ENZYME TECHNOLOGY

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DEFINITION

- The inactive protein molecule of the enzyme is known as Apo enzyme
- The Inorganic non-protein molecules which bind with apo-enzyme to make the enzyme in active state . Cofactor
- The organic non-protein molecules that bind with the apo-enzyme to make the enzyme in active state Coenzyme
- Composite form of both cofactor and apoenzyme is known as Holo-enzyme
- A coenzyme that is tightly or even covalently bound with the apo-enzyme is termed as Prosthetic group

Concept of catalyst and enzyme

"*A substance that alter the rate of a reaction without modifying the overall standard Gibbs energy change in the reaction and the process is called catalysis. The catalyst is both a reactant and product of a reaction*"

QUANTIFICATION OF ENZYME ACTIVITY

Catalytic activity of enzyme is often referred as Unit (symbol U).

One “Unit” of enzyme can be defined as the amount of the enzyme that catalyzes the conversion of 1 micro mole of substrate per minute at predefined standard reaction conditions.

One katal is the amount of enzyme that converts 1 mole of substrate per second at pre-defined standard reaction conditions,

1 U = 1/60 micro katal.

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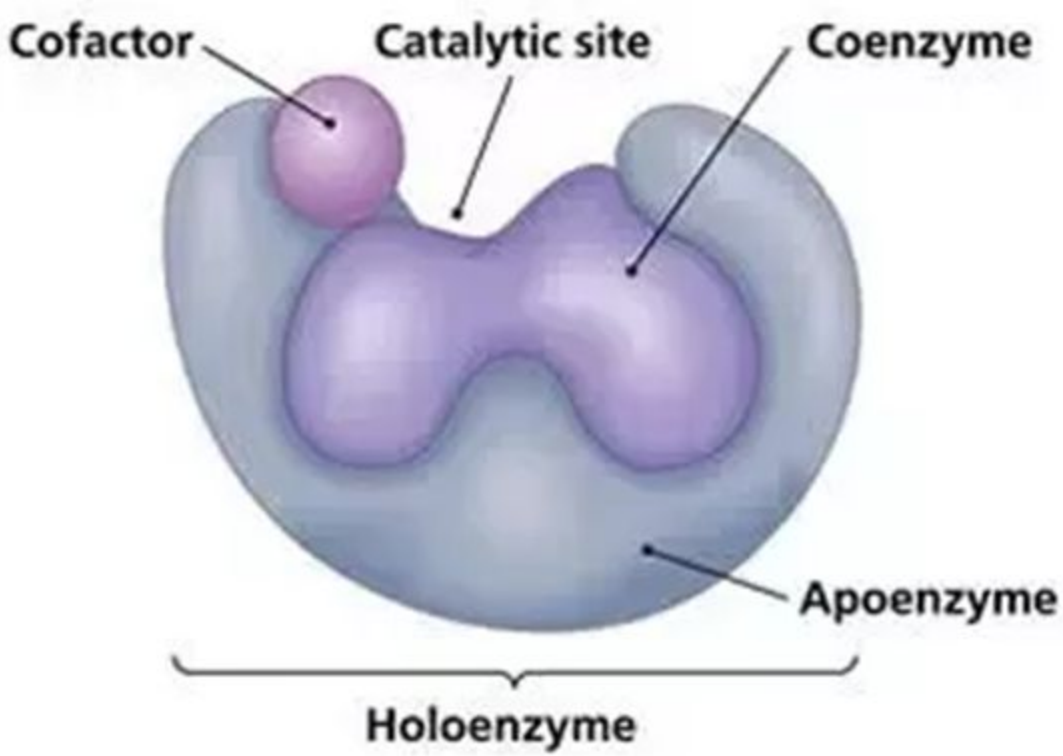


ENZYME

Prosthetic group	Function
Flavin mononucleotide	Redox reactions
Flavin adenine dinucleotide	Redox reactions
Pyrroloquinoline quinone	Redox reactions
Pyridoxal phosphate	Transamination, decarboxylation and deamination
Biotin	Carboxylation
Methylcobalamin	Methylation and isomerisation
Thiamine pyrophosphate	Transfer of 2-carbon groups, α cleavage
Heme	Oxygen binding and redox reactions
Molybdopterin	Oxygenation reactions
Lipoic acid	Redox reactions

Cofactor	Vitamin	Additional component	Chemical group(s) transferred
Thiamine pyrophosphate	Thiamine (B_1)	pyrophosphate	2-carbon groups, α cleavage
NAD^+ and $NADP^+$	Niacin (B_3)	ADP	Electrons
Pyridoxal phosphate	Pyridoxine (B_6)	None	Amino and carboxyl groups
Methylcobalamin	Vitamin B_{12}	Methyl group	acyl groups
Cobalamine	Cobalamine (B_{12})	None	hydrogen, alkyl groups
Biotin	Biotin (H)	None	CO_2
Coenzyme A	Pantothenic acid (B_5)	ADP	Acetyl group and other acyl groups
Tetrahydrofolic acid	Folic acid (B_9)	Glutamate residues	Methyl, formyl, methylene and formimino groups
Menaquinone	Vitamin K	None	Carbonyl group and electrons
Ascorbic acid	Vitamin C	None	Electrons
Flavin mononucleotide	Riboflavin (B_2)	None	Electrons
Flavin adenine dinucleotide	Riboflavin (B_2)	ADP	Electrons
Coenzyme F420	Riboflavin (B_2)	Amino acids	Electrons

Coenzymes and prosthetic groups

Nomenclature

- Cofactor: nonprotein component of enzymes
- Cofactor - a co-catalyst required for enzyme activity
- Coenzyme - a dissociable cofactor, usually organic

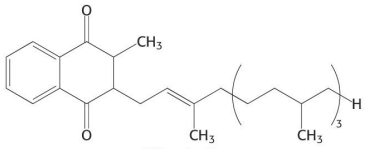
Nomenclature

- Prosthetic group - non-dissociable cofactor
- Vitamin - a required micro-nutrient (organism cannot synthesize adequate quantities for normal health - may vary during life-cycle).
  - water soluble - not stored, generally no problem with overdose
  - lipid soluble - stored, often toxic with overdose.
- Apoenzyme - enzyme lacking cofactor (inactive)
- Holoenzyme - enzyme with cofactors (active)

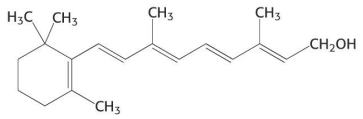
Vitamins are precursors of cofactors

TABLE 8.9 Water-Soluble Vitamins

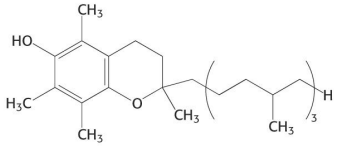
Vitamin	Coenzyme	Typical reaction type	Consequences of deficiency
Thiamine (B_1)	Thiamine pyrophosphate	Aldehyde transfer	Beriberi (weight loss, heart problems, neurological dysfunction)
Riboflavin (B_2)	Flavin adenine dinucleotide (FAD)	Oxidation–reduction	Cheliosis and angular stomatitus (lesions of the mouth), dermatitis
Pyridoxine (B_6)	Pyridoxal phosphate	Group transfer to or from amino acids	Depression, confusion, convulsions
Nicotinic acid (niacin)	Nicotinamide adenine dinucleotide (NAD^+)	Oxidation–reduction	Pellagra (dermatitis, depression, diarrhea)
Pantothenic acid	Coenzyme A	Acyl–group transfer	Hypertension
Biotin	Biotin–lysine complexes (biocytin)	ATP-dependent carboxylation and carboxyl-group transfer	Rash about the eyebrows, muscle pain, fatigue (rare)
Folic acid	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis	Anemia, neural-tube defects in development
B_{12}	5′-Deoxyadenosyl cobalamin	Transfer of methyl groups; intramolecular rearrangements	Anemia, pernicious anemia, methylmalonic acidosis
C (ascorbic acid)		Antioxidant	Scurvy (swollen and bleeding gums, subdermal hemorrhages)



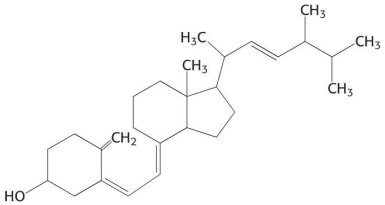
Vitamin K_1



Vitamin A (Retinol)

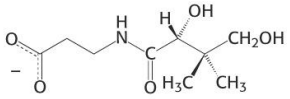


Vitamin E (α-Tocopherol)

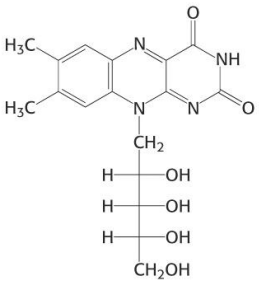


Vitamin D_2 (Calciferol)

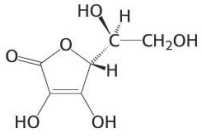
Why cofactors?



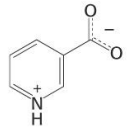
Vitamin B_5
(Pantothenate)



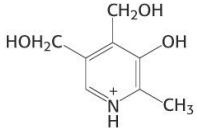
Vitamin B_2
(Riboflavin)



Vitamin C
(Ascorbic acid)



Vitamin B_3
(Niacin)



Vitamin B_6
(Pyridoxine)

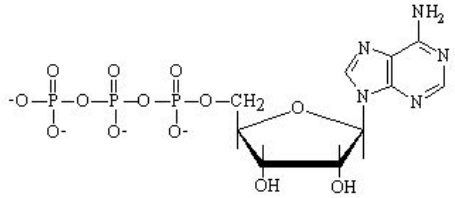
Adenine Nucleotide Coenzymes

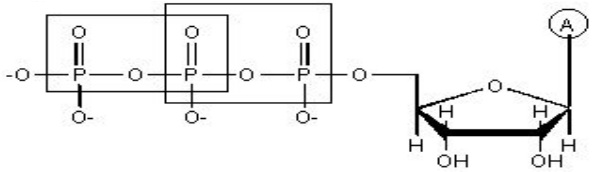
All use the adenine nucleotide group solely for binding to the enzyme!

- pyridine dinucleotides (NADH, NADPH)
- flavin mono- and dinucleotides (FMN, FADH)
- **coenzyme** A

Nucleotide triphosphates

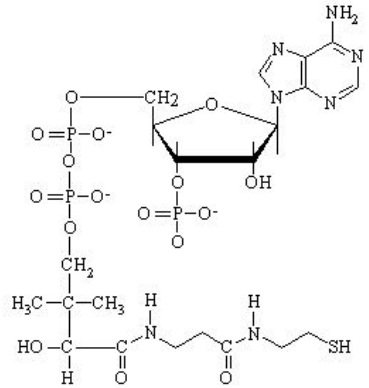
- ATP hydrolysis
  - resonance stabilizes products
  - reactants cannot be resonance stabilized because of competition with adjacent bridging anhydrides
  - charge density greater on reactants than products



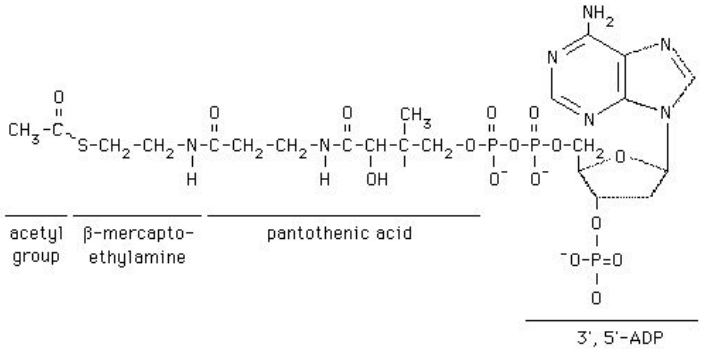


Coenzyme A

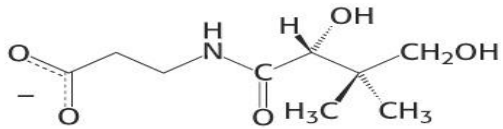
- Activation of acyl groups for transfer by nucleophilic attack
- activation of the alpha-hydrogen of the acyl group for abstraction as a proton
- Both these functions are mediated by the reactive -SH group on CoA, which forms thioesters



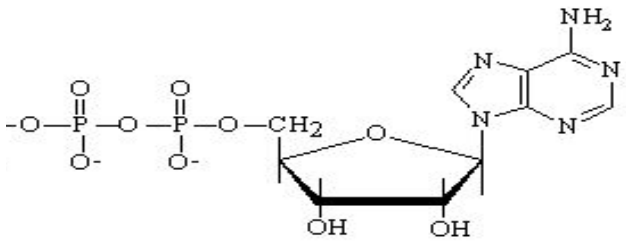
Coenzyme A



Acetyl coenzyme A, showing its constituents



Vitamin B_5 (Pantothenate)

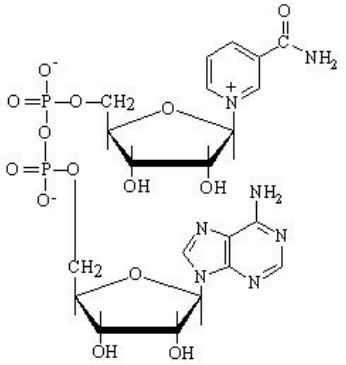


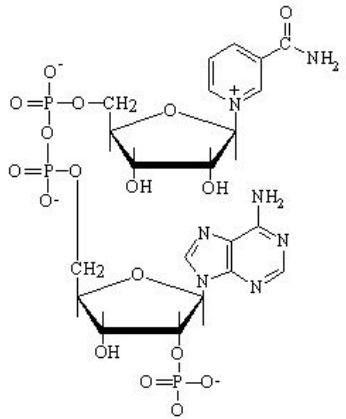
Nicotinic Acid/Nicotinamide Coenzymes

- These coenzymes are two-electron carriers
- They transfer hydride anion (H-) to and from substrates
- Two important coenzymes in this class:
- Nicotinamide adenine dinucleotide (NAD+)
- Nicotinamide adenine dinucleotide phosphate (NADP+)

NAD,NADP

- The quaternary nitrogen of the nicotinamide ring acts as an electron sink to facilitate hydride transfer
- The site (on the nicotinamide ring) of hydride transfer is a pro-chiral center!
- Hydride transfer is always stereospecific!

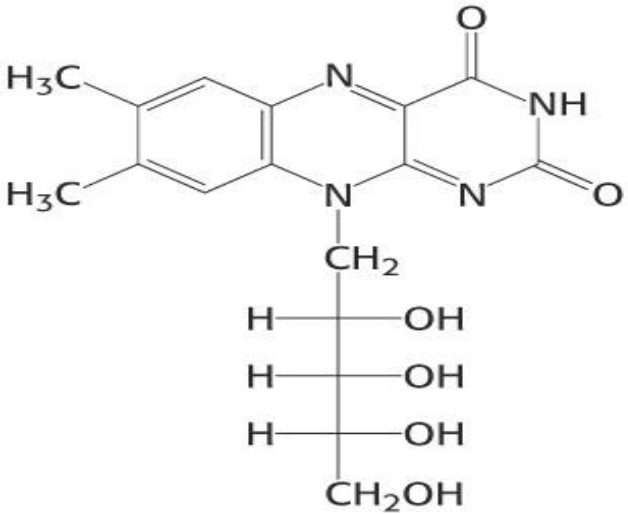




Riboflavin and the Flavins

Vitamin B2

- All these substances contain ribitol and a flavin or isoalloxazine ring
- Active forms are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)
- FMN is not a true nucleotide
- FAD is not a dinucleotide
- But the names are traditional and they persist!



Vitamin B_2 (Riboflavin)

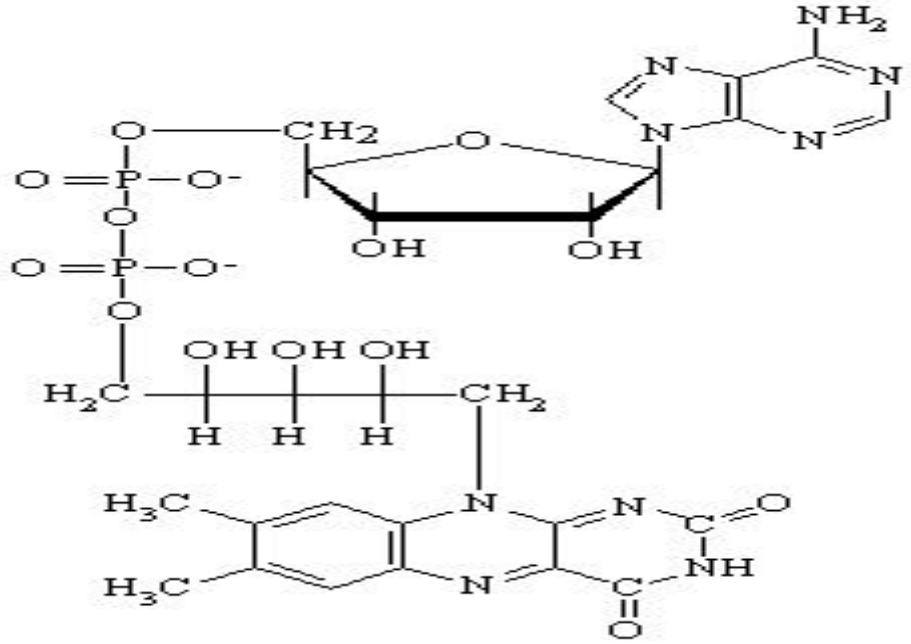
Flavin Mechanisms

Flavins are one- or two-electron transfer agents

- Name "flavin" comes from Latin flavius for "yellow"
- The oxidized form is yellow, semiquinones are blue or red and the reduced form is colorless

Flavin adenine dinucleotide

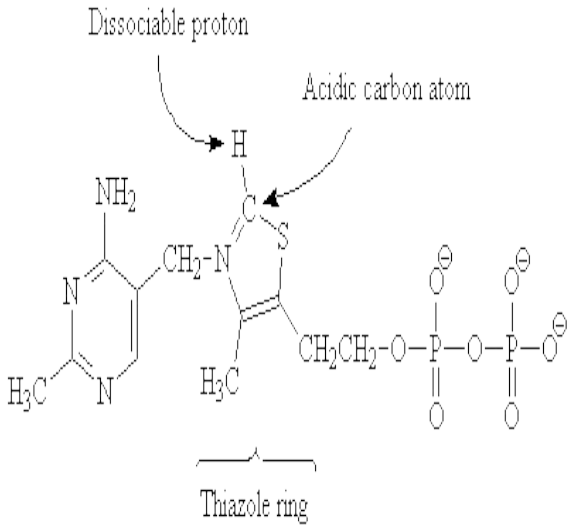
- FAD



Thiamine pyrophosphate

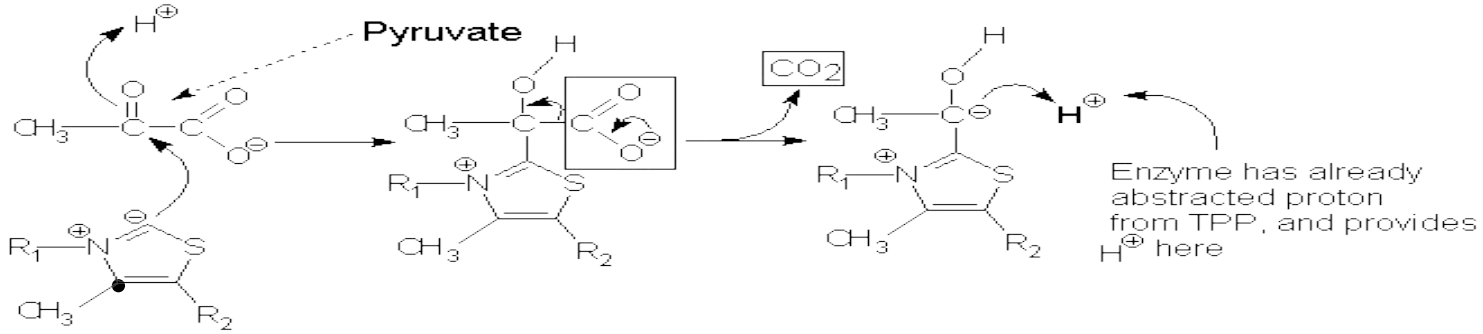
Vitamin B1

- Thiamine - a thiazole ring joined to a substituted pyrimidine by a methylene bridge
- Thiamine-PP is the active form
- TPP is involved in carbohydrate metabolism
- Catalyzes decarboxylations of α-keto acids and the formation and cleavage of α -hydroxyketones



THIAMINE PYROPHOSPHATE (TPP)

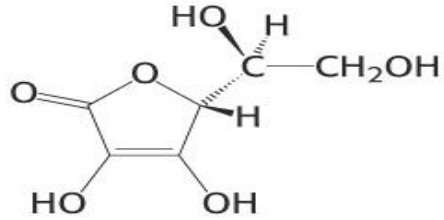
Thiamine pyrophosphate TPP



- All these reactions depend on accumulation of negative charge on the carbonyl carbon at which cleavage occurs!
- Thiamine pyrophosphate facilitates these reactions by stabilizing this negative charge
- The key is the quaternary nitrogen of the thiazolium group
  - provides electrostatic stabilization of the carbanion formed by removal of the C-2 proton
  - acts as an electron sink via resonance interactions

Ascorbic Acid

- Vitamin C
  - Most plants and animals make ascorbic acid - for them it is not a vitamin
  - Only a few vertebrates - man, primates, guinea pigs, fruit-eating bats and some fish (rainbow trout, carp and Coho salmon) cannot make it!
  - Vitamin C is a reasonably strong reducing agent
  - It functions as an electron carrier

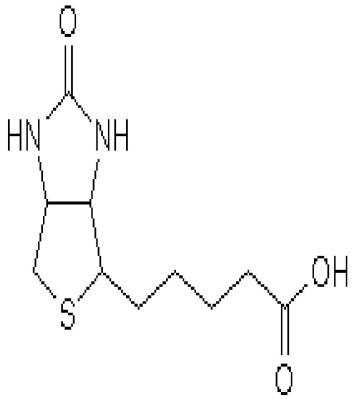


Vitamin C
(Ascorbic acid)

- Hydroxylations of proline and lysine (collagen)
- Metabolism of Tyr in brain
- Fe mobilization from spleen
- May prevent the toxic effects of some metals
- Ameliorates allergic responses
- Can stimulate the immune system

Biotin

- Mobile carboxyl group carrier
- Bound covalently to a lysine
- The biotin-lysine conjugate is called biocytin
- The biotin ring system is thus tethered to the protein by a long, flexible chain

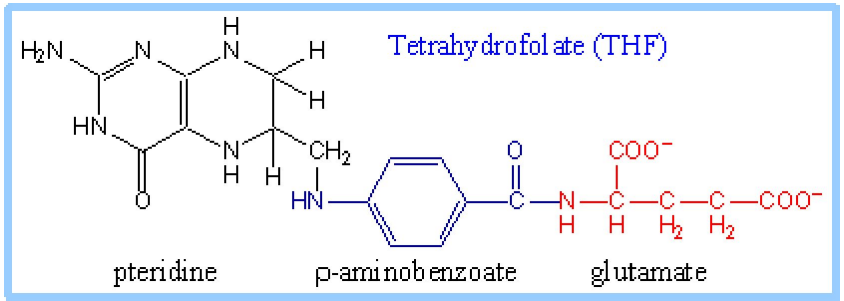


- Whenever you see a carboxylation that requires ATP and CO2 or HCO3-, think biotin!
- Activation by ATP involves formation of carbonyl phosphate (aka carboxyl phosphate)
- Carboxyl group is transferred to biotin to form N-carboxy-biotin

Folic Acid

Folates are donors of 1-C units for all oxidation levels of carbon except that of CO_2

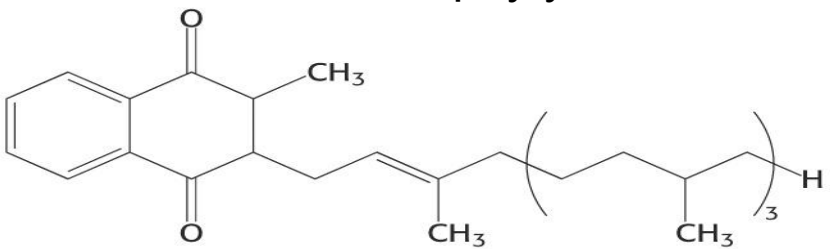
- Active form is tetrahydrofolate (THF)
- THF is formed by two successive reductions of folate by dihydrofolate reductase



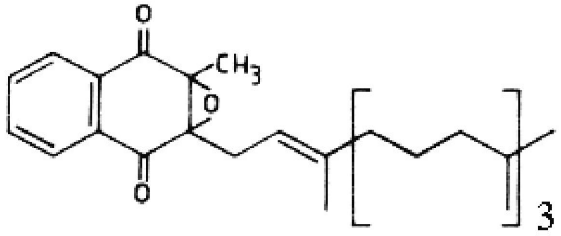
phytyl side chain

- Vitamin K
  - essential for blood clotting
- Carboxylation of 10 Glu on prothrombin (γ carboxy-Glu) is catalyzed by a vitamin K-dependent enzyme, liver microsomal glutamyl carboxylase
- Extra carboxyl enables calcium binding

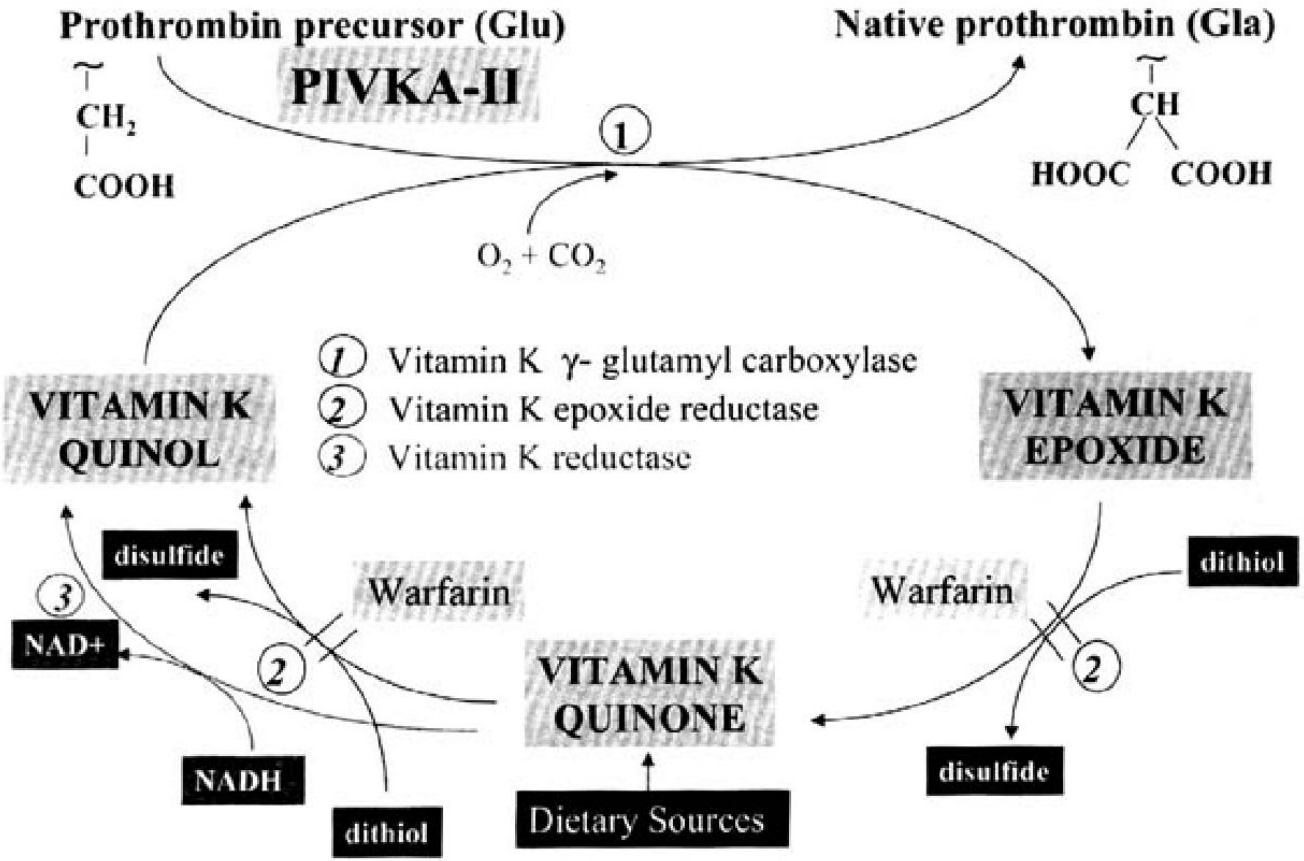
phytyl side chain



Vitamin K_1



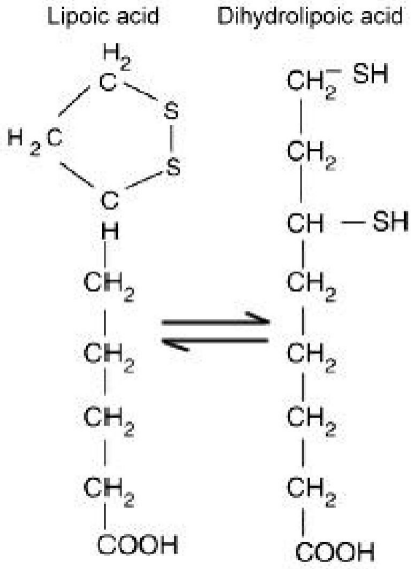
trans- vitamin $K_{1(20)}$ – 2,3–epoxide


Prothrombin precursor (Glu)
PIVKA-II
Native prothrombin (Gla)
CH2
COOH
CH
HOOC COOH
1
O2 + CO2
1 Vitamin K γ- glutamyl carboxylase
2 Vitamin K epoxide reductase
3 Vitamin K reductase
VITAMIN K QUINOL
VITAMIN K EPOXIDE
disulfide
dithiol
3
NAD+
Warfarin
Warfarin
2
2
VITAMIN K QUINONE
NADH
dithiol
disulfide
Dietary Sources

Lipoic Acid

Another example of "chemistry on a tether"!

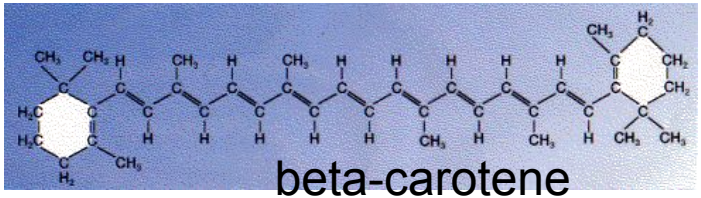
- Lipoic acid, like biotin, is a ring on a chain and is linked to a lysine on its protein
- Lipoic acid is an acyl group carrier
- Found in pyruvate dehydrogenase and α-ketoglutarate dehydrogenase
- Lipoic acid functions to couple acyl-group transfer and electron transfer during oxidation and decarboxylation of α-keto acids

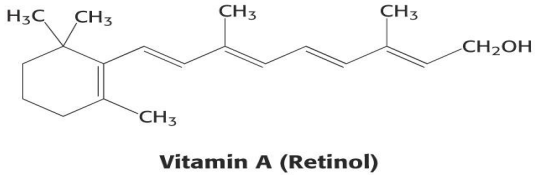


Retinol

Vitamin A

- Retinol-binding proteins (RBPs) help to mobilize and transport vitamin A and its derivatives
- Retinol is converted to retinal in the retina of the eye and is linked to opsin to form rhodopsin, a light-sensitive pigment protein in the rods and cones
- Vitamin A also affects growth and differentiation





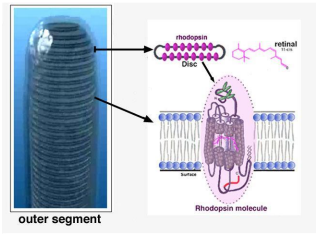
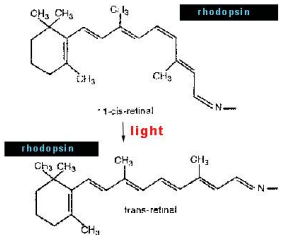
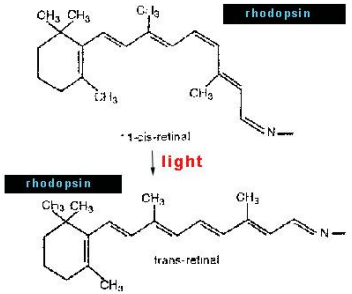
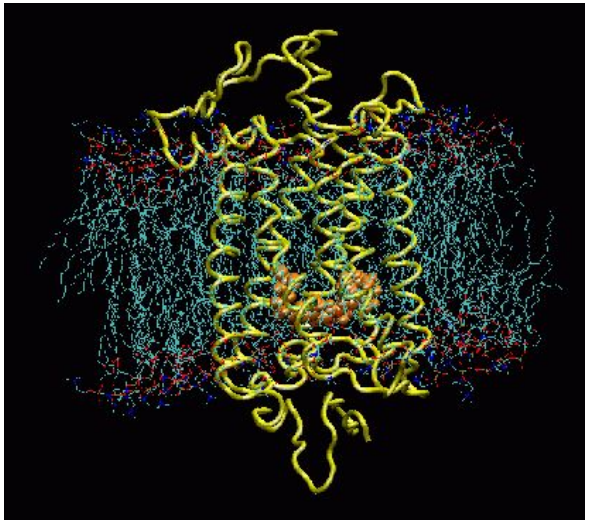


Fig 8. Schematic diagram of Rhodopsin in the outer segment discs.



Retinal in rhodopsin





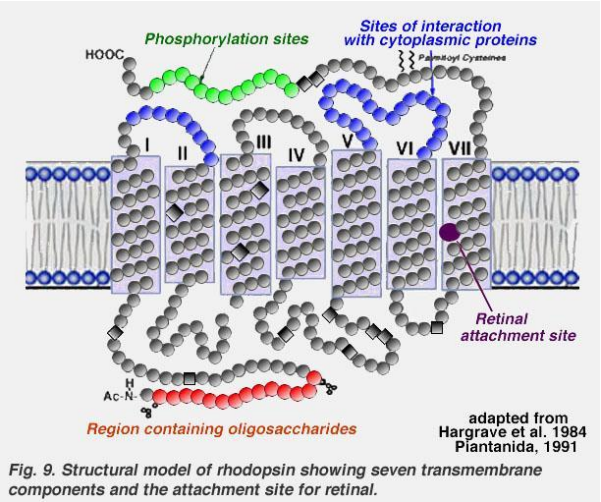
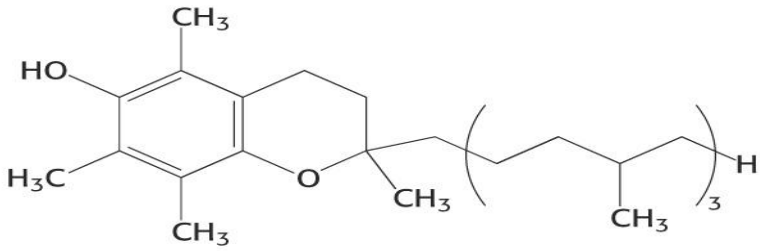


Fig. 9. Structural model of rhodopsin showing seven transmembrane components and the attachment site for retinal.

Tocopherol

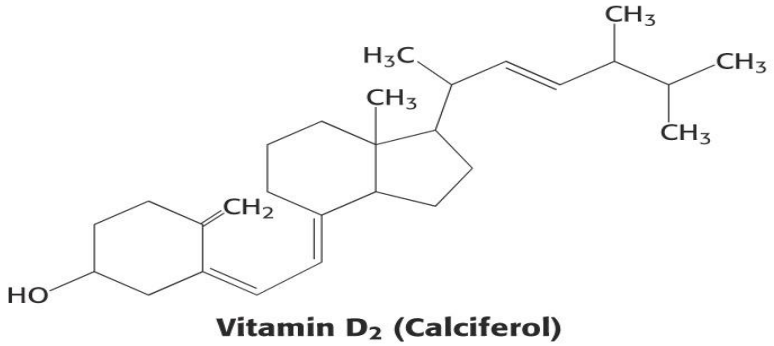
- Vitamin E
  - Potent antioxidant
  - Molecular details are almost entirely unknown
  - May prevent membrane oxidations



Vitamin E (α-Tocopherol)

Calciferol

- Vitamin D
  - Cholecalciferol is made in the skin by the action of UV light on 7-dehydrocholesterol
  - Major circulating form is 25-hydroxyvitamin D
  - 1,25-dihydroxycholecalciferol (1,25-dihydroxyvitamin D3) is the most active



Vitamin D_2 (Calciferol)

  - regulates calcium homeostasis
  - role in phosphorus homeostasis

Metal cofactors

- Single metal sites
  - mostly structural sites Ca^{2+}, Zn^{2+}
  - exceptions Cu^{2+}
- Metal clusters
  - Fe,S (Fe_4S_4, Fe_2)
  - FeMoCo
  - Mn_4, Mn_2, Cu_2, mixed metal clusters
- Organometallic cofactors
  - Porphyrins
  - Cobalamin

Metal chelation by amino acids

Ligands are determined by electronic affinity and geometrical constraints

Small, “hard” metals prefer “hard” ligands

e.g. Ca^{2+} --- $^{-}OOC–R$ (Asp, Glu)

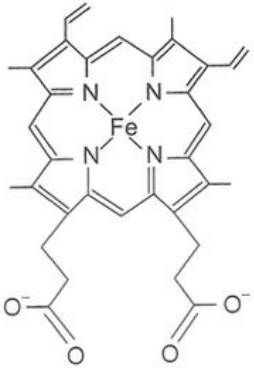
Large “soft” metals prefer “soft” ligands

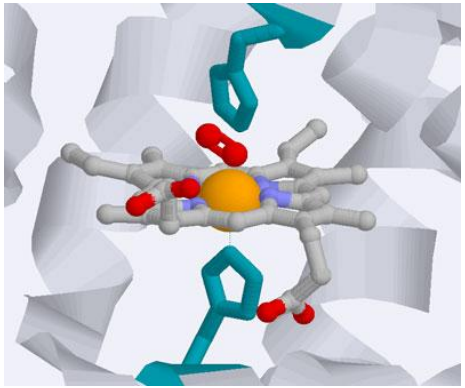
e.g. Hg^{2+} --- S–R (Cys)

Iron and copper in between

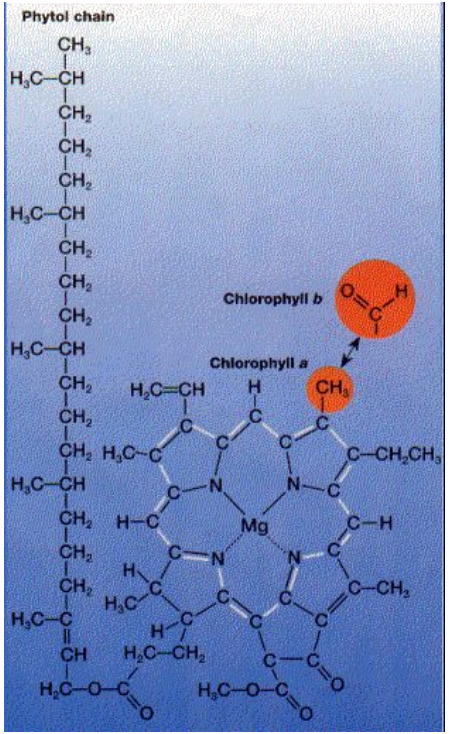
Heme iron complexes

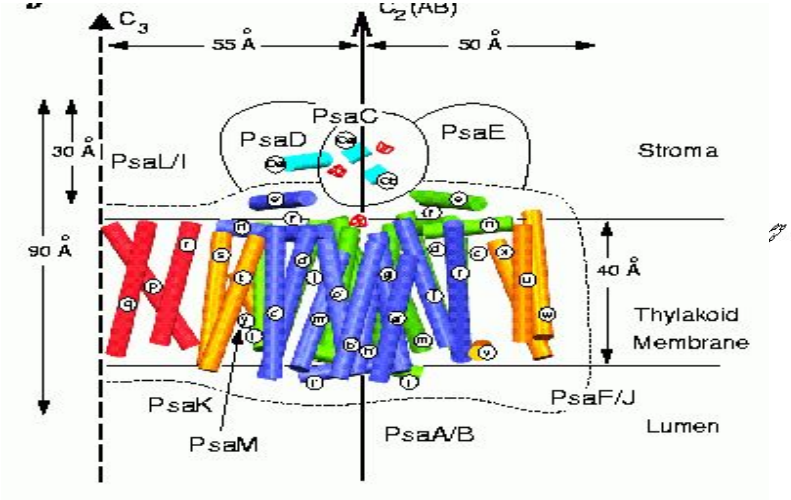
- porphyrin (pyrrole) ring
- iron prefers hexacoordination
- 5th coordinate position protein amino acid (usually His)
- 6th coordinate substrate binding or protein binding





Chlorophyll





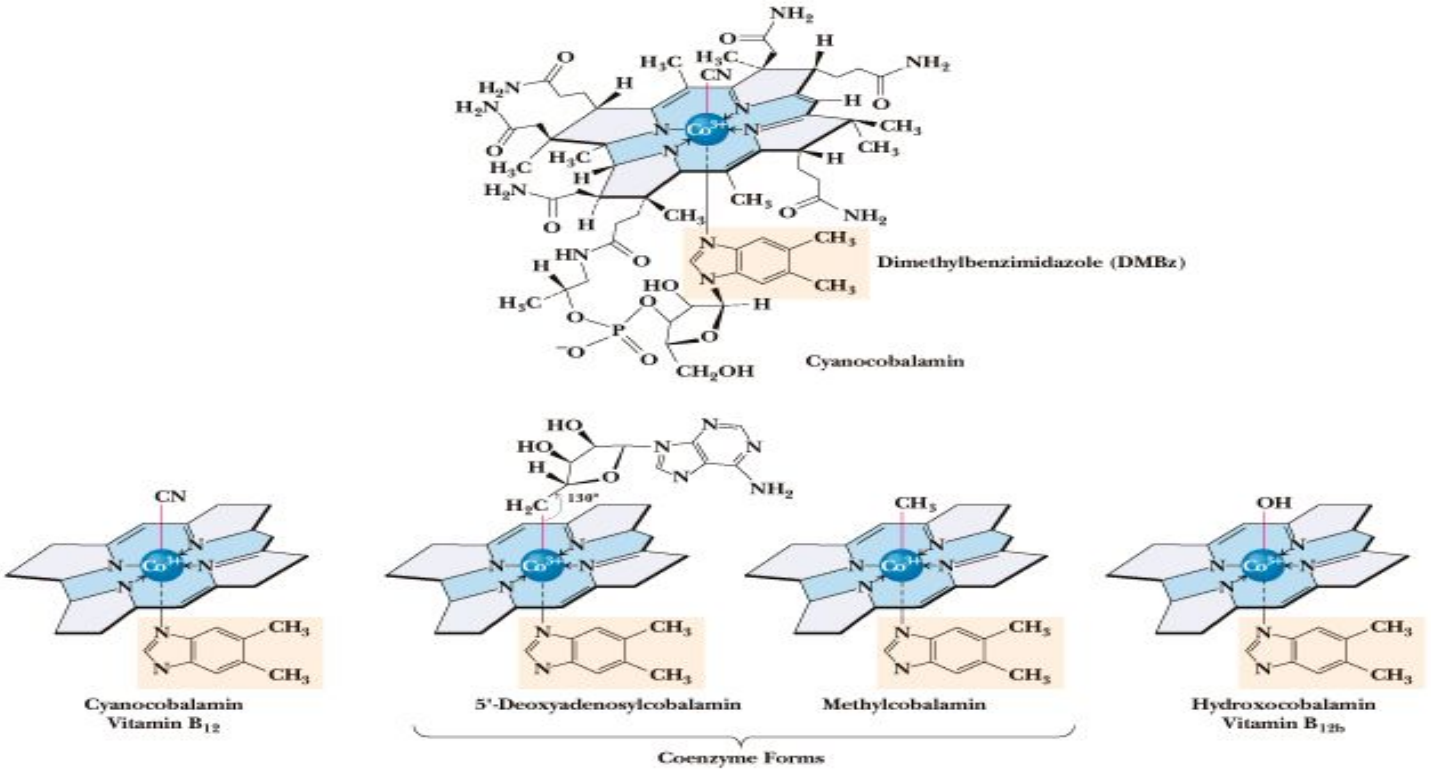
photosystem I contains 100 chlorophyll molecules, three different types of Fe-S clusters and phylloquinones

Cobalamin (B12)

- B12 is converted into two coenzymes in the body
  - 5'-deoxyadenosylcobalamin
  - methylcobalamin
- Catalyzes three reaction types
  - Intramolecular rearrangements
  - Reductions of ribonucleotides to deoxyribonucleotides
  - Methyl group transfers (assisted by tetrahydrofolate)

- Cobalamin is needed in the maturation of red blood cells and is used in carbohydrate metabolism and DNA synthesis
- Only found in animal products...not made by plants!

Cobalamin


Dimethylbenzimidazole (DMBz)
Cyanocobalamin
Cyanocobalamin
Vitamin B12
5'-Deoxyadenosylcobalamin
Methylcobalamin
Hydroxocobalamin
Vitamin B12b
Coenzyme Forms