BCH 209: INTRODUCTORY ENZYMOLOGY

LECTURER:

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Course Outline:

- Discovery, Naming and Classification of Enzymes.
- Properties of Enzyme
- Steady State Enzyme Kinetics
- Michaelis-Menten Equation
- Lineweaver-Bulk plot
- Estimation of Kinetic parameters--- Km, Vmax, Ki etc.
- Enzyme Activation & Inhibition
- Concept of active site, vitamins & coenzymes.
- Zymogen activation, Digestive enzymes, Muscle actins, ATPase, Troponin, & Allosterism
- Isolation, Purification & Characterisation of enzymes.

INTRODUCTION

- Enzymology
- What is an enzyme?
- Mention some sources of enzymes known to you.

Enzymes

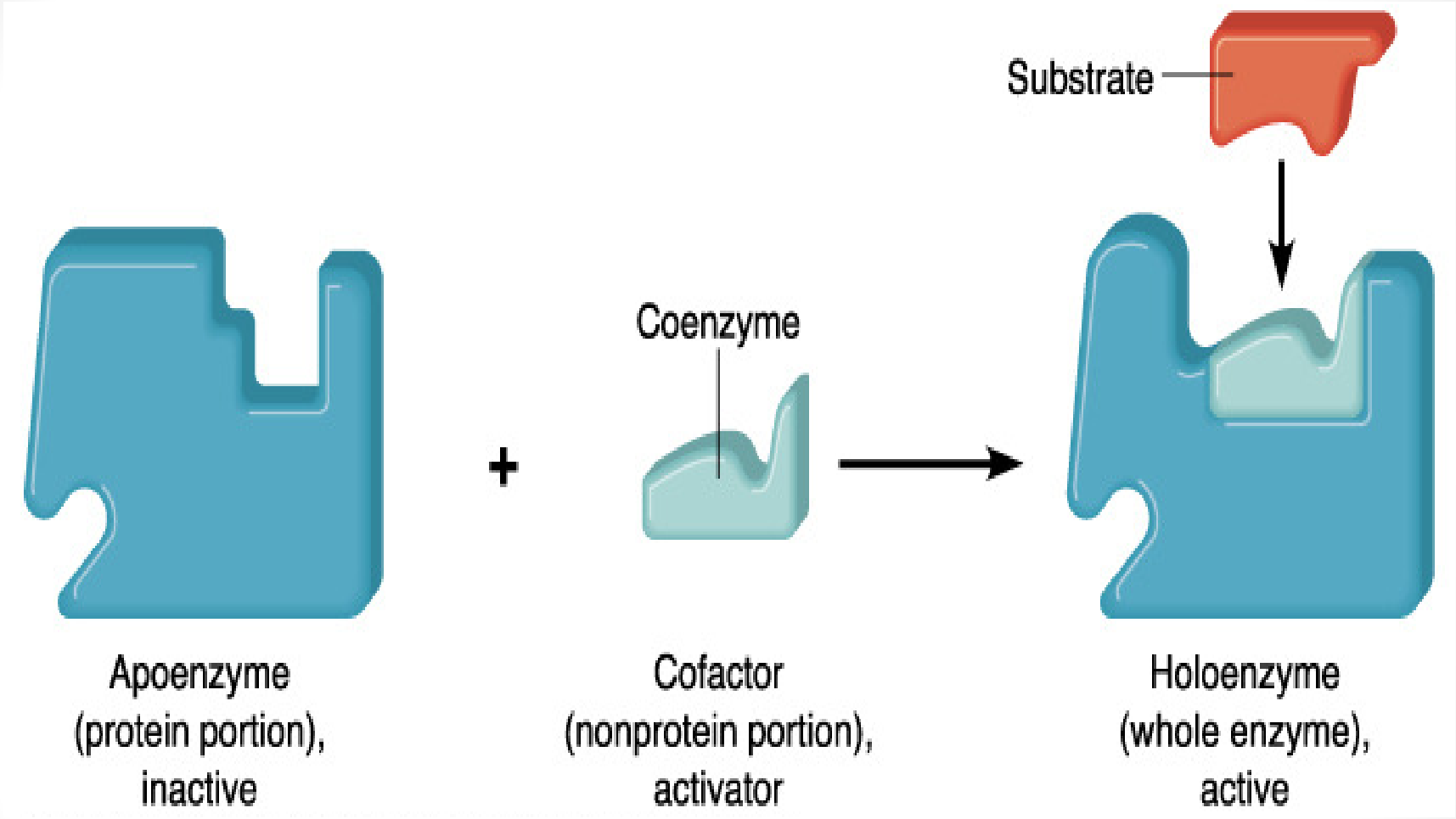
- Macromolecular components composed of protein. They are known as biological catalysts responsible for supporting almost all of the chemical reactions that maintain life processes
- Enzymes are found in all tissues and fluids of the body.
- Enzymes have a high degree of specificity for types of reaction catalized and for their substrate
- Enzymes are also stereospecific catalysts for specific stereoisomers (L & D)

- All enzymes are proteins except ribozymes;
- ribozymes are certain RNA molecules that act as catalysts

- ribozymes catalyzing the cleavage and synthesis of phosphodiester bond in RNA at specific sites in RNA

Some Common Terminologies

- **Apoenzyme;** protein portion of an enzyme (i.e., lacking a coenzyme)
- **Enzyme;** protein which catalyzes a biochemical reaction, an enzyme name ends in -ase (e.g., amylase and carbonic anhydrase)
- **Holoenzyme;** complete active enzyme (i.e., protein + coenzyme)
- **Allosteric enzyme;** a regulatory enzyme whose affinity for its substrate is affected by the presence or absence of other molecules.


Substrate
Coenzyme
+
Apoenzyme
(protein portion),
inactive
Cofactor
(nonprotein portion),
activator
Holoenzyme
(whole enzyme),
active

Discovery of Enzymes: A Brief History.

- In 1833, a French chemist Anselme Payen discovered the first enzyme, diastase.

- In 1878, German physiologist Wilhelm Kuhne, first used the term enzymes.

- **Some Importance or Applications of Enzymes.**

- **Biomarkers for diagnosis of diseases**

- **As components of analytical reagents**

e.g Randox kits for Glucose oxidase.

- **Food industries e.g for bread making etc**
- **Pharmaceutical industries**

- **Breweries**
- **Textile industries**
- **Paper industries etc.**

➢ ENZYME NOMENCLATURE

- What is nomenclature?
- How have names been given to enzymes in the past? And…….
- How should we give names to new enzymes?

➢ CLASSES OF ENZYMES

- ➢1) Oxidoreductases
- ➢2) Transferases
- ➢3) Hydrolases
- ➢4) Lyases
- ➢5) Isomerases
- ➢6) Ligases

- CLASSES AND SUBCLASSES OF ENZYMES

- 1) OXIDOREDUCTASES

Subclasses: Dehydrogenases, Oxidases, Reductases, Peroxidases, Catalase, Oxygenase, and Hydroxylases.

- 2) TRANSFERASES

Subclasses: Transaldolase, transketolase; Acyl, methyl, glycosyl, and phosphoryltransferases; Kinases, and Phosphomutases

➢3) HYDROLASES

Subclasses: Esterases, Glycosidases, Peptidases, phosphatases, Thiolases, Phospholipase, Amidases, Deaminases, Ribonucleases.

➢4) LYASES

Subclasses: Decarboxylases, Aldolases, Hydratases, Dehydratases, Synthases, Lyases.

➢5) ISOMERASES

Subclasses: Racemases, Epimerases, Isomerases, Mutases (not all)

➢6) LIGASES

Subclasses: Synthetases, Carboxylases.

➢OTHER PROPERTIES OF ENZYMES

(a) Solubility:

Enzymes as proteins are soluble in water or dilute salt solution

(b)Molecular weight

Enzymes have high ↑Mw (varying from 10000 -several thousands)

(c) Enzymes are charged molecules:

Due to the presences of amino acids, each enzyme has a charge.
The charge depends on the pH of the solution.
At very low pH the amino acids are fully protonated and there is a positive charge on the proteins; as pH is increased, the protein losses a proton to neutralize the OH- group and becomes a zwitter ion (a charged molecule with equal number of +ve and –ve charges. As more alkali is added, the NH_3^+ group gives its H+ and protein becomes positively charged.

(c) Enzymes have buffering capacity (acid-base).

They are amphotericmolecules i.e behave both as acids and bases. (due to presence of both free amino group and free carboxyl group) -they act as buffer. At pKa they make the most efficient buffer.

(d)Each enzyme has a specific Isoelectric pH (pI):

It is the pH at which the net charge on protein equal to zero –so they do not move in an electric field.
[It is the pH at which the protein molecule carries an equal positive and negative charges]

Above PI -negatively charged can move in an electric field

Below PI -positive charged and can move under an electric field.

- **(e)Denaturation: When proteins are heated , or subjected to extremes of temperature, high salt, organic solvents etc, the non-covalent bonds break, changing the native structure to random coil. This unfolding of protein is due to loss of secondary, tertiary and quaternary structure.**
- **It does not affect primary structure.**
- **Effect of Denaturation: Loss of activity due to loss of shape and active site. (Protein become insoluble & precipitates).**
- **Denaturing Factors:**
- **•Heat**
- **•Change in pH (↑,↓)**
- **•Radiation**
- **•Heavy metals**
- **•Detergents**
- **•Digestive enzymes**
- **•Urea (8M)**
- **•Repeated freezing and thawing (which cause disruption of hydrogen or other weak bonds)**

Assignment:

Write a short note on the six classes of enzymes. Give an example each of an enzyme-catalyzed reaction under each class.

Active site of an enzyme

- The catalytic activity of enzyme involves the binding of their substrates to form an enzyme-substrate complex (*ES*). In an enzymatic reaction, the substrate binds to a specific region of the enzyme called the active site.
- While bound to the active site, the substrate is converted into the product of the reaction, which is then released from the enzyme.

$$S + E \rightleftharpoons ES \rightleftharpoons E + P$$

The active site of an enzyme lower E_A and speed the chemical reaction barrier by;-

- 1-orienting substrates correctly
- 2-strain substrate bonds
- 3-providing a favorable micro-environment
- 4-covalently bonding to the substrate

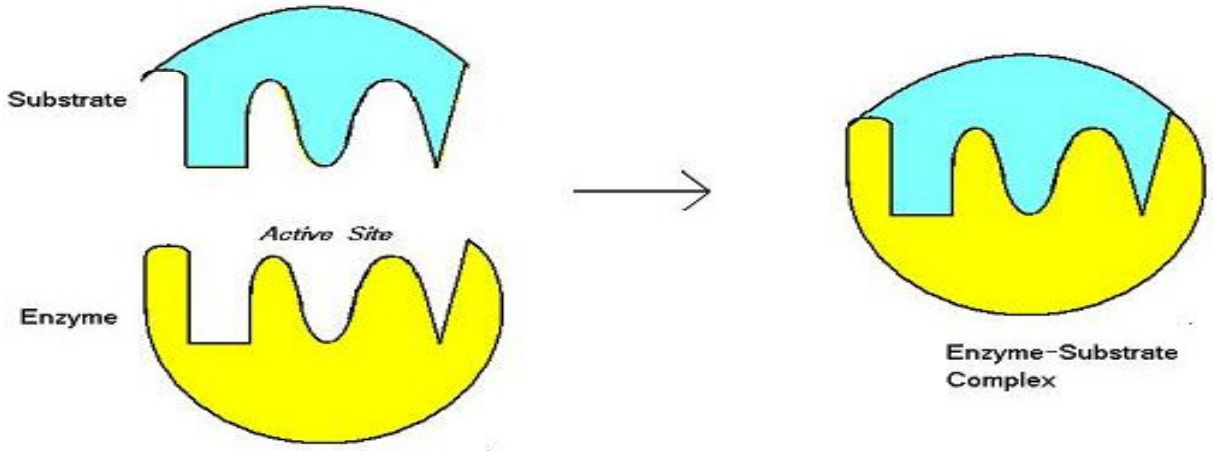
Proposed models or Hypotheses of substrate binding

- Two model of substrate binding to the active site of the enzyme;
- 1) Lock and key model or hypothesis
- 2) Induced fit model or hypothesis.

Lock and Key Hypothesis or model

--This model assumes that the substrate and enzyme active site have complementary shapes in which the substrate fits exactly into the active site.

-- The enzyme's active site represents the 'lock' while the substrate denotes the 'key'. Catalysis therefore only takes when a substrate with the right shape complements the active site shape.

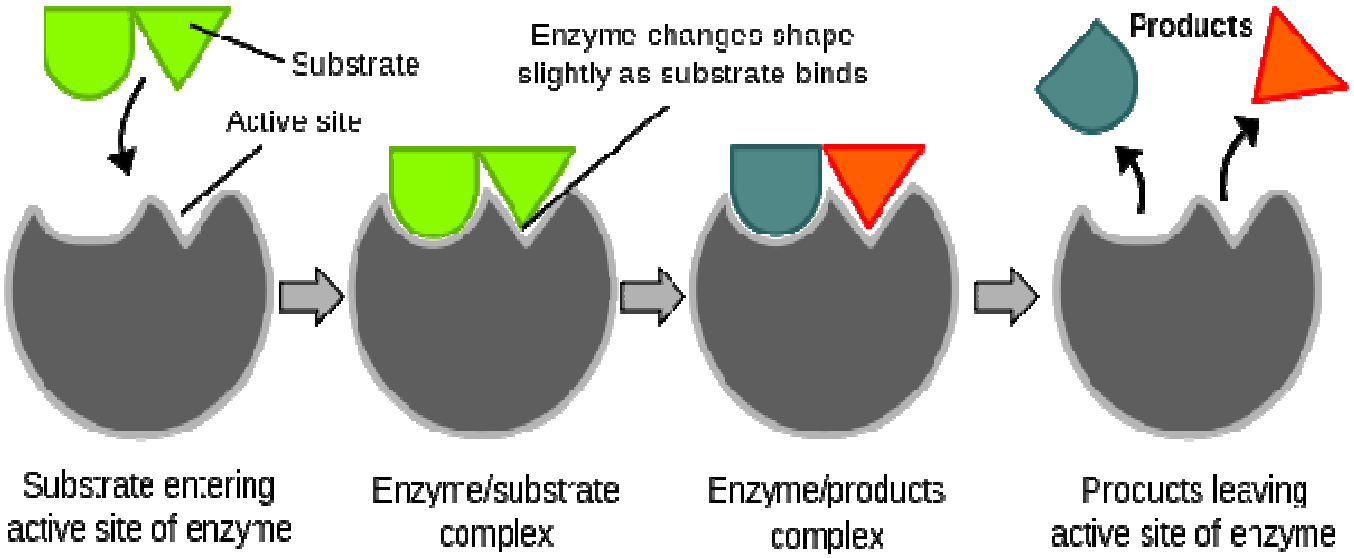


Lock-and-key Model.- The substrate and enzyme active site have complementary shapes

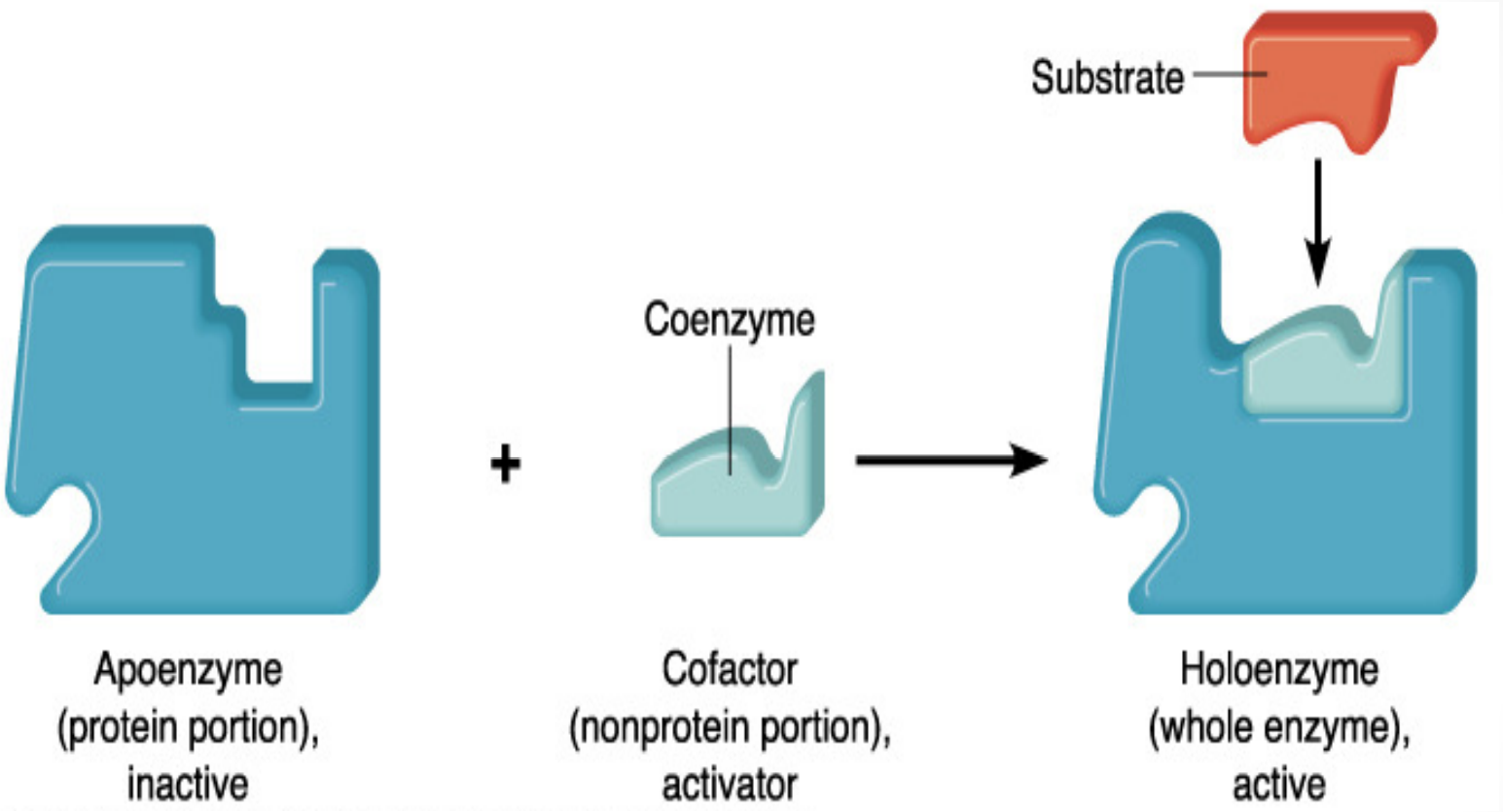
The Induced Fit Hypothesis or model

--This model proposes that the initial interaction between enzyme and substrate is relatively weak, but that these weak interactions rapidly induce conformational changes in the enzyme that strengthen binding.

--Hence, the configurations of both the enzyme and substrate are modified by substrate binding and it is this conformational change that leads to product formation.



BCH 209: VITAMINS, COENZYMES AND COFACTORS



EXAMPLES OF COFACTORS & SOME ENZYMES THAT REQUIRE THEM FOR CATALYTIC ACTIVITY

TABLE 6–1 Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

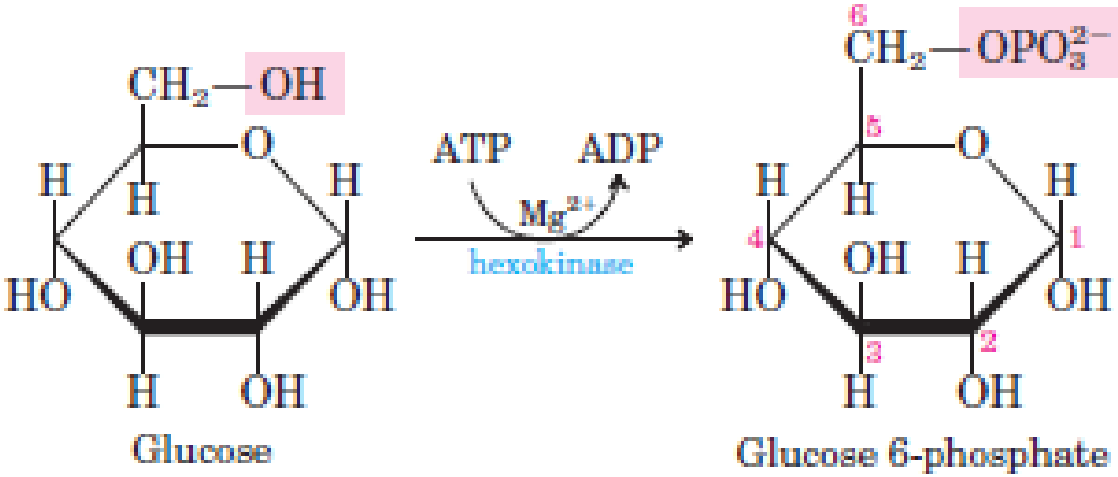
VITAMINS AND COENZYMES

#-- A vitamin is an organic compound and an essential nutrient that an organism requires in limited amounts.

#-- Essential & non essential vitamins.

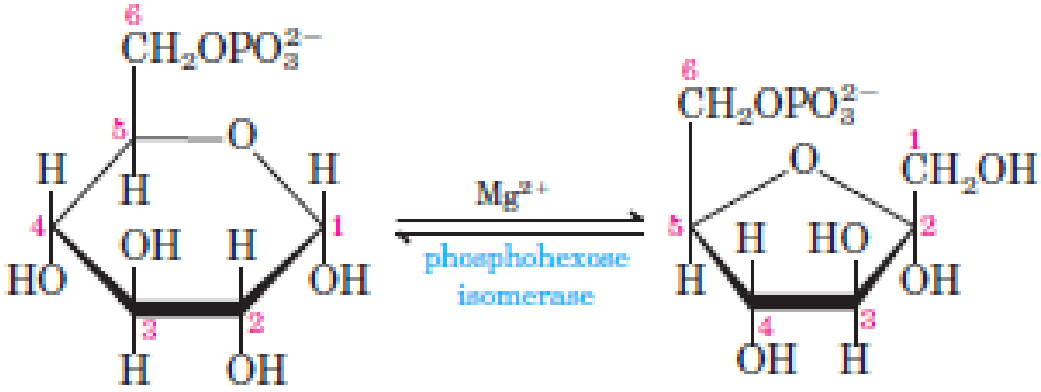
#-- Majority of vitamins serves as coenzymes.

EXAMPLES OF REACTIONS INVOLVING COFACTORS



Glucose

Glucose 6-phosphate



Glucose 6-phosphate

Fructose 6-phosphate

VITAMIN-BASED COENZYMES

Table 1.1 The vitamins.

Vitamin	Chemical name	Deficiency disease	Biochemical function	Coenzyme chemistry
A	Retinol	Night blindness	Visual pigments	—
B_1	Thiamine	Beriberi	Coenzyme (TPP)	Decarboxylation of α-keto acids
B_2	Riboflavin	Skin lesions	Coenzyme (FAD, FMN)	$1e^-/2e^-$ redox chemistry
Niacin	Nicotinamide	Pellagra	Coenzyme (NAD)	Redox chemistry
B_6	Pyridoxal	Convulsions	Coenzyme (PLP)	Reactions of α-amino acids
B_{12}	Cobalamine	Pernicious anaemia	Coenzyme	Radical re-arrangements
C	Ascorbic acid	Scurvy	Coenzyme, anti-oxidant	Redox agent (collagen formation)
D	Calciferols	Rickets	Calcium homeostasis	—
E	Tocopherols	Newborn haemolytic anaemia	Anti-oxidant	—

VITAMIN-BASED COENZYMES

H	Biotin	Skin lesions	Coenzyme	Carboxylation
K	Phylloquinone	Bleeding disorders	Coenzyme, anti-oxidant	Carboxylation of glutamyl peptides
	Folic acid	Megaloblastic anaemia	Coenzyme (tetrahydrofolate)	1-carbon transfers
	Pantothenic acid	Burning foot syndrome	Coenzyme (CoA, phosphopantotheine)	Acyl transfer

CoA, coenzyme A; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; NAD, nicotinamide adenine dinucleotide; PLP, pyridoxal-5′-phosphate; TPP, thiamine pyrophosphate.

Examples of Coenzymes, Structures and Some Specific Biochemical Reactions they are involved in.

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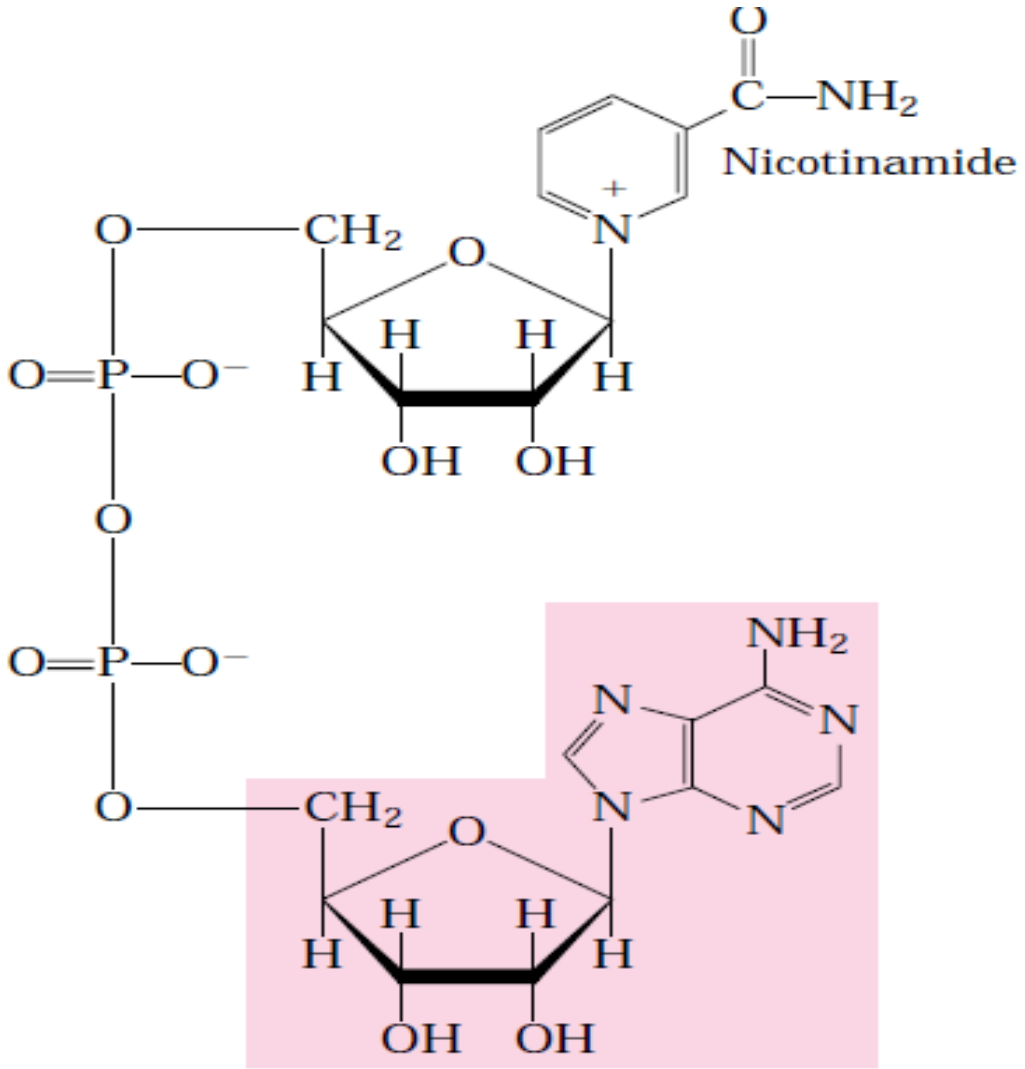
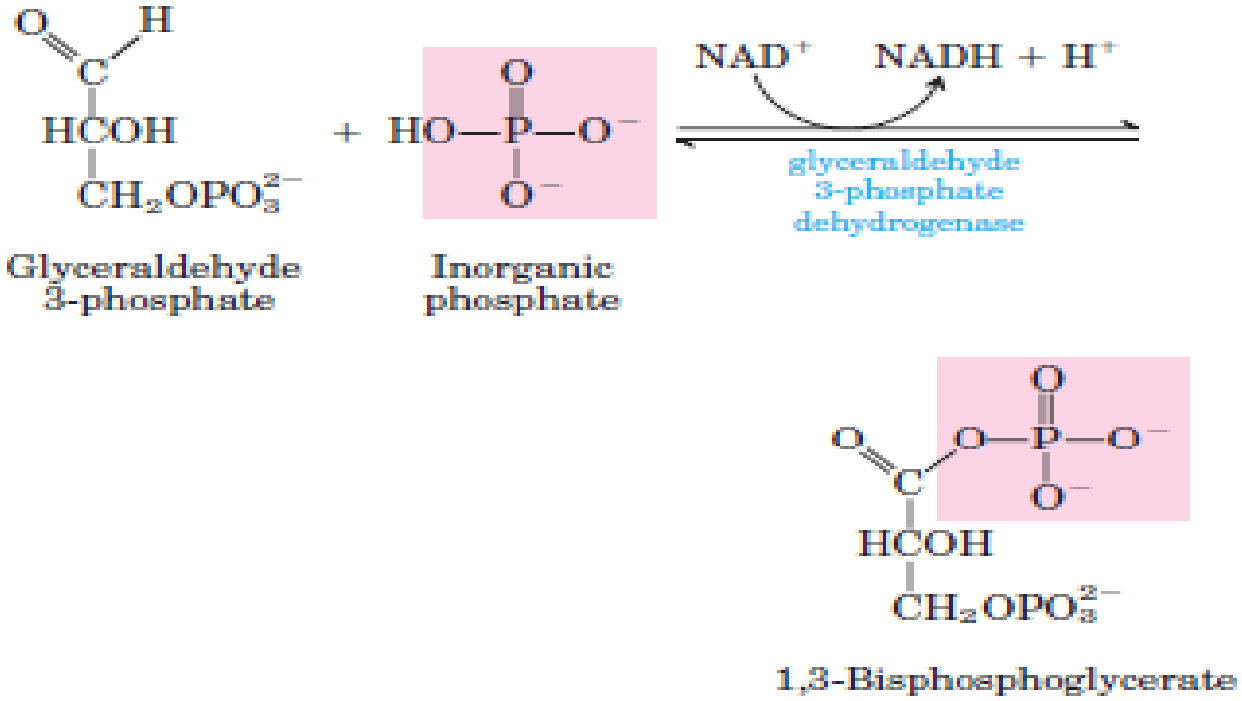


Nicotinamide adenine dinucleotide (NAD^+)

Examples of a biochemical reaction that involved NAD^+:



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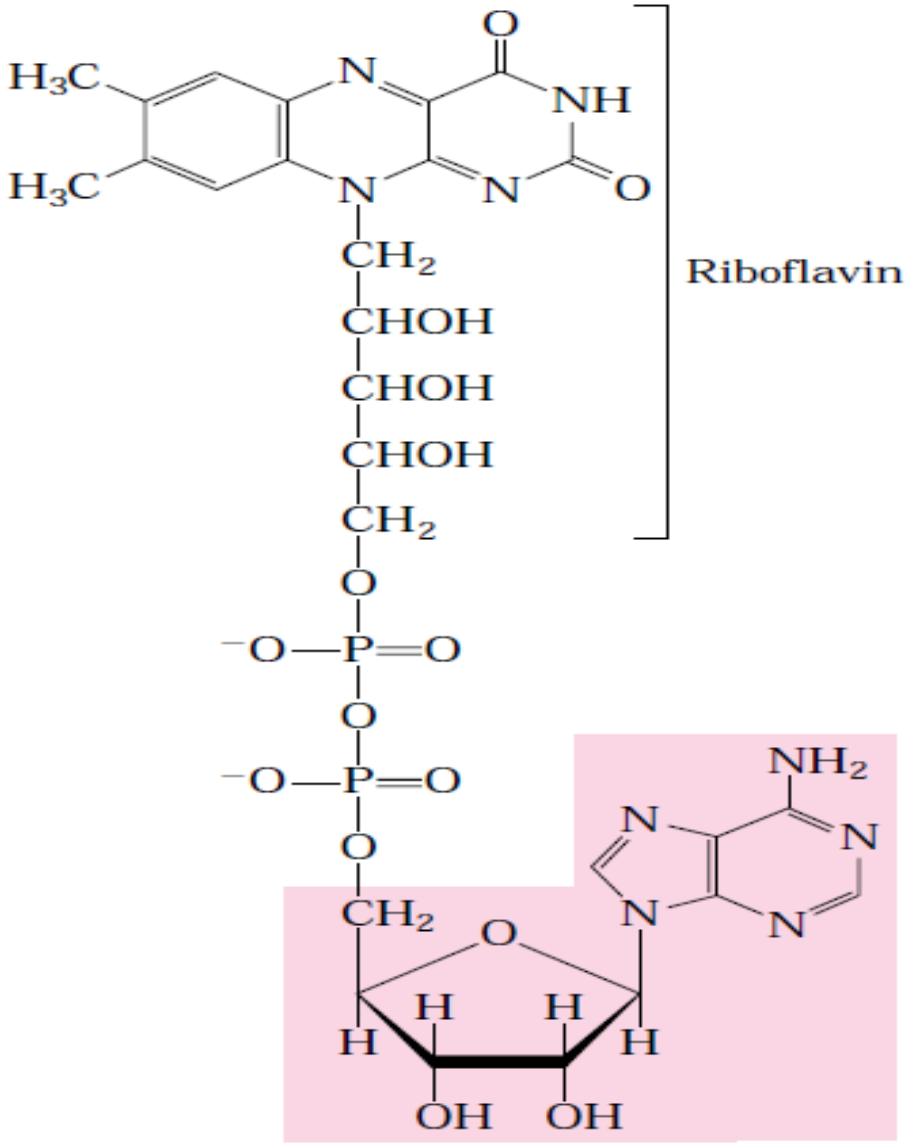
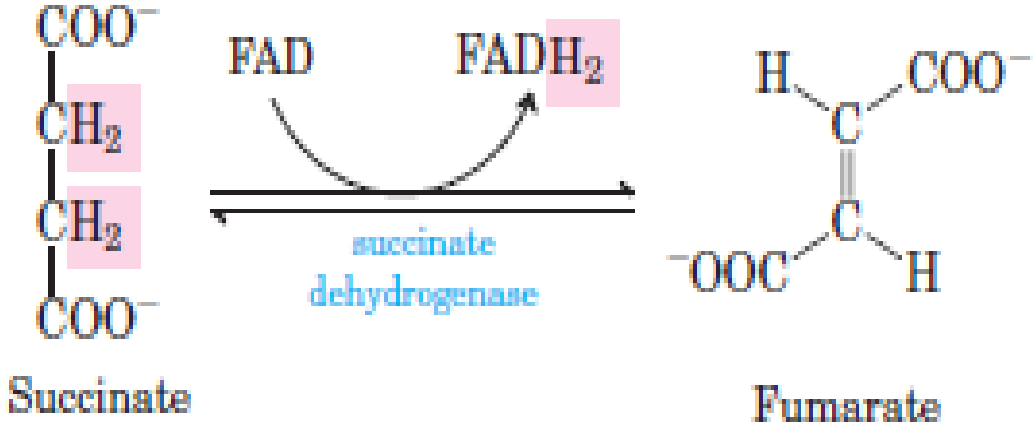


Flavin adenine dinucleotide (FAD)

Examples of a biochemical reaction that involved FAD:



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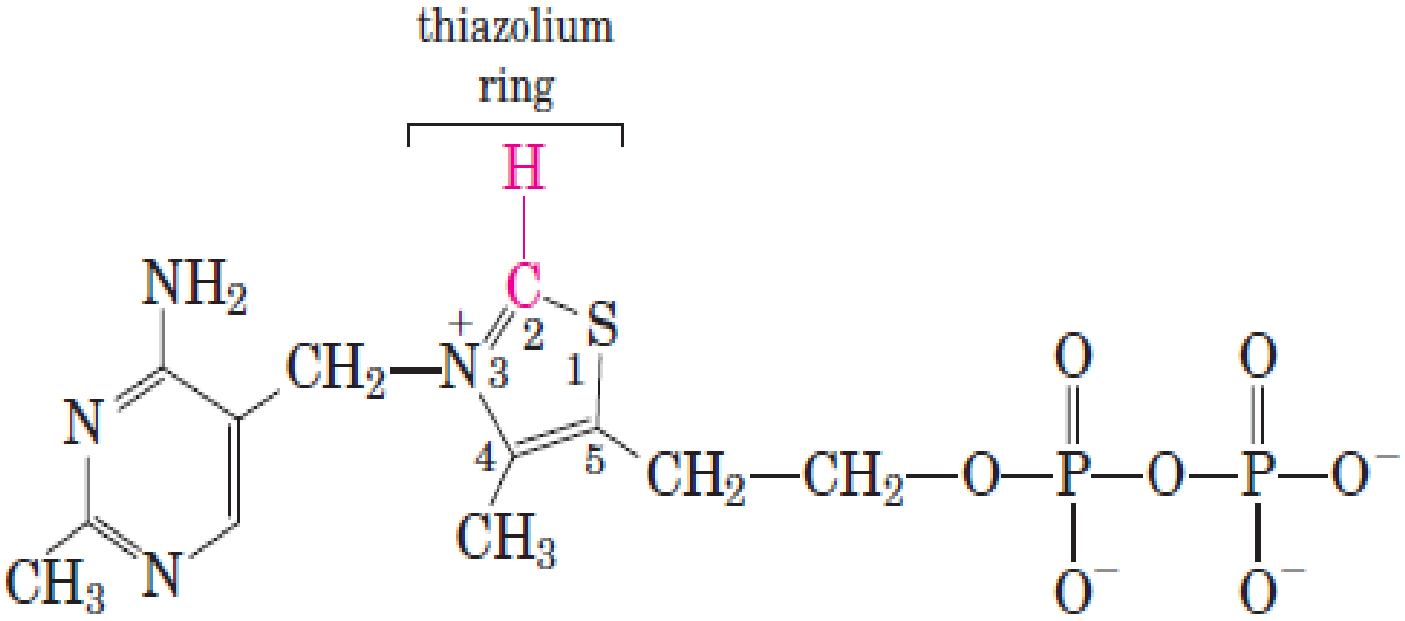
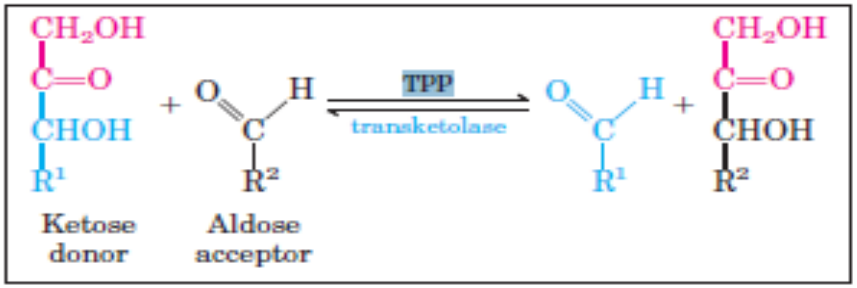


Thiamine pyrophosphate (TPP)

Examples of a biochemical reaction that involved TPP:



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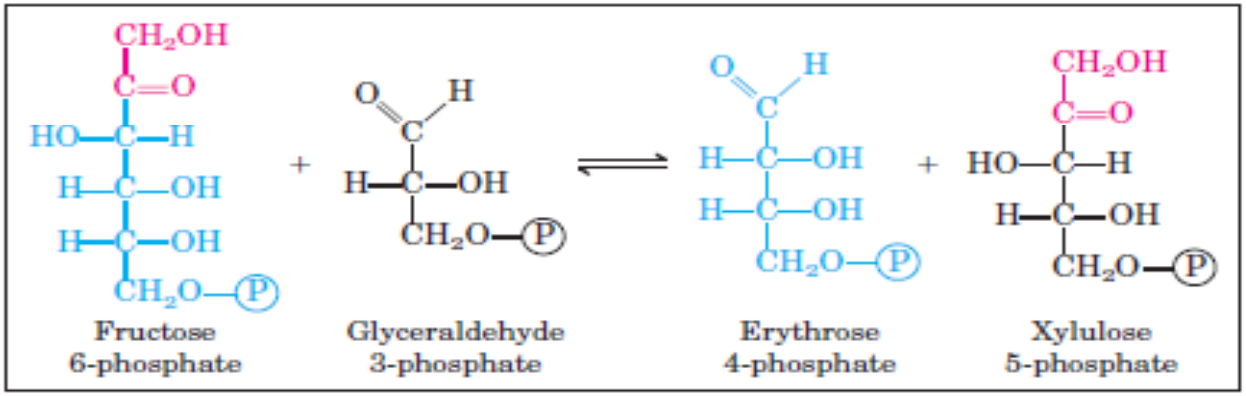

(b)

Assignment 1

Draw the structure of the following coenzymes and write an equation of a biochemical reaction they are involved in:

i) Coenzyme A (CoA)
ii) Biotin
iii) Tetrahydrofolate.

Recommended Textbooks

for further insight

- Lehninger Principles of Biochemistry by Nelson and Cox.

- Textbook of Biochemistry with Clinical Correlations by Thomas M. Devlin.
- Harper's Biochemistry
- Visit the Library for Encyclopædia Britannica, Journals, Periodicals etc.