COENZYMES & VITAMINS

Course: Biochemistry I (BIOC 230)
Textbook:
Principles of Biochemistry, 5th Ed., by L. A. Moran and others. 2014, Pearson. . Chapter 7

What is a cofactor?

- Living cells require catalysts other than enzymes
- Other chemical species, called cofactors often participate in catalysis
- **Cofactors:** are required by inactive **apoenzymes** (proteins only) to convert them to active **holoenzymes**

Two types of cofactors

- Essential ions: mostly inorganic metal ions
- Coenzymes: organic compounds
- Both inorganic and organic essential portions of the active sites of certain enzymes

Essential ions

- Many of the minerals required by all organisms are essential because they are cofactors
- Some essential ions called activator ions, are reversibly bound, often participate in the binding of substrates. While some CATIONS are tightly bound and frequently participate directly in catalytic reactions

Coenzymes

- Coenzymes are group-transfer reagents
- They are specific for the chemical groups that they accept and donate; for some coenzymes, the group is hydrogen or an electron, others carry larger covalently attached larger groups

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

- Over a quarter of all known enzymes require metallic cations to achieve full catalytic activity
- These enzymes can be divided into two groups:
  - Metal-activated enzymes
  - Metalloenezymes

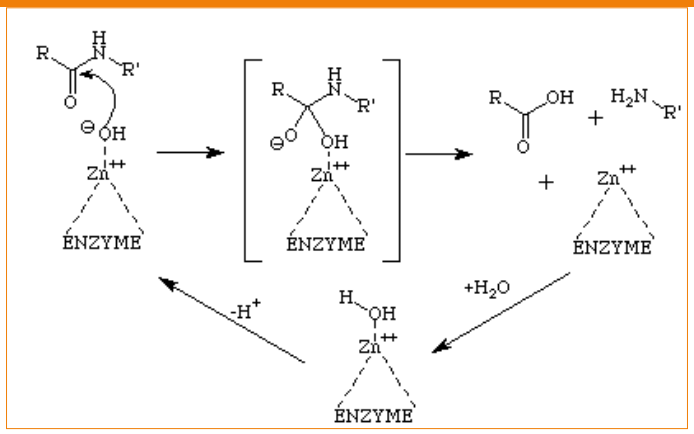
Metal-activated enzymes

- Metal-activated enzymes have either an absolute requirement for added metal ions or are stimulated by the addition of metal ions
- Some enzymes require monovalent cations such as K^+ and other require divalent cations such as Ca^{2+} or Mg^{2+}
- E.g., Kinases require Mg^{2+} for the Mg^{2+}-ATP complex they use as a phosphoryl-group donating substrate

Metalloenzymes

- Metalloenzymes contain firmly bound metal ions at their active sites
- The ions most commonly found in metalloenzymes are the transition metals such as iron and zinc and less often copper and cobalt

Metal ions can function as electrophiles in active site



Zinc protease (angiotensin converting enzyme)

Coenzyme classification

- Coenzymes can be classified into two groups based on how they interact with the apoenzyme: cosubstrates & prosthetic groups
- **Cosubstrates**: are actually substrates in enzyme-catalyzed reactions.
- Cosusbstrate is altered in the course of the reaction, dissociate from active site
- Regenerated in a subsequent reaction catalyzed by another enzyme.
- Shuttle mobile metabolic groups among different enzyme-catalyzed reactions

Prosthetic groups

- A prosthetic group remains bound to the enzyme during the course of the reaction
- Prosthetic grousp:
- Covalently attached to its apoenzyme while in other cases it is tightly bound to the active by means of weak interactions
- Must return to its original form during each full catalytic event or the holoenzyme will not remain catalytically active
- Both cosubstrates and prosthetic groups supply reactive groups not present on amino acid side chains

Coenzymes

- Metabolite coenzymes – synthesized from common metabolites
- Nucleoside triphosphates – (ATP) can donate phosphates, pyrophosphates, adenosyl groups
- S-adenosylmethionine (SAM) – donates methyl groups
- Nucleotide sugars (uridine diphosphate glucose = UDP-glucose) - transfer sugars in carbohydrate metabolism

Vitamins

- Most species are capable of synthesizing their coenzymes from simple precursors. This is specially true for 4 kingdoms: prokaryotes, protists, fungi and plants
- Animals in general have lost the ability to synthesize some coenzymes
- Mammals including human, need a source of coenzymes or their immediate precursors, in order to survive
- These coenzymes are usually supplied in nutrients, usually in small amounts
- These essential compounds are called VITAMINS

Some vitamins and their associated nutritional-deficiency diseases

Vitamin	Disease
Ascorbate (C)	Scurvy
Niacin	Pellagra
Riboflavin (B2)	Growth retardation
Pantothenate (B5)	Dermatitis in chickens
Thiamin (B1)	Beriberi
Pyridoxal (B6)	Dermatitis in rats
Biotin	Dermatitis in humans
Folate	Anemia
Cobalamin (B12)	Pernicious anemia

Vitamins

- Two classes of vitamins
  - **Water soluble:** required daily in small amounts because they are excreted in urine and cellular stores are unstable
  - **Fat soluble or lipid vitamins:** such as A, D, E, K; are stored by animals and excessive intake can result in toxic conditions known as hypervitaminoses
-

Vitamin derived coenzymes

- Must be obtained from diet
- Synthesized by microorganisms and plants
- Vitamin deficiencies lead to disease state
- Most vitamins must be enzymatically transformed to function as a coenzyme

Major coenzymes (1)

Coenzyme	Vitamin source	Major metabolic roles	Mechanistic role
Adenosine triphosphate (ATP)	-	Transfer of phosphoryl or nucleotidyl groups	Cosubstrate
S-adenosylmethionine	-	Transfer of methyl groups	Cosubstrate
Uridine diphosphate glucose	-	Transfer of glucosyl groups	Cosubstrate
NAD+ & NADP+	Niacin	Oxidation-reduction reactions involving two-electrons transfer	Cosubstrate
FMN & FAD	Riboflavin (B2)	Oxidation-reduction reactions involving one & two-electrons transfers	Prosthetic group

Major coenzymes (2)

Coenzyme	Vitamin source	Major metabolic roles	Mechanistic role
Coenzyme A (CoA)	Pantothenate (B5)	Transfer of acyl groups	Cosubstrate
Thiamine pyrophosphate TPP)	Thiamine (B1)	Transfer of two-carbon fragments containing a carbonyl group	Prosthetic group
Pyridoxal phosphate (PLP)	Pyridoxine (B6)	Transfer of groups to and from amino acids	Prosthetic groups
Biotin	Biotin	ATP-dependent carboxylation of substrates or carboxyl-group transfer between substrates	Prosthetic group

Major coenzymes (3)

Coenzyme	Vitamin source	Major metabolic roles	Mechanistic role
Tetrahydofolate	Folate	Transfer of one carbon substituents; especially formyl and hydroxymethyl groups; provides the methyl group for thiamin in DNA	Cosubstrate
Adenosylcobalamin	Cobalamin (B12)	Intramolecular rearrangements	Prosthetic group
Methylcobalamin	Cobalamin (B12)	Transfer of methyl groups	Prosthetic group

Major coenzymes (4)

Coenzyme	Vitamin source	Major metabolic roles	Mechanistic role
Lipoamide	-	Transfer of Oxidation of a hydroxyalkyl group from TPP and subsequent transfer as an acyl group	Prosthetic group
Retinal	Vitamin A	Vision	Prosthetic group
Vitamin K	Vitamin K	Carboxylation of some glutamate residues	Prosthetic group
Ubiquinone (Q)	-	Lipid-soluble electron carrier	Cosubstrate

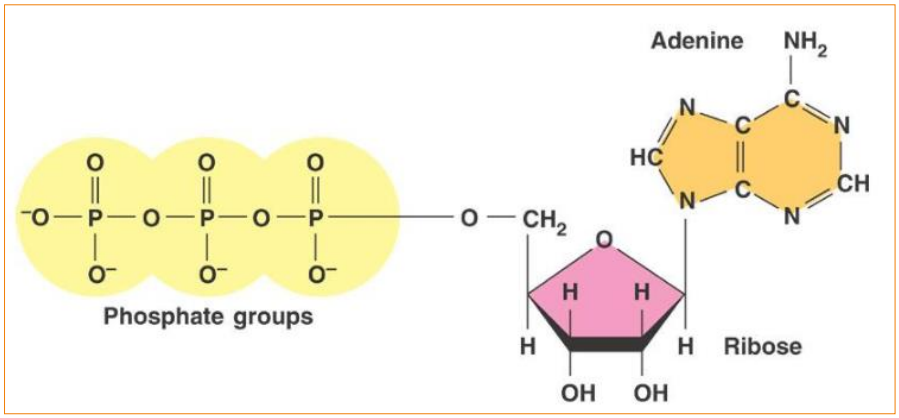
Vitamins vs. coenzymes: summary

Vitamin	Coenzyme
Ascorbic acid	Not a coenzyme
Niacin	NAD(P)+/ NAD(P)H
Riboflavin (B2)	FMN & FAD
Thiamin (B1)	Thiamin-pyrophosphate
Pyridoxal (B6)	Pyridoxal phosphate
Biotin	Biotin
Folate	Tetrahydrofolate
Cobalamin (B12)	Adenosyl- & Methyl-cobalamin
Vitamin A	Retinol
Vitamin K	Vitamin K
Pantothenate (B3)	Coenzyme A

ATP & other nucleotide cosubstrates

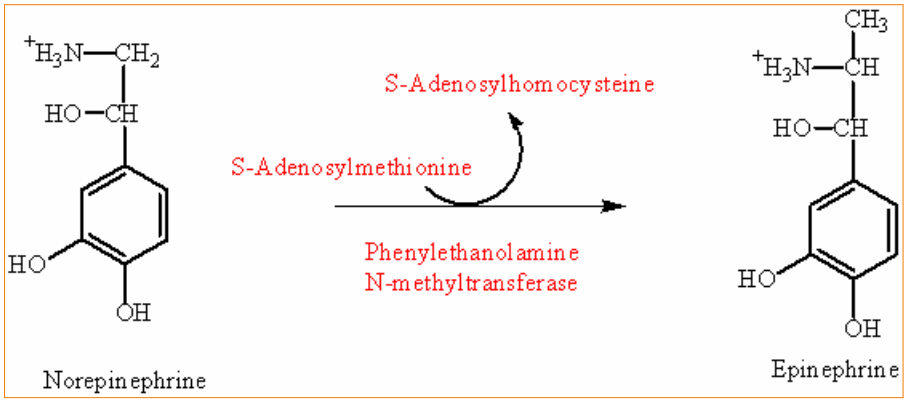
- ATP is most abundant
- Other include: GTP, S-adenosylmethionine, nucleotide sugars such as UDP-glucose
- ATP:
  - A versatile reactant that can donate its phosphoryl, pyrophosphoryl, adenylyl (AMP), or adenosyl groups in group transfer rxns
  - Most commonly involved in phosphoryl-group transfer rxns catalyzed by kinases
  - Second most common rxn is nucleotidyl-group transfer (transfer of AMP moiety), leaving PPi
  - ATP plays a central role in metabolism

ATP



ATP

- ATP is the source of several other metabolite coenzymes, like S-adenosylmethionine, synthesized by the rxn:
- Methionine + ATP → S-adenosylmethionine + Pi + PPi

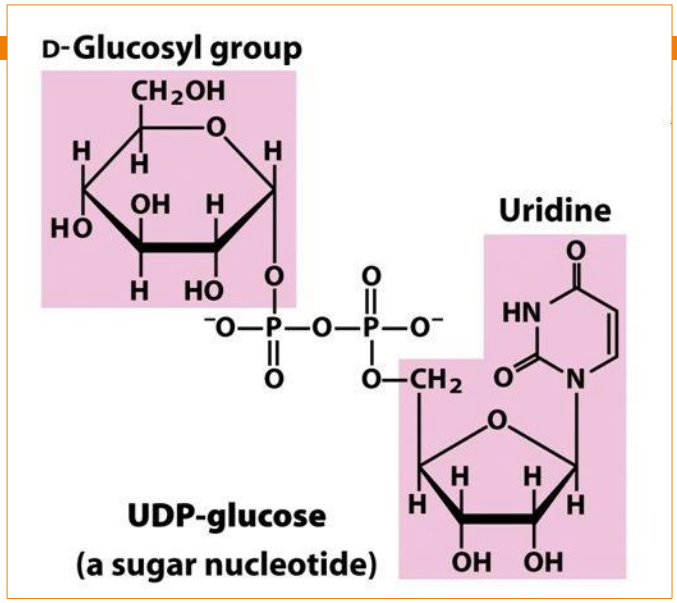


S-adenosylmethionine

- S-adenosylmethionine is donor of virtually all the methyl groups used in biosynthetic rxns, for example it is required for conversion of Norepinephrine to Epinephrine
- S-adenosylmethionine is involved in methylation of phospholipids,, proteins, DNA and RNA

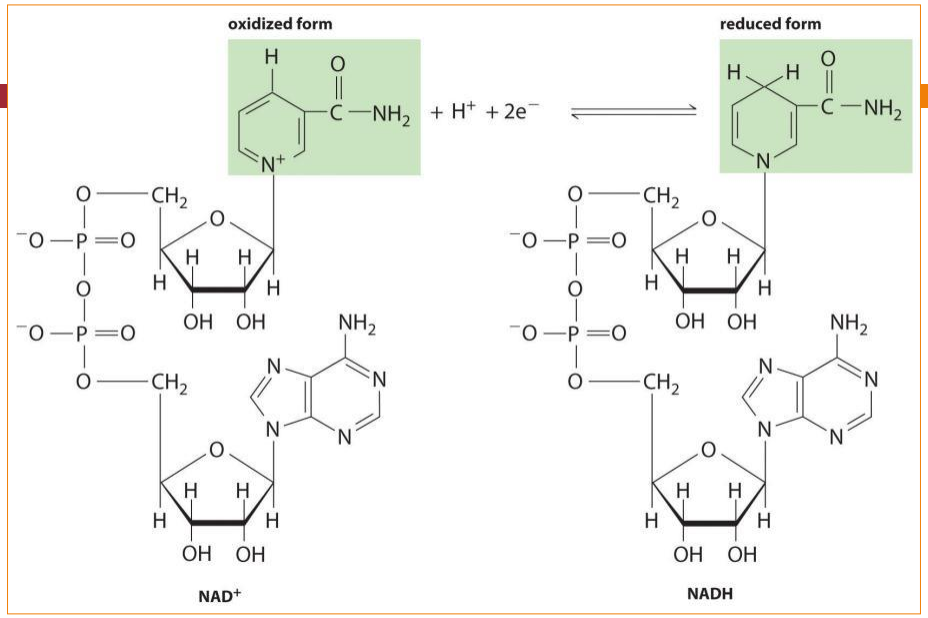
Nucleotide sugars

- Nucleotide sugars are involved in carbohydrate metabolism
- Most common is UDP-glucose
- UDP-glucose can donate its glycosyl group to a suitable receptor



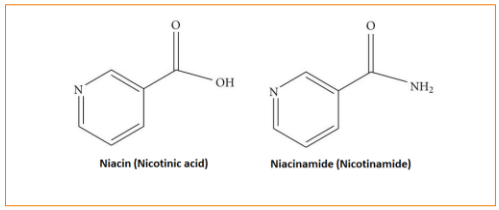
NAD+ / NADP+

- Serve as cofactors in oxidation/reduction reactions
- Act as co-substrates for dehydrogenases
- Reduction of NAD+/NADP+ and oxidation of NADH/NADPH occurs 2 e- at a time.
- Function in hydride ion transfer
- Rxns forming NADH/NADPH are catabolic
- NADH is coupled with ATP production in mitochondria
- NADPH is an impt reducing agent in biosynthetic reactions
- Reduced forms (NADH/NADPH) absorb light at 340 nm, oxidized forms (NAD+/NADP+) do not



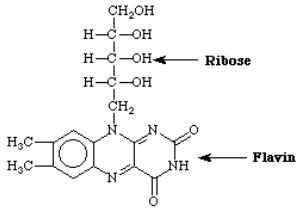
Niacin (nicotinic acid)

- Deficiencies lead to pellagra (dermatitis, diarrhea, dementia)
- Required in relatively high amounts compared to other vitamins
- Not true vitamin because can be synthesized from tryptophan in the liver



Riboflavin (B2)

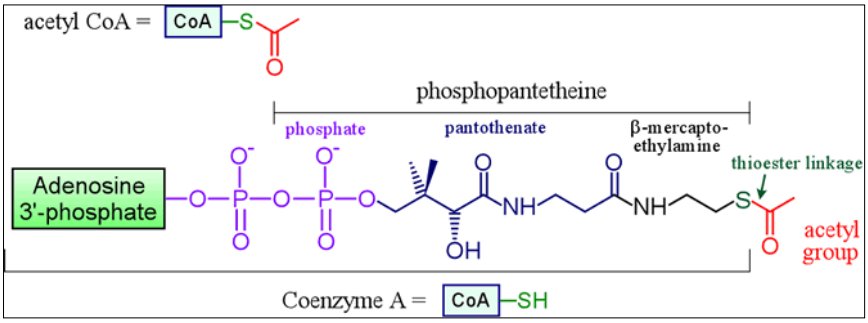
- Water soluble vitamin
- Severe deficiencies lead to growth retardation, reproductive problems and neural degeneration
- Meat, dairy products and dark green vegetables, legumes and grains are good sources



Vitamin B_2
(Riboflavin)

Coenzyme A

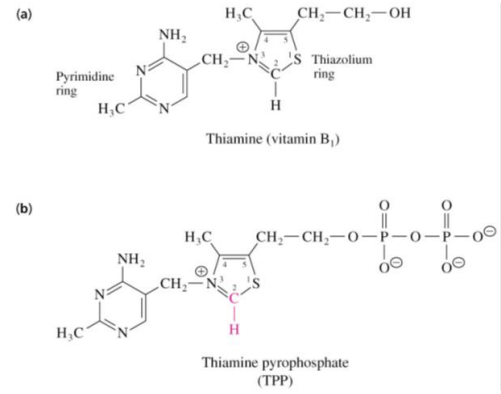
- Many metabolic processes depend on coenzyme A (CoA, HS-CoA) including oxidation of fuel molecules and the biosynthesis of some carbohydrates and lipids
- It is involved in the acyl-group transfer reactions in which simple carboxylic acids and a fatty acids are the mobile metabolic groups
- Acetyl-CoA is an energy rich compound due to high energy of the thioester linkage



Thiamine pyrophosphate

- Thiamine (Vitamin B1) is the first Vitamin discovered (Vital amine = Vitamin)
- Deficiencies lead to disease called Beriberi (neurological disorders, heart problems, anorexia)
- Beriberi prevalent in undeveloped countries where polished grains make up the majority of the diet.
- Associated with alcohol related disorders (Wernickes-Korskoff syndrome – memory loss, unstable walk)

Fig 7.14 Thiamine (Vitamin B_1) and TPP



Pyridoxal phosphate (B6)

- The B6 family of water soluble vitamins consists of 3 closely related molecules that differ in the state of oxidation or amination of the carbon bound to position 4 of the pyridine ring
- Vitamin B6-most often pyridoxal or pyridoxamine-is widely available from plant and animal sources
- B6 deficiency in human is rare

- Important in amino acid metabolism
- Bound to enzyme as a Schiff base thru rxn with Lysine
- PLP functions in transamination, decarboxylation, racemization, isomerization, side-chain elimination rxns involving amino acids

Biotin

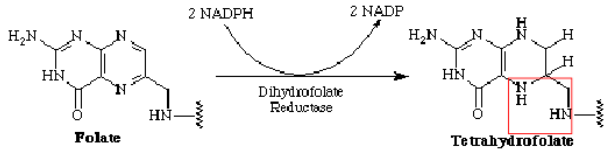
- Biotin is the prosthetic group for enzymes that catalyze carboxyl group transfer reactions and ATP-dependent carboxylation reactions
- Water soluble Vitamin
- Produced by gut microflora which supplies ½ RDA
- Deficiencies are rare
- Consuming 6 raw eggs a day can cause deficiencies due to the presence avidin (biotin binding protein).

Folate

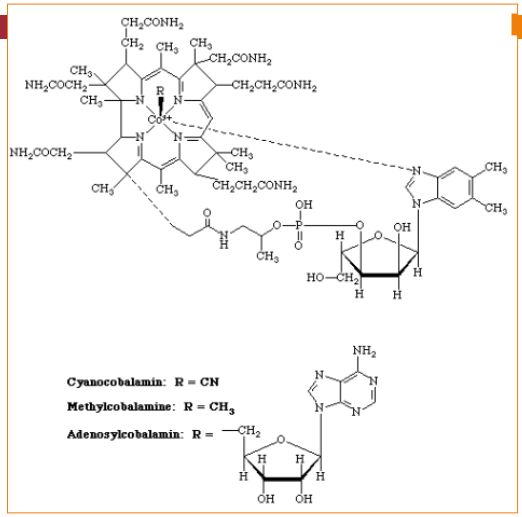
- Water soluble vitamin
- Folate is important during pregnancy to prevent neural tube defects in fetus (I.e. spina bifida)
- Vitamin B12 deficiencias cause folate deficiencias
- Has a poly-glutamate tail formed by gamma-carboxy and alpha amino groups (unusual peptide bond)

Tetrahydrofolate (THF)

- Folate is converted to THF by the addition of 4 hydrogens to the pterin ring.
- Impt. in transfer of one-carbon units
- Pterin ring impt. functional group
- The reaction below is impt in biosynthesis of DNA



Cobalamin (B12)



- Water soluble Vitamin
- Corrin ring with Cobalt cation
- Involved in intramolecular rearrangements, methyl group transfer, reduction of ribonucleotides to deoxyribonucleotides.
- Forms radical species

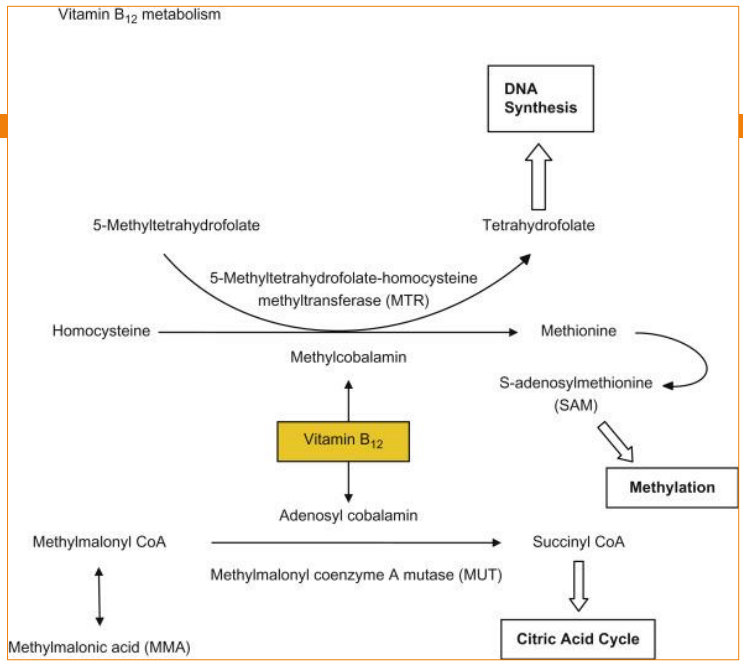
Cobalamin (B12)

- It is the largest B vitamin
- In the coenzyme form, the R group is either a methyl group (methylcobalamin) or 5'-adenosyl group (adenosylcobalamin)
- It is synthesized by some bacteria
- Deficiency of B12 cause pernicious anemia, which in turn causes neurological disorders
- B12 absorption in the intestine require a protein called intrinsic factor

Reactions involving Vitamin B12

- Adenosylcobalamin is a coenzyme for methylmalonyl-CoA mutase that converts odd-chain fatty acid/ Methylmalonyl CoA to succinyl CoA

- Methylcobalamin (Tetrathydrofolate is also involved) is a coenzyme for the enzyme Homocysteine methyltransferase that converts Homocysteine to methionine



Lipoic acid/Lipoamide

- Not a vitamin
- Important reactive groups are the sulfur atoms
- Disulfide can be reduced to form 2 sulfhydryl groups
- Involved in acyl group transfer reactions
- Co-factor covalently attached to enzyme through amide linkage with lysine residue

Fat soluble Vitamins

- Structure of four lipid vitamins (ADEK) contain rings and long chain aliphatic side chains
- Highly hydrophobic, also each one has at least one polar group
- Ingested lipid vitamins are absorbed in the intestine by a process similar to the absorption of other lipid nutrients. After digestion or proteins carrying them, they are carried to the cellular interface of the intestine as micelles formed with bile salts

Vitamin A

- Vitamin A (retinol) derived from ß-carotene impt for vision, regulation of gene expression during cell differentiation, teratogenic
- Obtained from diet either directly or indirectly as B-carotene

- Nutrients rich in Vitamin A: carrots and other yellow vegetables
- Exist in 3 forms that differ in oxidation state: the stable alcohol retinol, the aldehyde retinal and retinoic acid.
- All 3 forms have important biological functions

- Roles of vitamin A
  - Retinoic acid: signal compound that binds to receptor proteins inside cells. Electron carrier in mitochondria
  - Aldehyde retinal: a light sensitive compound with an impt role in vision. Retinal is the prosthetic group of protein rhodopsin
  - Retinol: play an essential role in metabolic functioning of the retina, the growth of and differentiation of epithelial tissue.

Vitamin D

- A collective name for a group of related lipids
- Vitamin D – impt in Ca absorption, regulates intestinal absorption and deposition in bones
- Exposure of humans to sunlight leads to formation of vitamin D3 (cholecalciferol) that is formed non-enzymatically in skin from steroid 7-dehydrocholesterol
- Vitamin D2 (related to D3 but with additional methyl group) is the additive in fortified milk
- The active form of vitamin D is 1,25-dihydroxycholecalciferol, is formed from D3 by two hydroxylation reactions
- Vitamin D deficiency causes rickets in children and osteomalacia in adults

Vitamin E (a-tocopherol)

- Vitamin E – antioxidant
- Phenol group of vitamin E can undergo oxidation to a stable free radical
- It functions as a reducing agent that scavenges oxygen and free radicals which in turn prevent damage to fatty acids in biological membranes
- Deficiency of vit E: rare but may lead to fragile red blood cells and neurological damage

Vitamin K (Phylloquinone)

- Lipid vitamin from plants
- Required for synthesis of proteins involved in blood coagulation
- Vitamin K is a cofactor for the enzyme that carboxylates certain glutamate residues on prothrombin to gamma-carboxyglutamate residues
- Ca+ binds g-carboxyglutamate residues causes protein to adhere to platelet surface

Vitamin K (Phylloquinone)

- Only fat soluble cofactor that functions as a cofactor
- Vitamin K analogs are used as drugs that inhibit blood coagulation by acting as competitive inhibitors for enzymes that catalyze regeneration of reduced form of vitamin K from Oxidized form

cytochromes

- Protein coenzyme
- Heme containing proteins
- Fe3+ can undergo reversible one electron reduction
- Impt in redox rxns
- Classified as a, b and c based on the basis of their visible absorbance spectra