Vitamins, Cosubstrates, and Coenzymes

Objectives:
I. Define the terms cofactor, activator ion, metalloenzymes, coenzyme, cosubstrate, prosthetic group, vitamin, apoenzyme, and holoenzyme.
II. Describe the structure; precursor vitamin, if present; biological function; and deficiency state for the cosubstrates / coenzymes:
   A. Nicotinamide adenine dinucleotide (NAD+)
   B. Nicotinamide adenine dinucleotide phosphate (NADP+)
   C. Flavin mononucleotide (FMN)
   D. Flavin adenine dinucleotide (FAD)
   E. Coenzyme A (CoA)
   F. Pyridoxal phosphate
   G. Thiamine pyrophosphate
   H. Tetrahydrofolate
   I. Biotin
   J. Lipoic acid
   K. Ascorbic acid
   L. Vitamin B_{12}

Background - Review

Some enzymes require nonprotein components to attain full enzymatic activity. These nonprotein prosthetic groups can be metal ions or small organic molecules. Necessary metal ions are called Cofactors or Essential Ions. Cofactors tightly bound to the protein form Metalloenzymes. Those that are loosely associated with the protein are termed Activator Ions. The small organic molecules that are tightly bound or covalently linked to the protein are called Coenzymes. Cosubstrates are diffusible, they diffuse to and bind to the protein during the catalytic cycle and then diffuse away to be used by other enzymes.

The APOENZYME is the protein part of the enzyme devoid of its required cofactor, cosubstrate, or coenzyme. The HOLOENZYME is the active functional enzyme, the protein and its necessary cofactor, cosubstrate, or
Coenzymes and Cosubstrates are often the metabolically active form of the vitamins. The word **VITAMIN** comes from **VITAL AMINE**. The first vitamins isolated were amines. Vitamins are small organic molecules that must be obtained from an outside source. Animals obtain vitamins from their diet and/or from the bacteria that colonize their gastrointestinal tracts.

There are numerous cosubstrates and coenzymes necessary for the proper functioning of cellular enzymes. A brief description of the most commonly encountered cosubstrates and coenzymes follows:

**The Important Cosubstrates and Coenzymes**

**Nicotinamide Adenine Dinucleotide (NAD⁺) and Nicotinamide Adenine Dinucleotide Phosphate (NADP⁺).** These cosubstrates are composed of an AMP molecule covalently linked to a Nicotinamide Mononucleotide, hence dinucleotide. The nicotinamide part of the molecule comes from the vitamin **Niacin**. NADP⁺ differs from NAD⁺ by the presence of an additional phosphate group on carbon two of the ribose ring of AMP. These molecules are diffusible cosubstrates that take part in oxidation / reduction reactions. NAD⁺ and NADP⁺ are the oxidized forms of the cosubstrate. When reduced, carbon four of the nicotinamide ring accepts a **Hydride (H⁻)** ion, a proton and two electrons. NAD⁺/NADP⁺ always undergo two electron oxidations or reductions. NADH and NADPH are the abbreviation for the reduced forms.

The NAD⁺/NADH pair participates in oxidative reactions involved in energy production. NAD⁺ accepts electrons, is the oxidizing agent, during a particular metabolic reaction, the NADH formed during the first reaction is then used to reduce a substrate during a subsequent metabolic reaction. The NADP⁺/NADPH pair participates in reductive biosynthetic reactions. NADPH acts as the reducing agent.
If a vitamin is present at insufficient quantities or is completely lacking in the diet a deficiency disease often results. The effects of a diet lacking a single nutrient were determined using laboratory animals. The results were then extrapolated to the human animal. Whether a deficiency disease due to the lack of a single nutrient is possible in the human animal other than induced by a laboratory formulated diet is unclear. With that said, when Niacin is lacking the disease is called Pellagra. This condition is characterized by dermatitis on exposed areas; stomatitis; atrophic, sore, magenta tongue; impaired digestion; diarrhea; and disturbances of the CNS.

**Flavin Mononucleotide (FMN) and Flavin Adenine Dinucleotide (FAD)** {below} are both derived from the vitamin Riboflavin (B2). FMN is riboflavin linked to a modified form of the sugar ribose (the sugar alcohol ribitol) with a phosphate group esterified to the hydroxyl group of carbon five. FAD is FMN linked to an AMP molecule. FMN and FAD take part in oxidation / reduction reactions. The riboflavin ring usually undergoes a two electron oxidation or reduction. The flavin ring when reduced accepts a pair of hydrogen atoms, one at N-5 and the second at N-1 of the flavin ring. The reduced form of these molecules are FMNH2 or FADH2. This coenzyme can, under certain conditions, undergo one electron oxidations or reductions. For example, it can accept two electrons (2 H atoms) from a substrate in the first step of the reaction and then pass these two electrons (H atoms), one at a time to two different electron acceptor molecules. These molecules are usually covalently linked to their apoenzyme, hence they are considered coenzymes.
Flavin Ring
(Vit - Riboflavin)

Ribitol

Flavin mononucleotide
[oxidized form] (FMN)

Flavin adenine dinucleotide
[oxidized form] (FAD)

2 Hydrogen Atoms

Flavin mononucleotide
[reduced form] (FMNH₂)

Flavin adenine dinucleotide
[reduced form] (FADH₂)
Riboflavin is not known to be the prime factor in a human deficiency disease. Riboflavin deficiency is characterized by a magenta tongue, fissuring at the corners of the mouth, seborrheic dermatitis, and corneal vascularization; symptoms similar to Pellagra. It is unclear whether this deficiency disease is due to a lack of riboflavin or due to a lack of several water soluble vitamins including niacin.

Coenzyme A (abbreviated CoA or CoA-SH) is composed of an ADP molecule, linked to a molecule of pantothenic acid (Vit B3) that is then linked to a molecule of 2-mercaptoethylamine. During the biosynthesis of CoA, the amino acid cysteine is amide linked to pantothenic acid and subsequently decarboxylated to form the 2-mercaptoethylamine. The active end of CoA is the free thiol (–SH) group of the 2-mercaptoethylamine. CoA carries carboxylic acids in thioester linkage. Since thioesters are high energy chemical bonds, the carboxylic acids carried by CoA are “activated” to undergo a variety of metabolic reactions. This molecule is a diffusible cosubstrate. No known deficiency states.

Pyridoxal phosphate is the biochemically active form of the vitamin pyridoxal (Vit B6). Pyridoxal phosphate is the prosthetic group for a large number of enzymes that catalyze a wide variety of reactions involving amino acids. These reactions include isomerization, decarboxylation, side chain elimination, and/or amino group transfer (transamination). The formation of a Schiff base between the amino group on the substrate and the carbonyl group on pyridoxal phosphate is the first step in the reactions utilizing this coenzyme. The deficiency state is characterized by growth failure, dermatitis, edema, hypochromic anemia, convulsions, and CNS changes.
THIAMINE PYROPHOSPHATE (TPP) is the metabolically active form of the vitamin THIAMINE (B1). Carbon two of the thiazolium ring reacts with and carries molecules containing a ketone functional group. It is a necessary prosthetic group for enzymes that catalyze decarboxylation reactions of 2-ketocarboxylic acids (∋-ketocarboxylic acids). It is also a prosthetic group for enzymes that transfer ketone groups between molecules (Transketolase reactions). The deficiency disease is BERIBERI. It is characterized by weight loss, muscle weakness, muscle wasting, and peripheral neuritis. Sensory changes, anxiety states, and mental confusion can occur.

TETRAHYDROFOLATE is the biologically active form of the vitamin FOLIC ACID (FOLATE). The folate molecule is composed of a conjugated PTERIN RING system coupled to 4-AMINOBENZOIC ACID which is linked to from one to six GLUTAMATE molecules. Tetrahydrofolate (TH4) is important in one carbon metabolism. It accepts and donates a variety of one carbon fragments bonded to N-5, N-10, or both. These one carbon fragments are donated to a variety of acceptor molecules. The one carbon fragments carried by TH4 are at the oxidation levels of methyl, methylene, methanol, formaldehyde, or formic acid. The deficiency state is characterized by growth failure, anemia, leukopenia, or pancytopenia.
BIOTIN was originally called vitamin H. Biotin serves as a coenzyme for enzymes that catalyze carboxyl-group-transfer reactions and ATP dependent carboxylation reactions (the addition of CO₂). The cofactor is usually covalently linked to the enzyme by an amide bond to the amino group on a lysine side chain. The usually substrate for the carboxylation reaction is the bicarbonate ion (HCO₃⁻). Mechanistically, the reaction involves at least two steps. In the first step bicarbonate ion is activated by reaction with ATP and covalent linkage to N-1 of the biotin ring system. The lysine side chain tether then moves the activated biotin-carboxyl complex to a different region of the active site where the carboxyl group is passed to an acceptor molecule.

LIPOIC ACID can be synthesized in adequate amounts by mammals, so by the strictest definition it is not a vitamin. Lipoic acid is 6, 8-DITHIOOCTANOIC ACID. The thiols can exist in a five membered oxidized disulfide ring system or in a reduced (open) form. When active it is covalently linked to the enzyme by an amide bond to the side chain of a lysine residue. Lipoic acid is involved in oxidation / reduction reactions and it carries carboxylic acids to and from active sites in multi-enzyme complexes.
VITAMIN C or ASCORBIC ACID has been discussed in the context of collagen biosynthesis. It is a necessary coenzyme for the hydroxylation of proline (Prolyl Hydroxylase) and the hydroxylation of lysine (Lysyl Hydroxylase) during collagen biosynthesis. Vitamin C also functions as an anti-oxidant within the cell.

The deficiency state is SCURVY. The symptoms of scurvy in adults include spongy gums, loosening of the teeth, bleeding into the skin and mucous membranes due to increased capillary fragility, and decreased immunocompetence. Bone development in children is abnormal resulting in bowed legs and/or stunted growth. With a severe or prolonged deficiency of vitamin C there is decreased wound healing, osteoporosis, hemorrhaging, and anemia.

VITAMIN B\textsubscript{12} or COBALAMINE takes part in only two reactions in humans. It is necessary for the Methionine Synthase a transferase reaction which methylates homocysteine to methionine and it is necessary for L-Methylmalonyl-CoA Mutase which converts L-methylmalonyl-CoA to succinyl-CoA. Vitamin B\textsubscript{12} deficiency results in the accumulation of both homocysteine and L-methylmalonyl-CoA. B\textsubscript{12} deficiency presents with megaloblastic anemia with neurological deterioration caused by progressive demyelination. Vitamin B\textsubscript{12} is widespread in foods of animal origin, especially meats. Since the liver can store up to a six year supply, deficiencies are rare except in older individuals and in long term vegetarians.