Vitamins in the Enzyme-Catalyzed

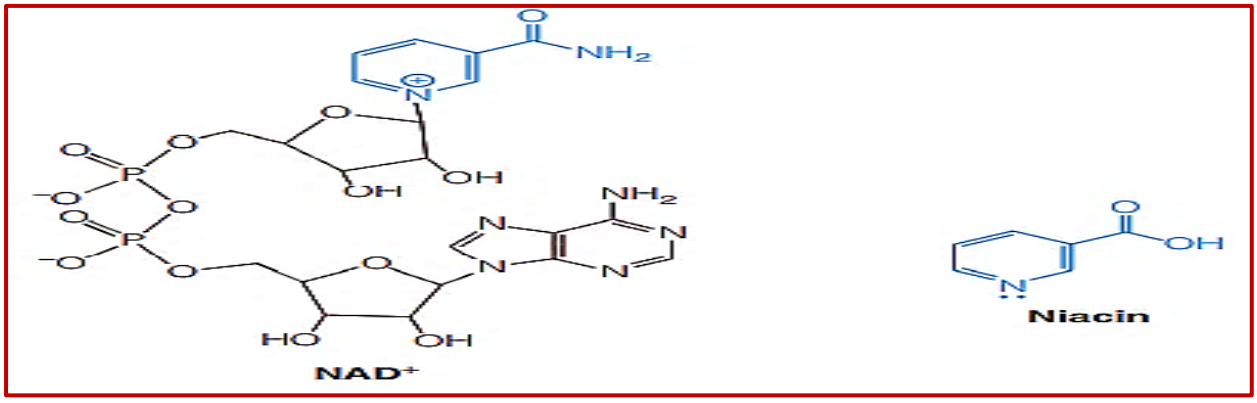
Introduction:

- Coenzymes are organic compounds required by many enzymes for catalytic activity; They are often vitamins or derivatives of vitamins. Sometimes they can act as catalysts in the absence of enzymes, but not as effectively as in the presence of an enzyme.
- As with metal enzyme links, there is a range of strengths for coenzymes.
- The point of distinction between tightly bound cofactor (prosthetic group) and loosely bound cofactor: Coenzymes, which are prosthetic groups, form an integral part of the active site of an enzyme and undergo no net change as a result of acting as a catalyst; loosely bound coenzymes can be regarded as co-substrates since they often bind to the enzyme-protein together with the other substrates at the start of a reaction and are released in an altered form at the end.
- They are regarded as coenzymes since they usually bind to the enzyme before the other substrates are bound. They participate in many reactions and may be reconverted to their original form by the enzymes present within the cells.

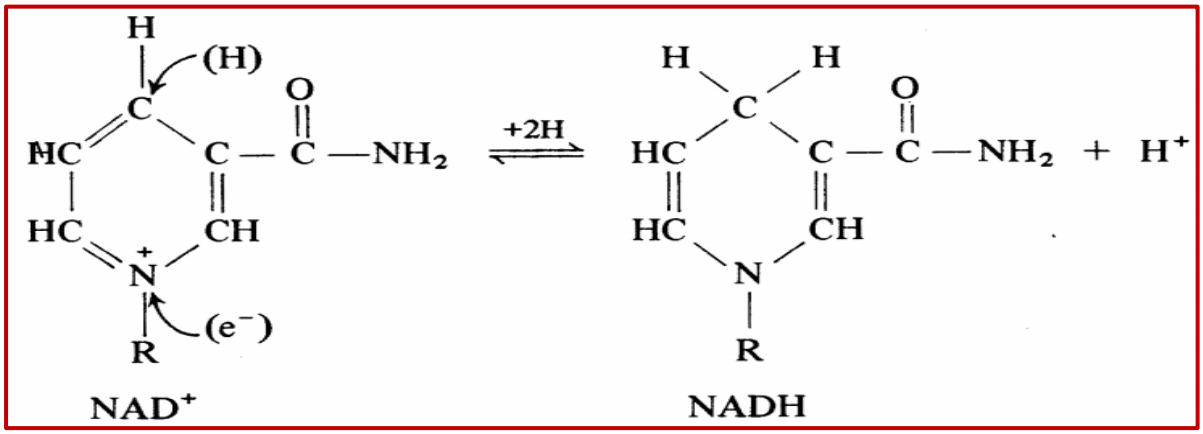
Some important coenzymes are discussed below.

1- Nicotinamide nucleotides (NAD^+ and $NADP^+$)

Nicotinamide nucleotides (NAD^+ and $NADP^+$) are derived from the vitamin **niacin,** which is nicotinamide or nicotinic acid. The structure of nicotinamide adenine dinucleotide (NAD^+) in its oxidized and reduced forms is given below: Nicotinamide **adenine dinucleotide (NAD^+)** is derived from the vitamin **niacin (VIT. B_3).**



The reduction of **NAD^+** to **NADH** requires two reducing equivalents per molecule: one electron (e-) and one hydrogen atom (H=H+ +e-) added to the pyridine ring of nicotinamide as shown below. The pyridine ring is conjugated, so the positive charge may be delocalized, making several points vulnerable to nucleophilic attack. However, the exact mechanism of the reaction is not known.



Where: R represents the remainder of the molecule.

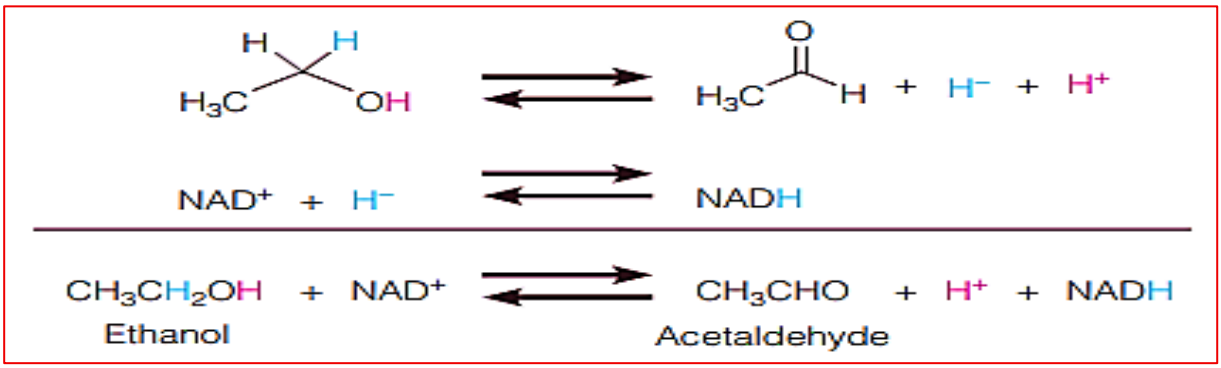
Show: Nicotinamide Adenine Dinucleotide Phosphate **($NADP^+$)** is identical to **NAD^+**, except that the 2′-position of the **D-ribose** unit attached to the adenine is phosphorylated**.**

This structural difference **does not affect** the **oxidation–reduction properties** of the molecule, but it causes **NAD^+** and **$NADP^+$** to function as **coenzymes for different enzymes**:

- **Enzymes that utilize NAD^+** usually have a **catabolic role**, where the **NADH produced** serves as an **energy source** for the cell.
- **Anabolic enzymes**, in contrast, commonly use **NADPH** as a coenzyme.

❖ Both **NAD^+** and **$NADP^+$** act as **coenzymes** in **oxidation–reduction reactions**. They are **loosely bound** to enzymes and **leave them in a chemically changed form** at the end of the reaction.

❖ A typical reaction in which **NAD^+ acts as an oxidizing agent** is the **conversion of alcohols to aldehydes or ketones**,
as catalyzed by the **alcohol dehydrogenase** enzyme of the liver.



- The C-linked H, not the O-linked H, is transferred to NAD^+, as can be demonstrated by studies using deuterated compounds.
- Sometimes, it isn't easy to distinguish between a true cofactor and the second substrate in a reaction.
- The dehydrogenase enzymes, such as alcohol dehydrogenase, each have a strong binding site for the oxidized form of the cofactor, NAD^+.
- After oxidation of the substrate, the reduced form, NADH, leaves the enzyme and is reoxidised by other electron-acceptor systems in the cells.
- ***Yet*** NAD^+ and NADH differ from most substrates in that they are continually recycled in the cell and used repeatedly. Because of this behavior, we consider their cofactors.

Stereospecificity in Enzyme–Coenzyme Reactions:

- Many enzyme-catalyzed reactions involving NAD^+ or $NADP^+$ are **highly stereospecific.**
- The enzyme's active site is **asymmetric,** which means that even symmetrical substrates such as ethanol bind in **a specific orientation**.
- As a result, **only one of the two hydrogen** atoms attached to the substrate carbon is transferred to the coenzyme. This occurs because the enzyme constrains the substrate to interact at defined points, creating a ***prochiral* environment** in which the two hydrogens are no longer equivalent.
- As illustrated in **Figure 11.37**, the asymmetric surface of the enzyme (orange region) binds the substrate molecule at multiple specific sites. The carbon atom (gray circle, C) is attached to two equivalent groups (X and X), but only one of them (pink X) can interact correctly with the enzyme surface. The blue group (Y) and the yellow region (Z) represent additional binding sites that help orient the substrate properly.
- Such stereochemical control explains why alcohol dehydrogenase and other dehydrogenases catalyze the transfer of hydrogen to a particular face of the nicotinamide ring in NAD^+, ensuring precise and reproducible reaction outcomes.
- ***Note:*** **Prochiral environment** occurs when a non-chiral atom (like carbon with two identical groups) becomes effectively chiral due to the **asymmetric surface of an enzyme,** which differentiates between the two identical (similar) groups. This allows only one group to react, giving **stereospecificity** in enzyme-catalyzed redox reactions.

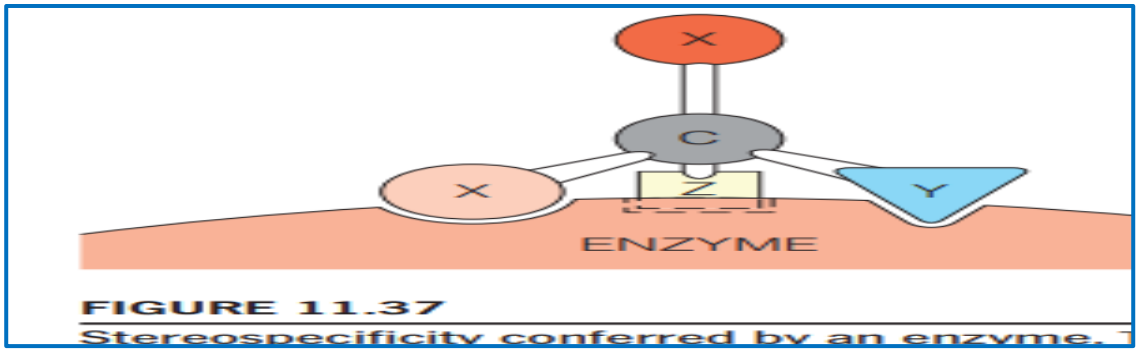


FIGURE 11.37
Stereospecificity conferred by an enzyme.

An example of NAD^+ **behaving as a cofactor:**

Is the reaction catalyzed by **UDP-galactose 4-epimerase** shown in **Figure 11.38.** This enzyme facilitates the synthesis of complex polysaccharides by interconverting **UDP-glucose** and **UDP-galactose** (see Chapters 9 and 13) in the following mechanism:

1) **Step 1:**
   UDP-galactose is bound to the enzyme, which carries the coenzyme NAD^+.
2) **Step 2**:
   Hydride is transferred to NAD^+ from C4 of the galactose ring to produce the carbonyl intermediate.
3) **Step 3:**
   Then, it (**Hydride**) is transferred back to C4 to give the opposite stereochemistry.
4) **Step 4:**
   Then, the product, **UDP-glucose**, is released.

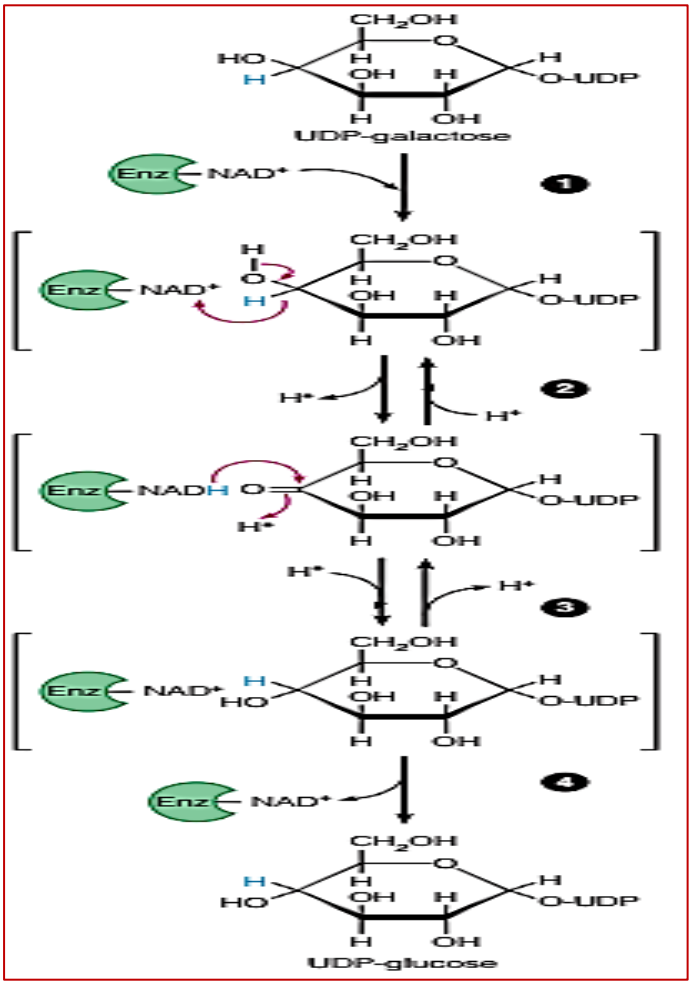


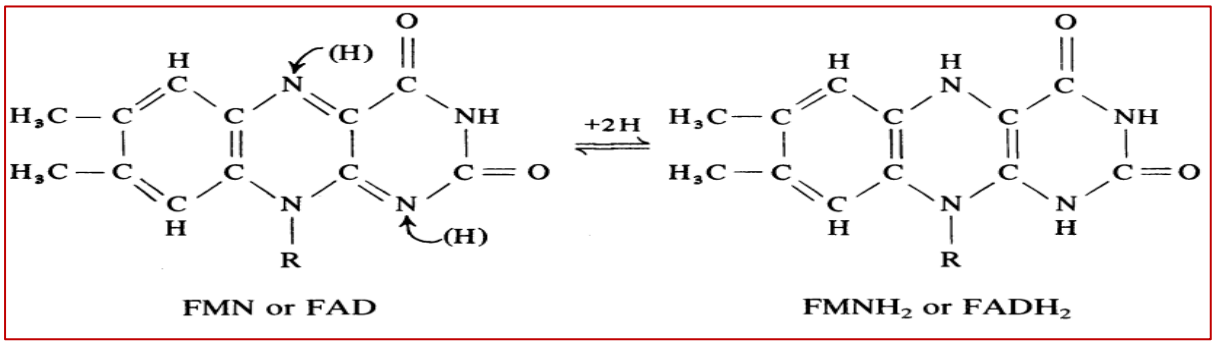
FIGURE 11.38 Proposed mechanism for **UDP-galactose epimerase**.

Note: A **hydride** is a compound formed when **hydrogen bonds with another element**, usually a metal or nonmetal.

In enzyme reactions, a **hydride ion (H^-)** transfer often occurs during **oxidation–reduction (redox) reactions**, where the hydride moves from one molecule (donor) to another (acceptor), as seen in reactions involving **NAD^+/NADH** or **$FAD/FADH_2$**.

2- Flavin nucleotides (FMN and FAD)

Flavin nucleotides (FMN and FAD) are derived from riboflavin (vitamin B_2). Like the nicotinamide nucleotides (NAD and NADP), **flavin nucleotides** function in **oxidation–reduction reactions**, where the reducing equivalents are carried by the fused three-ring system of the flavin molecule, as shown below.



Here, **R** represents the remainder of the molecule.

Example:

$$\text{D-glucose} + O_2 \rightleftharpoons \text{D-glucono-}\delta\text{-lactone} + H_2O_2$$

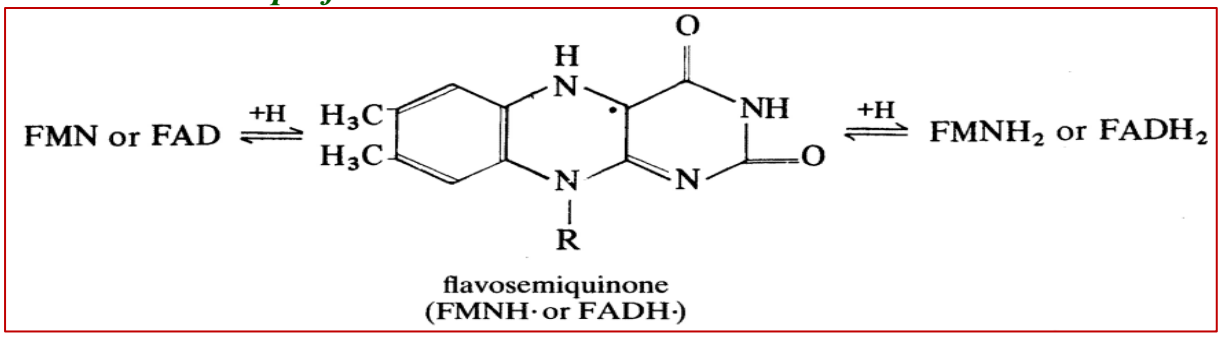
This enzyme in this reaction utilizes **FAD** as a prosthetic group and **O_2** as a hydrogen acceptor. Where the reaction proceeds in two stages:

$$\text{E–FAD} + \text{D-glucose} \rightleftharpoons \text{E–FADH}_2 + \text{D-glucono-}\delta\text{-lactone}$$

$$\text{E–FADH}_2 + O_2 \rightleftharpoons \text{E–FAD} + H_2O_2$$

$$\text{D-glucose} + O_2 \rightleftharpoons \text{D-glucono-}\delta\text{-lactone} + H_2O_2$$

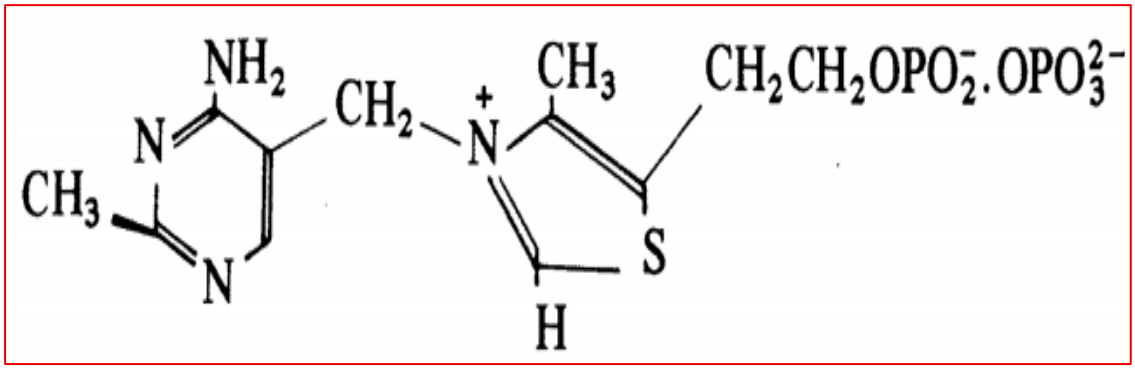
With some **flavoproteins**, the reduction of the flavin has been shown to be a two-step process, involving an unstable free radical **semiquinone** as intermediate:



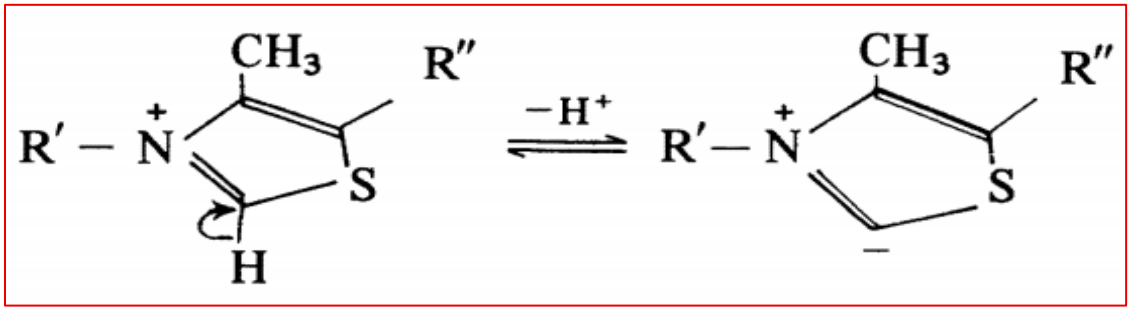
Note: **Flavoproteins** are **enzymes that contain a flavin nucleotide (FAD or FMN)** as a prosthetic group and participate in **oxidation–reduction (redox) reactions** by transferring electrons or hydrogen atoms.

3- Thiamine pyrophosphate (TPP), vitamin B_1

Thiamine pyrophosphate (TPP) is derived from **vitamin B_1 (thiamine)** and has the following structure:



The **thiazole ring** of TPP can lose a proton to generate a **negatively charged carbon atom**.



This carbon acts as a **strong nucleophile**, allowing TPP to participate in **covalent**

catalysis, particularly in reactions catalyzed by **α-keto (oxo) acid decarboxylases, α-keto acid oxidases, transketolases,** and **phosphoketolases**.

Table 11.8 lists several important **enzyme cofactors**, their **related vitamins**, and the **types of reactions** in which they participate.

Table 11.8 – Important Enzyme Cofactors and Their Related Vitamins

Vitamin	Cofactor	Reactions Involving These Cofactors
Thiamine (Vitamin B_1)	Thiamine pyrophosphate (TPP)	Activation and transfer of aldehyde groups
Riboflavin (Vitamin B_2)	Flavin mononucleotide (FMN); Flavin adenine dinucleotide (FAD)	Oxidation–reduction reactions
Niacin (Vitamin B_3)	Nicotinamide adenine dinucleotide (NAD^+); Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	Oxidation–reduction reactions
Pantothenic acid (Vitamin B_5)	Coenzyme A (CoA)	Acyl group activation and transfer
Pyridoxine (Vitamin B_6)	Pyridoxal phosphate (PLP)	Various reactions involving amino acid activation
Biotin (Vitamin B_7)	Biotin	CO_2 activation and transfer (carboxylation reactions)
Lipoic acid	Lipoamide	Acyl group activation; oxidation–reduction reactions
Folic acid (Vitamin B_9)	Tetrahydrofolate (THF)	Activation and transfer of single-carbon functional groups
Cobalamin (Vitamin B_{12})	Adenosylcobalamin; Methylcobalamin	Isomerization and methyl group transfer reactions