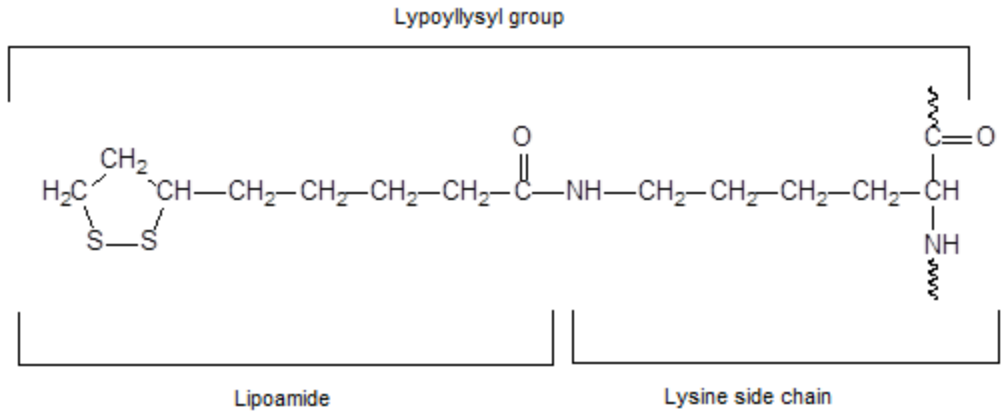


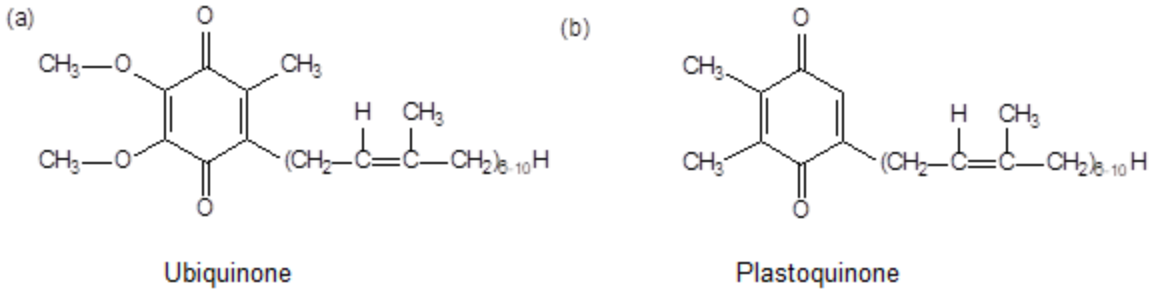
Figure 13. Lipoamide

It is believed for lipoamide to function as a pendulum which carries acyl groups between active centers in multi-enzymatic complexes. For example, in complex of pyruvate dehydrogenase, disulfide ring of lipoamide prosthetic group reacts with HETPP, binding its acetyl group on sulfur atom attached to C-8 of lipoamide and creating thioester. Then, the acyl group is moved to the sulfur atom of coenzyme A giving reduced (dihydrolipoamide) form of prosthetic group.

The last step catalyzed by pyruvate dehydrogenase complex is the oxidation of dihydrolipoamide. In this reaction, NADH is formed by the action of the flavoprotein component of the complex. The actions of multiple coenzymes of pyruvate dehydrogenase complex show how coenzymes supplying reactive groups and thus increasing catalytic diversity of proteins are used for storing energy and carbon building blocks (Horton et al., 2006; Shen et al., 2011).

Ubiquinone

Ubiquinone (coenzyme Q) (Figure 14a) is a coenzyme soluble in fats synthesized by all species. In the membrane, ubiquinone transports electrons between enzymatic complexes situated in the membrane. Some bacteria use menaquinone instead of ubiquinone. Ubiquinone analog called plastoquinone (Figure 14b) has a similar function in the photosynthetic transport of electrons in chloroplasts (DiNicolantonio et al., 2015).



(c)

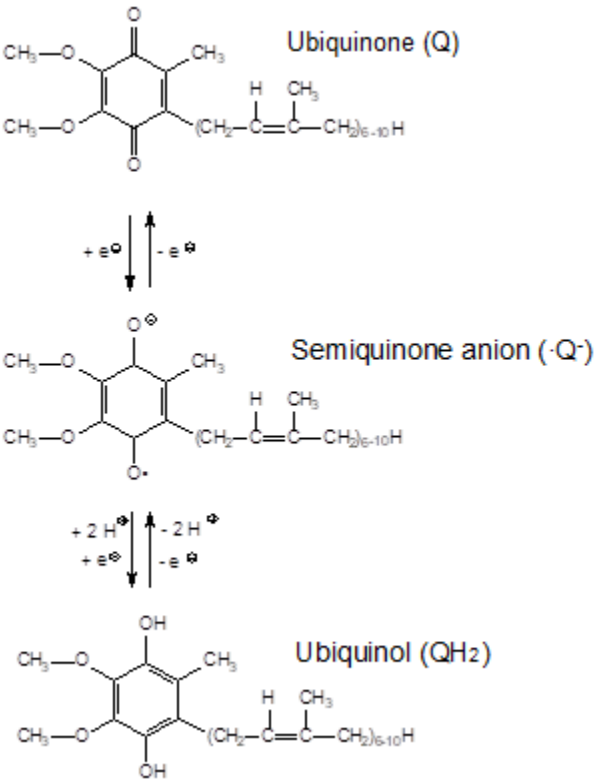


Figure 14. (a) Ubiquinone; (b) Plastoquinone; (c) Three oxidation states of ubiquinone

Ubiquinone is a stronger oxidation reagent than both NAD^+ and flavin coenzymes. Similarly to FMN and FAD, ubiquinone can accept or donate electrons (one or two) because it has three oxidation states: oxidized Q, partially reduced semiquinone free radical and completely reduced QH_2 (ubiquinol) (Figure 14c) (Sohal, 2004).

Coenzyme Q plays the leading role in the electron transport connected to the membrane. It is responsible for the moving of protons from one side of the membrane to another by the process known as cycle Q. The created protein gradient leads to ATP synthesis (Lenaz et al., 2007).

Protein coenzymes

Some proteins behave as coenzymes. They do not catalyze reactions, but they are necessary from specific enzymes. These enzymes are called either protein for groups transfer or protein coenzymes. They contain functional group either as a part of their protein skeleton or as a prosthetic group (Horton et al., 2006).

Metal ions, iron-sulfur clusters, and heme groups are reactive centers usually found in these protein coenzymes. Several protein coenzymes have two reactive centers. Thioredoxins are observed as reduction agents (cycle of citric acid, photosynthesis, and synthesis of deoxyribonucleotides). Disulfide reactive center of thioredoxin is on the surface of the protein, so it is available to the active centers of corresponded enzymes (Horton et al., 2006; Johnson et al., 2014).

Cytochromes

Cytochromes are protein coenzymes containing heme where Fe (III) atoms undergo reversible one-electron reduction. They are classified as *a*, *b* and *c* based on their visible absorption spectra. Heme of cytochrome *b* type is the same as that of hemoglobin and myoglobin. Heme of cytochrome *a* has a hydrophobic chain of 17 carbons on C-2 porphyrin ring and formyl group on C-8, while heme of type *b* has a vinyl group attached on C-2 and methyl group on C-8. In cytochromes of type *c*, the heme is covalently bound to apoprotein with two thioester bonds formed by the addition of thiol groups of two cysteine residues for vinyl groups of the heme (Heldt and Piechulla, 2011).

The tendency to transfer an electron to another substance, measured as a reductive potential, also varies among cytochromes. The range of reduction potentials among prosthetic groups is an

essential property of membrane-connected electron transferred cycles and biosynthesis (Heldt and Piechulla, 2011).

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