

Chapter 7. "Coenzymes and Vitamins"

Reading Assignment: pp. 192-202, 207-208, 212-214

Problem Assignment: 3, 4, & 7

I. Introduction

Many complex metabolic reactions cannot be carried out using only the chemical mechanisms available to the side-chains of the 20 standard amino acids. To perform these reactions, enzymes must rely on other chemical species known broadly as cofactors that bind to the active site and assist in the reaction mechanism. An enzyme lacking its cofactor is referred to as an apoenzyme whereas the enzyme with its cofactor is referred to as a holoenzyme. Cofactors are subdivided into essential ions and organic molecules known as coenzymes (**Fig. 7.1**). Essential ions, commonly metal ions, may participate in substrate binding or directly in the catalytic mechanism. Coenzymes typically act as group transfer agents, carrying electrons and chemical groups such as acyl groups, methyl groups, etc., depending on the coenzyme. Many of the coenzymes are derived from vitamins which are essential for metabolism, growth, and development. We will use this chapter to introduce all of the vitamins and coenzymes. In a few cases--NAD⁺, FAD, coenzyme A--the mechanisms of action will be covered. For the remainder of the water-soluble vitamins, discussion of function will be delayed until we encounter them in metabolism. We also will discuss the biochemistry of the fat-soluble vitamins here.

II. Inorganic cation cofactors

Many enzymes require metal cations for activity. Metal-activated enzymes require or are stimulated by cations such as K⁺, Ca²⁺, or Mg²⁺. Often the metal ion is not tightly bound and may even be carried into the active site attached to a substrate, as occurs in the case of kinases whose actual substrate is a magnesium-ATP complex. In metalloenzymes, the metal ion is tightly bound and participates in the catalytic mechanism more directly. A good example is the Zn²⁺ ion in the active site of carbonic anhydrase (**Fig. 7.2**). Here, the zinc promotes ionization of a water molecule in the active site to form a hydroxyl group. Formation of the hydroxyl group is favored because it is stabilized by electrostatic interaction with the zinc. The hydroxyl group subsequently attacks a CO₂ molecule that has entered the active site, yielding bicarbonate as the final product of this reaction. In other

metalloenzymes, metal ions participate directly in electron transfers during oxidation-reduction reactions.

III. Coenzyme classification

Coenzymes are part of the active site of the enzyme; they supply reactive groups that are not available on the side chains of the amino acids.

Coenzymes that can be synthesized from common metabolites are referred to as metabolite coenzymes. Those that cannot be synthesized and are derived from vitamins are known as vitamin-derived coenzymes.

Coenzymes can also be further classified based on whether they remain bound to an enzyme at all times or not. Many come on and off enzymes, carrying electrons, etc. from one enzyme to another. These are referred to as cosubstrates. Cosubstrates are actually substrates in the reactions: they are altered in the course of the reaction and dissociate from the active site. The original cosubstrate structure has to be regenerated in a subsequent independent reaction catalyzed by another enzyme. Thus, cosubstrates are continuously recycled in the cell, whereas the actual substrates typically undergo further transformations.

Coenzymes that remain bound, either covalently or noncovalently (via numerous weak interactions) to an enzyme are called prosthetic groups. Prosthetic coenzymes must return to their original form during each catalytic cycle.

A. Metabolite coenzymes

Examples of metabolite coenzymes are ATP, and nucleotide sugars such as uridine diphosphate glucose (UDP-glucose). ATP (**Fig. 7.4**) has a central function in phosphoryl-group transfer, as occurs in reactions catalyzed by kinases. Pyrophosphoryl and adenylyl (AMP) groups also can be transferred from ATP to substrates. Nucleotide-sugar coenzymes such as UDP-glucose (**Fig. 7.6**) are involved in carbohydrate metabolism. UDP-glucose is the carrier of glucose units used in glycogen synthesis whereas ADP-glucose is the carrier of glucose units used in starch synthesis.

B. Vitamin-derived coenzymes and nutrition

Vitamins are required for the synthesis of the vitamin-derived coenzymes. Thus, vitamins must be obtained daily from sources which are able to synthesize them such as fruits and vegetables. Nutritional

deficiency diseases result when daily consumption of vitamins is inadequate. Nine diseases, including some of classic renown, are listed in **Table 7.1**. While these diseases are not common in developed countries like the USA thanks to ample food supply and government-supported food supplementation programs, they still are common in many third world and developing countries. Vitamins can be classified into the water-soluble (the B vitamins and vitamin C) and fat-soluble (vitamin A, D, E, and K) groups. The consumption of excess water-soluble vitamins simply results in their excretion in the urine. Consumption of excess fat-soluble vitamins can lead to toxic conditions known as hypervitaminoses. The vitamin sources and the roles of the major coenzymes in metabolism, including both metabolite coenzymes and vitamin-derived coenzymes, are listed in **Table 7.2**.

IV. NAD^+ and NADP^+

These compounds were the first coenzymes to be recognized. The nicotinamide coenzymes are nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate (NADP^+). Both are derived from the vitamin niacin (nicotinic acid) (**Fig. 7.7**), the deficiency of which causes the disease pellagra. Niacin is present in many cereals, meat, and legumes. Humans can make a small amount of niacin from the amino acid tryptophan, but not enough to meet requirements. These coenzymes also are commonly called pyridine nucleotides due to the resemblance of the niacin ring to the organic compound pyridine.

The structures of the oxidized (NAD^+ & NADP^+) and reduced (NADH & NADPH) forms of the pyridine nucleotides are shown in **Fig. 7.8**. Each molecule contains 2 nucleotide moieties linked by a phosphoanhydride linkage, hence the dinucleotide designation. One of these nucleotides is adenosine monophosphate (AMP) which is a common component of many coenzymes. The NADP^+ and NADPH forms contain a phosphate attached to the 2'-hydroxyl group of the AMP group. The phosphate is important in enzyme recognition. Namely, NAD^+ is used only in oxidative reactions of catabolism whereas NADPH is used in the reductive biosynthesis reactions of anabolism.

The pyridine nucleotides function as electron carriers in oxidation-reduction reactions. In general, NAD^+ is used in reactions where an alcohol group is converted to a ketone or aldehyde group. The oxidized forms pick up electrons from substrates in the form of a hydride ion (H^-) which is carried at position-4 of the nicotinamide ring. The hydride can then be transferred to another acceptor regenerating the oxidized forms of the pyridine nucleotides. The participation of

NAD⁺ in metabolism is illustrated in the lactate dehydrogenase reaction (**Reaction 7.3 & Fig. 7.9**). During the reaction, a hydride is transferred from lactate carbon-2 to NAD⁺ forming NADH and pyruvate. A proton also is removed from lactate by a histidine in the active site, and this proton is eventually released into the cytoplasm.

V. FAD and FMN

The flavin nucleotides, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are derived from riboflavin (vitamin B₂). Vegetables, grains, and meats such as liver are rich sources of riboflavin. Riboflavin itself is composed of an isoalloxazine ring and a molecule of ribitol (**Fig. 7.10**). A phosphate is attached to the ribitol in FMN, and the phosphate plus AMP is attached in the dinucleotide FAD (**Fig. 7.10**). Riboflavin and the flavin nucleotides are colored bright yellow because the isoalloxazine ring absorbs light strongly. In contrast to NAD⁺ and NADP⁺, FMN and FAD are prosthetic groups which remain tightly bound to their enzymes and do not shuttle back and forth between enzymes. Enzymes that contain these coenzymes are called flavoenzymes or flavoproteins.

FMN and FAD also carry electrons in oxidation-reduction reactions. They are more powerful oxidants than NAD⁺ and NADP⁺ and often participate in reactions where a carbon-carbon single bond is oxidized to a double bond. Flavin nucleotides can accept and donate electrons either one or two at a time. The fully reduced forms that carry 2 electrons are FMNH₂ and FADH₂. The partially reduced forms that carry one electron are relatively stable free radicals called semiquinones. The oxidized quinone (FMN & FAD), reduced hydroquinone (FMNH₂ & FADH₂), and semiquinone (FMNH[•] & FADH[•]) forms of the isoalloxazine ring are shown in **Fig. 7.11**.

VI. Coenzyme A

Coenzyme A is an important coenzyme that is used to carry acyl groups in numerous reactions of carbohydrate and lipid metabolism. Coenzyme A (abbreviated CoA or HS-CoA) is composed of 2-mercaptoethylamine, which contains a free -SH group, the vitamin pantothenate (vitamin B₃), and a molecule of ADP in which the ribose 3'-hydroxyl is phosphorylated (**Fig. 7.12**). Acyl groups are carried in thioester linkage by the -SH group of 2-mercaptoethylamine. These compounds are referred to acyl-CoAs.

VII. Biotin

Biotin is a prosthetic coenzyme that catalyzes carboxyl-group transfer and ATP-dependent carboxylation reactions. It is covalently linked to the active site of its host enzyme by an amide bond to the ϵ -amino group of a lysine residue (**Fig. 7.19**).

Biotin deficiency is rare in humans, since very small quantities are required and since it is synthesized by intestinal bacteria (do not eat raw eggs!).

The high affinity of biotin to avidin is a useful tool in laboratory investigations (for purification, for attachment of substances to avidin-coated surfaces, etc.).

VII. Fat-soluble vitamins.

All fat-soluble vitamins are poorly soluble in water due to the presence of large nonpolar regions. They are absorbed in the intestines along with other dietary lipids and transported in the blood by specific carrier proteins or plasma lipoproteins.

A. Vitamin A (retinol)

Vitamin A is a 20-carbon lipid that can be derived by cleavage of β -carotene present in vegetables (**Fig. 7.27**). It also is prevalent in sources such as liver, egg yolk, and milk products. Vitamin A contains an alcohol group and is known as retinol. The retinol and retinoic acid forms are important regulators of gene expression, particularly during development. The aldehyde form retinal is the prosthetic group in the protein rhodopsin which captures photons in the eye. A deficiency of vitamin A causes night blindness.

B. Vitamin D₃ (cholecalciferol) and D₂

Vitamin D is the name for a group of related lipids that are the precursors of the hormone 1,25-dihydroxycholecalciferol which is important in calcium metabolism. Vitamin D₃ (cholecalciferol) is formed in the skin in people who get significant exposure to sunlight by a photoreaction in which a precursor of cholesterol is converted to the vitamin. Cholecalciferol subsequently is converted via 2 hydroxylation steps to 1,25-dihydroxycholecalciferol, the active hormone (**Fig. 7.28**). Vitamin D₂ is a different form of the vitamin that also can be converted to active hormone. Vitamin D₂ is produced in microorganisms such as yeast and is added to milk as a dietary supplement. People living in northern latitudes who received relatively little sunlight historically were prone to develop the disease known as rickets, which is noted for curvature of the long bones such as the

femur. It is believed that people living in tropical regions ultimately developed highly pigmented skin as protection against toxicity caused by the overproduction of vitamin D.

C. Vitamin E (α -tocopherol)

Vitamin E is an important lipid-soluble antioxidant that helps protect polyunsaturated fatty acids in membrane phospholipids from oxidative damage. Vitamin E, also known as α -tocopherol, contains a phenol group that can carry an electron as a stable free radical (**Fig. 7.29**). Vitamin E probably picks up electrons from lipid free radical species, quenching propagation of free radical damage. Interestingly, the free radical form of vitamin E is recycled to its original state by reaction with vitamin C, which also plays a role in protecting against oxidative damage. The reduced form of vitamin C is eventually excreted in the urine. Vitamin E is prevalent in fish oils.

D. Vitamin K (phylloquinone)

Vitamin K serves as a coenzyme in an important post-translational modification of some blood coagulation proteins. In this reaction, glutamate residues are carboxylated to their γ -carboxyglutamate form which bind calcium (**Fig. 7.29**). This divalent cation serves as a bridge by which coagulation proteins are anchored to platelets which home in to the area where clotting is needed. Vitamin K reversibly carries an electron in its quinone ring that is used in the carboxylation reaction. A number of medically important drugs block the recycling of vitamin K carriers and decrease coagulation protein carboxylation, thus reducing clotting in patients suffering from clotting disorders. One of the original vitamin K analogs developed, warfarin, also is used as a rat poison.