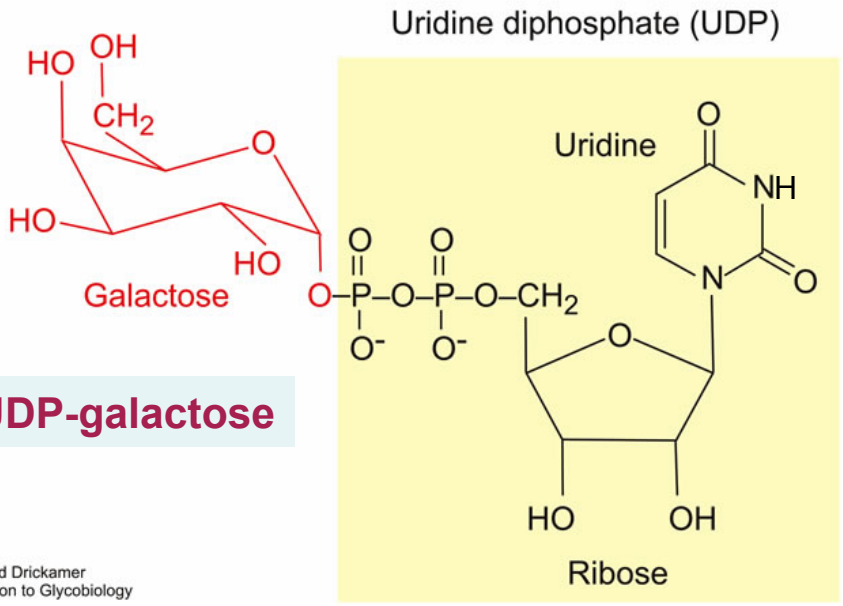


UDP-galactose

Taylor and Drickamer
Introduction to Glycobiology

A nucleotide sugar is a "biologically activated" monosaccharide. Sugar nucleotides are involved in sugar transformations and in the biosynthesis of complex carbohydrates (oligomers and polymers) *in vivo*. In the latter role, they serve as sugar donors in the sequential addition of monosaccharides to a growing oligomer or polymer chain catalyzed by glycosyltransferases.

Example reaction for NDP-sugar biosynthesis

$$\text{NTP + glycosyl 1-phosphate} \overset{E}{\rightleftharpoons} \text{NDP-sugar + } PP_i$$

e.g.: UTP + α-D-glucopyranosyl 1-P $\overset{E}{\rightleftharpoons}$ UDP-glucose + PP_i

dTDP-glucose 4,6-dehydratase: Cell wall biosynthesis

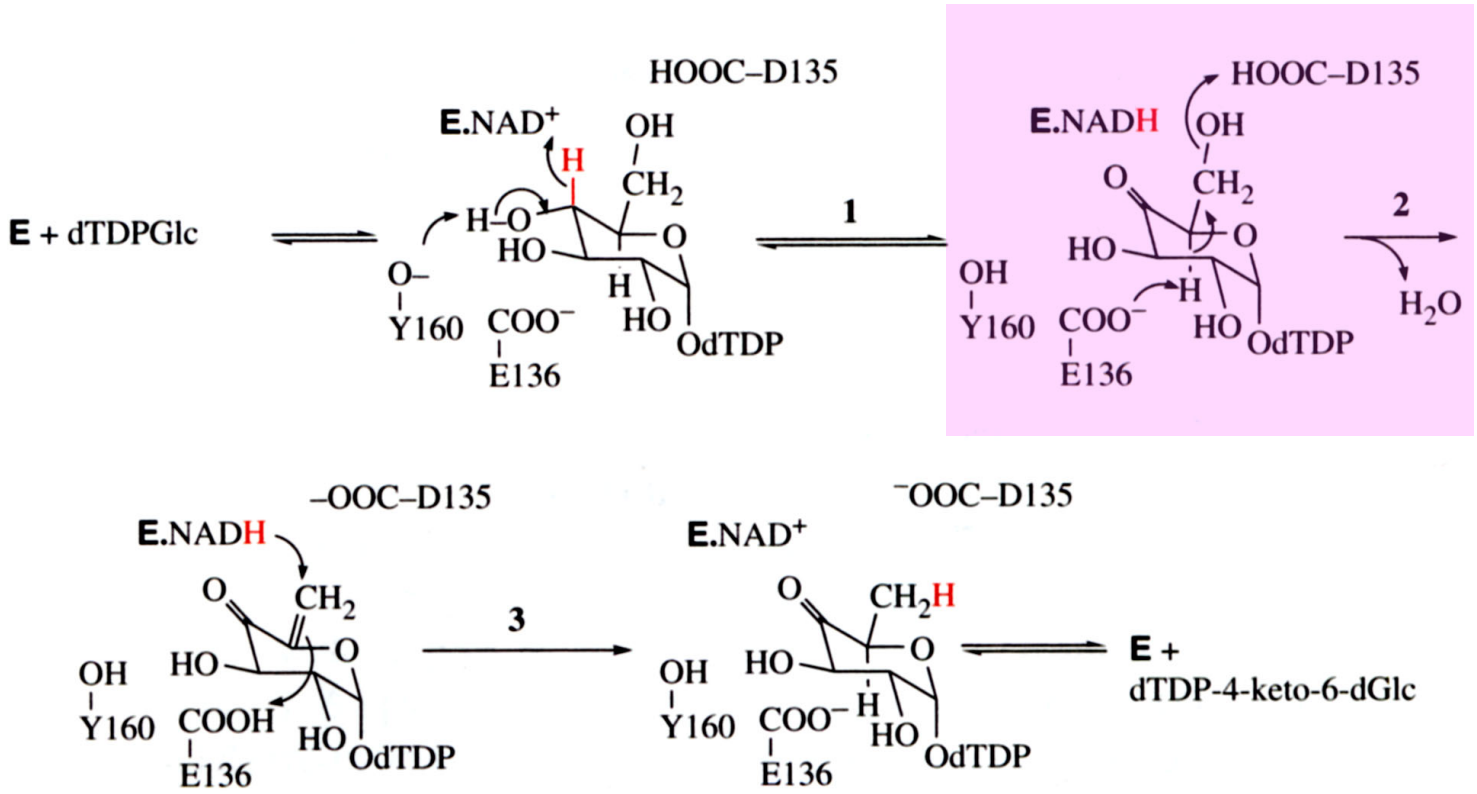


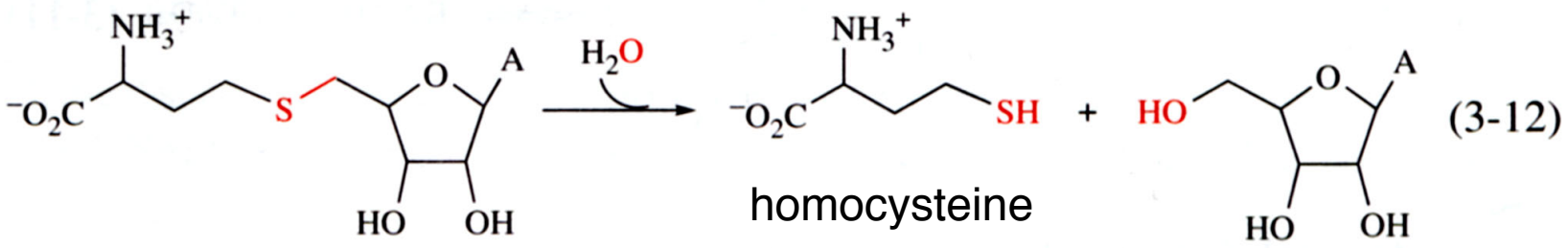
Fig. 3-4. A mechanism for the action of dTDP-glucose 4,6-dehydratase. The key steps are numbered 1 to 3 and include the dehydrogenation of glucosyl-C4 by NAD^+ in step 1 and the reduction of the 4-keto-Δ^5-glucosene at C6 by NADH in step 3. NAD^+, NADH, and all of the 4-ketosugar intermediates remain tightly bound at the active site throughout the reaction.

The NAD^+ coenzyme is tightly bound.

The acidity of H5 is increased by introduction of the carbonyl at C4.

The reaction catalyzed by S-adenosylhomocysteine hydrolase (SAH)

Overall reaction:



homocysteine

adenosine

S-adenosylhomocysteine (SAH)

The hydrolysis of sulfides is difficult chemically.

SAH contains a tightly bound NAD^+.

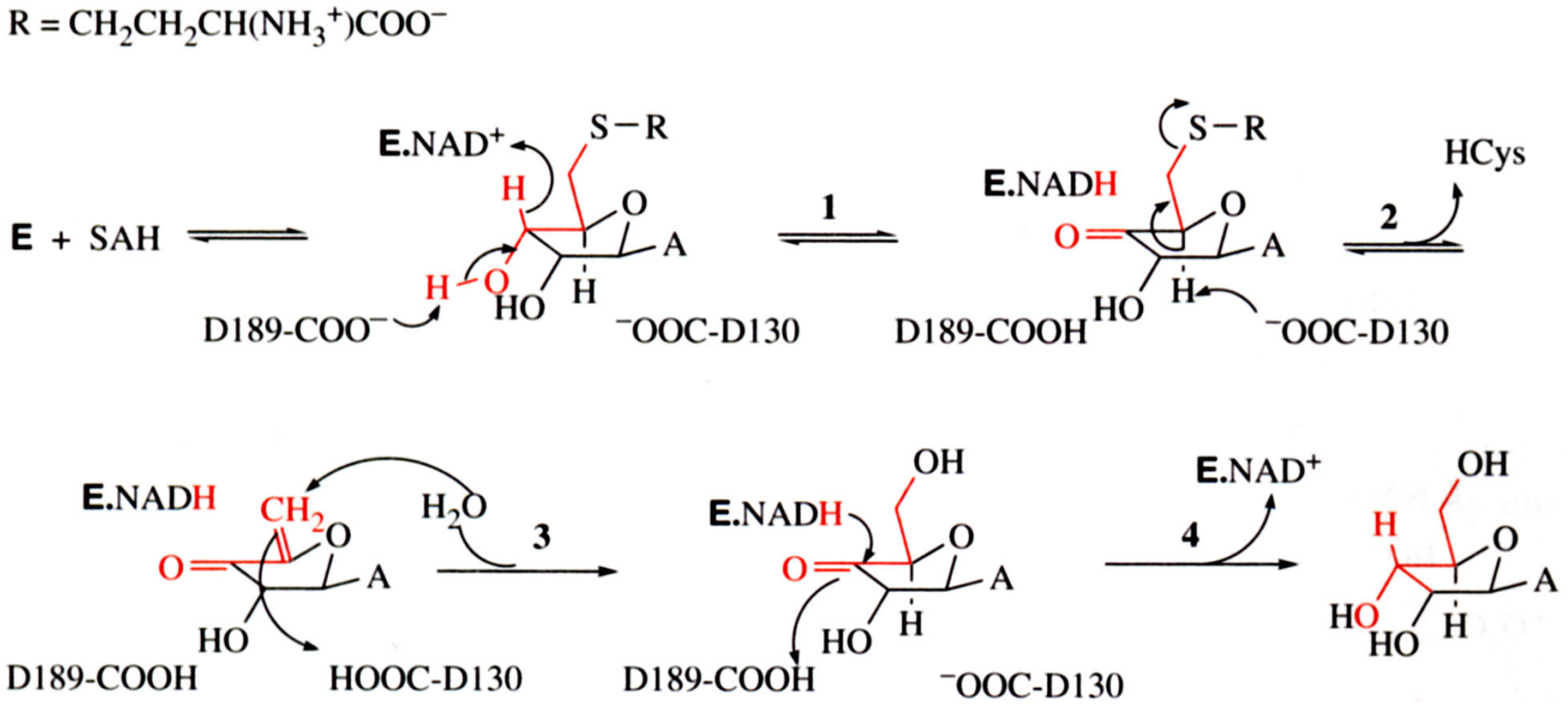


Fig. 3-5. A mechanism for the action of *S*-adenosylhomocysteine hydrolase. The key steps are numbered 1 to 4 and include the dehydrogenation of SAH at C3′ by NAD^+ in step 1 and reduction of the 3′-keto group by NADH in step 4. NAD^+, NADH, and all of the 3′-ketoadenosyl intermediates remain tightly bound at the active site throughout the reaction. The x-ray crystallographic structure and results of site-directed mutagenesis implicate Asp189 and Asp130 as the acid-base catalysts (Takata et al., 2002).

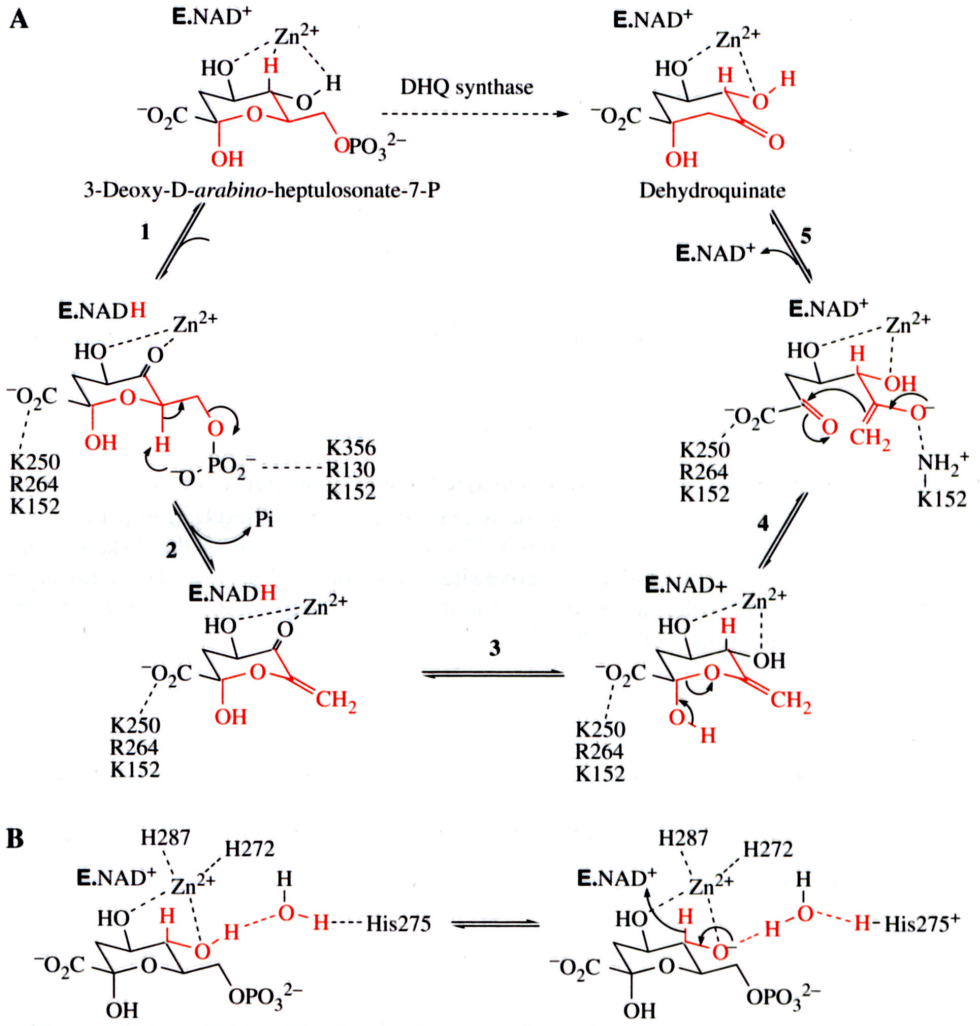


Fig. 3-6. Chemical mechanism for the reaction of dehydroquinate synthase (DHQ). (A) The overall transformation of 3-deoxy-D-*arabino*-heptulosonate-7-P into DHQ is indicated by the dashed arrow. The x-ray crystallographic structures reveal conformational changes attending the binding of the substrate to the open form of the purified complex of DHQ synthase and NAD^+ to generate the closed form of the enzyme-substrate complex in step 1 (Carpenter et al., 1998; Nichols et al., 2003). (B) The substrate is coordinated to Zn^{2+}, which is also ligated to His271, His275, and His287. The initial hydride transfer to NAD^+ in step 2 is promoted by Zn^{2+}-coordination, with expulsion of the proton to the solvent through an intervening water molecule hydrogen bonded to His275.

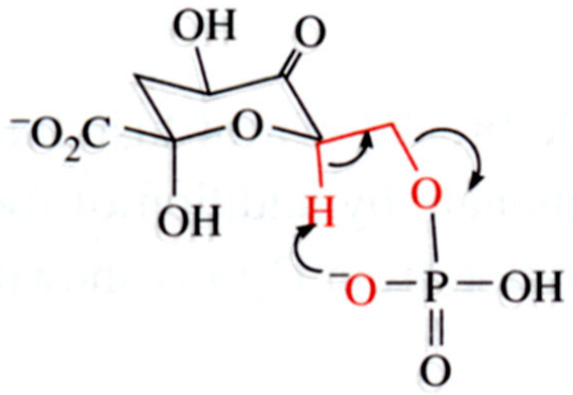
Dehydroquinate synthase

(shikimate pathway for the biosynthesis of aromatic amino acids in plants and microbes)

DHQS contains a tightly bound NAD^+.

DHQS is a zinc metalloenzyme.

Steps 4 and 5 are spontaneous chemical processes.



Scheme 3-1

Step 2 of the DHQS mechanism involves internal catalysis by phosphate - a β-elimination mechanism.

Ornithine cyclodeaminase
(biosynthesis of L-proline)

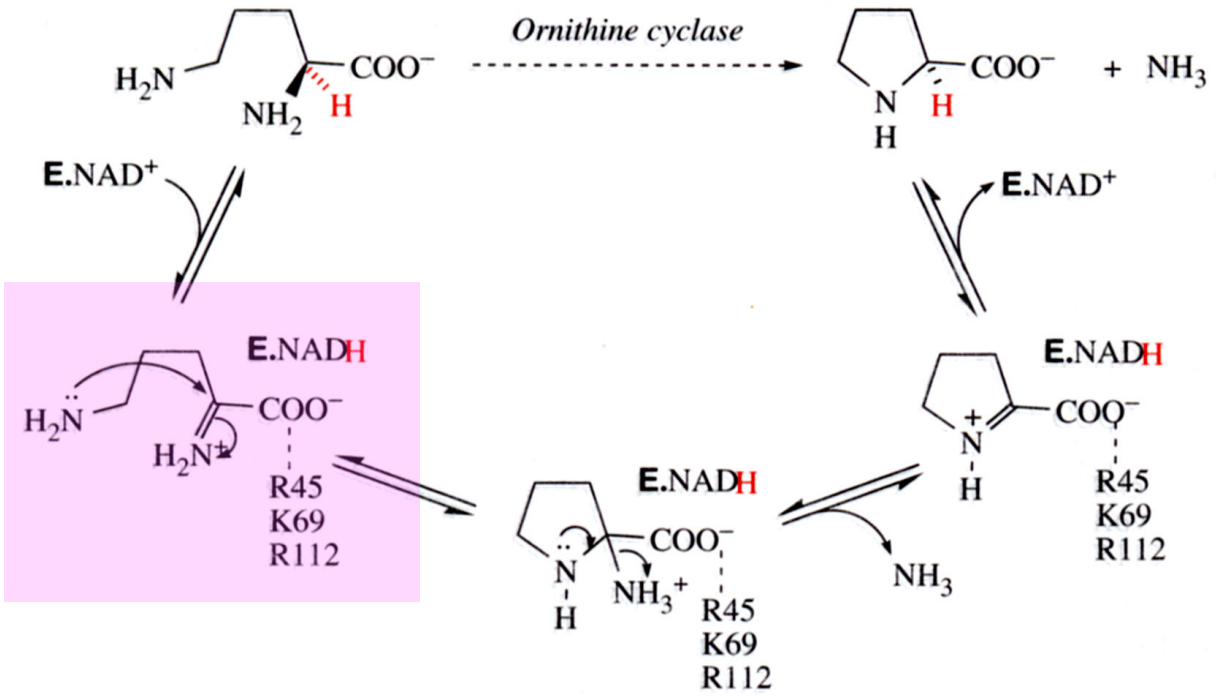
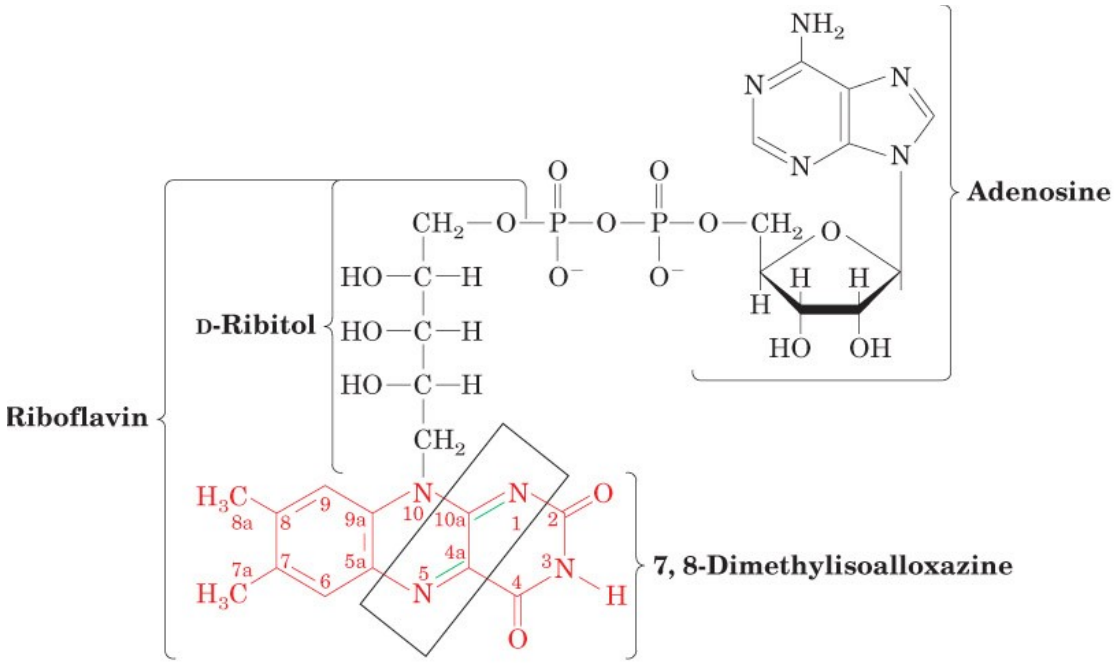


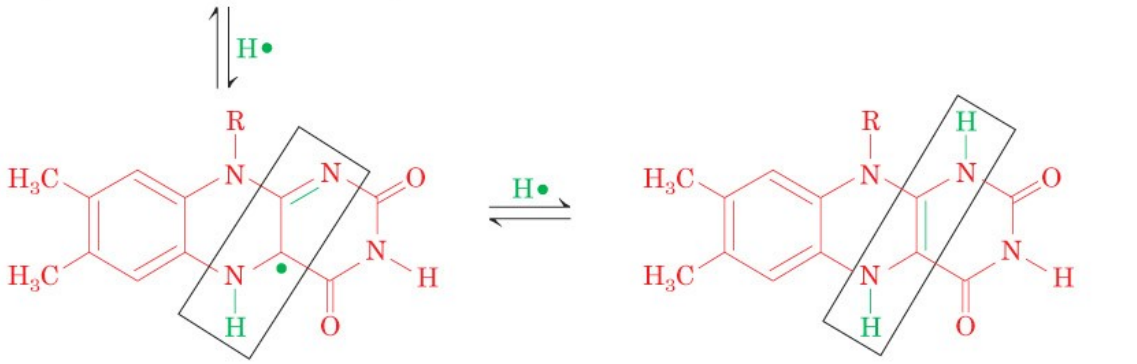
Fig. 3-7. Hypothetical mechanism for NAD$^+$-dependent cyclization of ornithine into proline.

The first step involves the formation of a protonated imine (iminium ion) in the active site.

The molecular formula and reactions of the coenzyme, flavin adenine dinucleotide (FAD and $FADH_2$)



Flavin adenine dinucleotide (FAD)
(oxidized or quinone form)

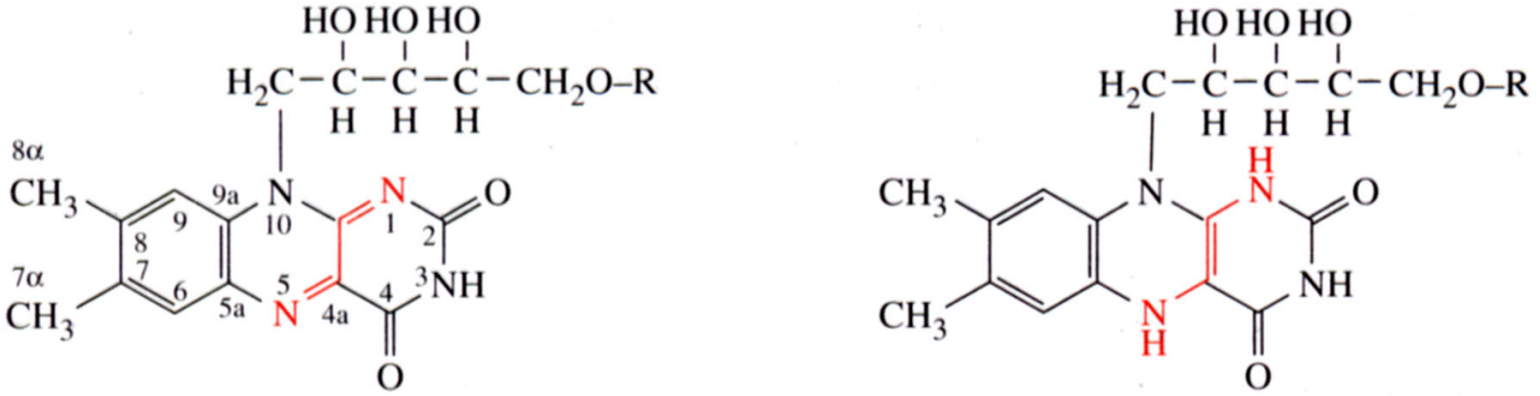


$FADH_2$ (reduced or hydroquinone form)

Vitamins are coenzyme precursors.

Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	*a*
Cobalamin (B_{12})	Cobalamin (B_{12}) coenzymes	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellagra
Pantothenate	Coenzyme A	*a*
Pyridoxine (B_6)	Pyridoxal phosphate	*a*
Riboflavin (B_2)	Flavin coenzymes	*a*
Thiamine (B_1)	Thiamine pyrophosphate	Beriberi

[a]No specific name; deficiency in humans is rare or unobserved.



R = H ; Riboflavin
= PO_3^{2-} ; FMN / $FMNH_2$
= ADP ; FAD/$FADH_2$

Fig. 3-27. Structures of flavin coenzymes. The heterocyclic ring characteristic of flavin coenzymes is isoalloxazine. In riboflavin, N1 is carries the 1-ribityl substituent; in FMN, the 1-ribityl moiety is phosphorylated at the 5′ position; and in FAD, the phosphoryl group is transformed into an ADP substituent.

Biosynthesis of FMN and FAD from riboflavin (vitamin B_2)

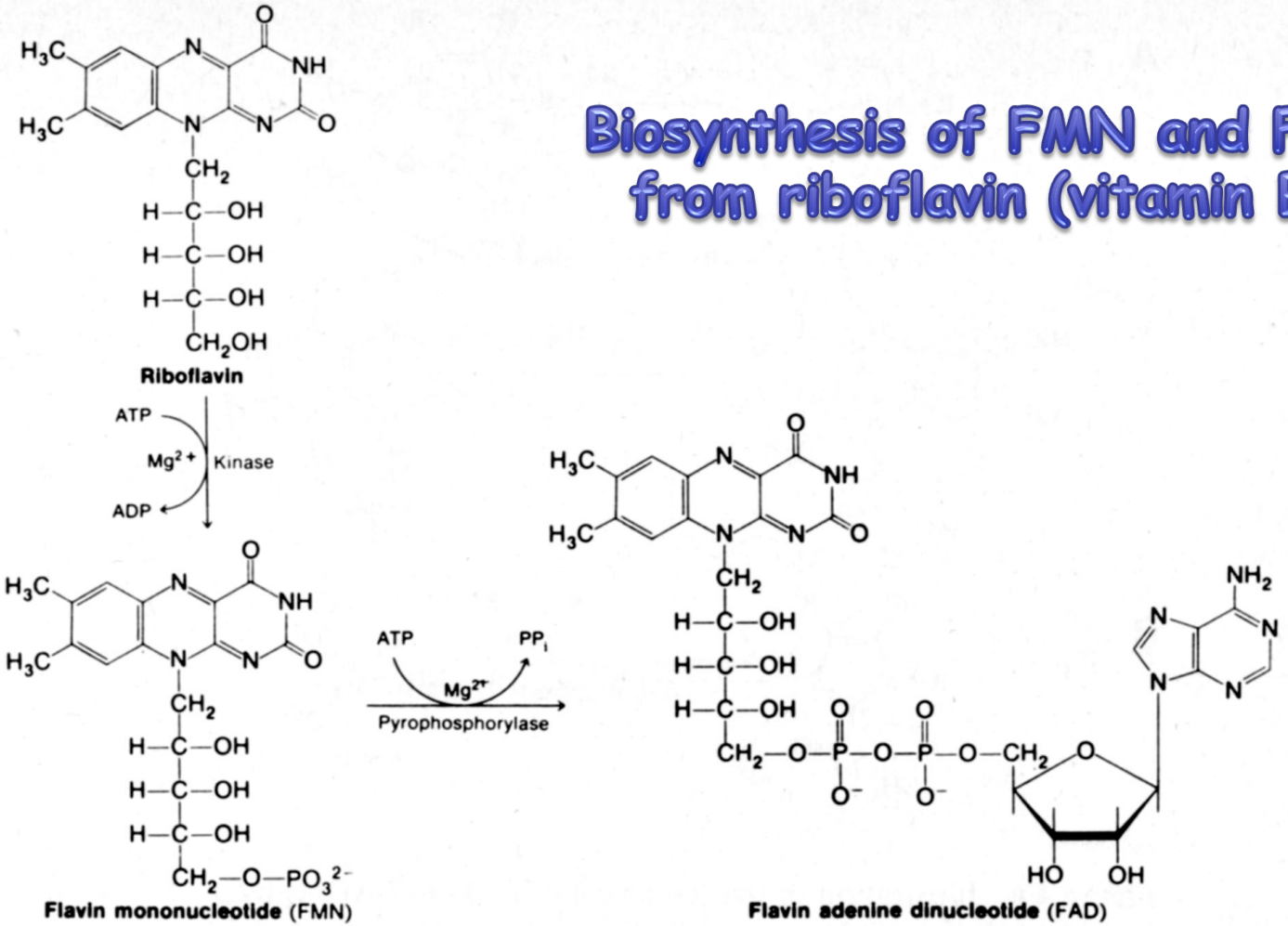


Figure 4.5 Biosynthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) from riboflavin (vitamin B_2). This figure illustrates the structures of the vitamin and its two coenzyme forms as well as the biosynthetic pathway. (Reproduced with permission from P. A. Frey, "Structure and Function of Coenzymes" in *Biochemistry,* G. Zubay, ed., Addison-Wesley, Reading, MA, 1983.)

FAD and FMN are tightly bound to most flavoproteins, and are sometimes covalently bound.

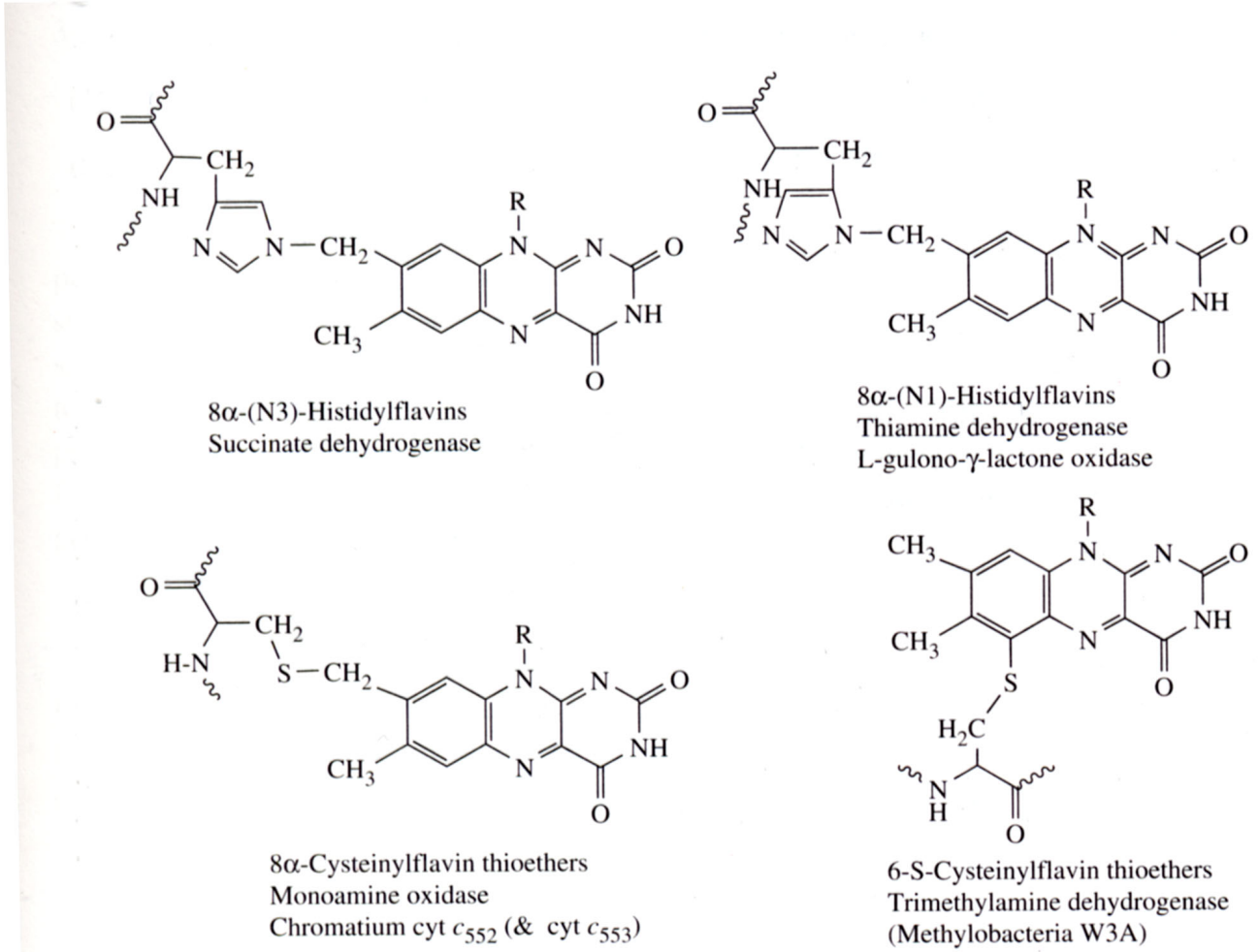


Fig. 3-28. Covalent linkages between the isoalloxazine rings of flavin coenzymes and certain enzymes. The covalent bonds to histidyl and cysteinyl side chains of proteins have characteristic effects on the visible absorption spectra of the flavins.

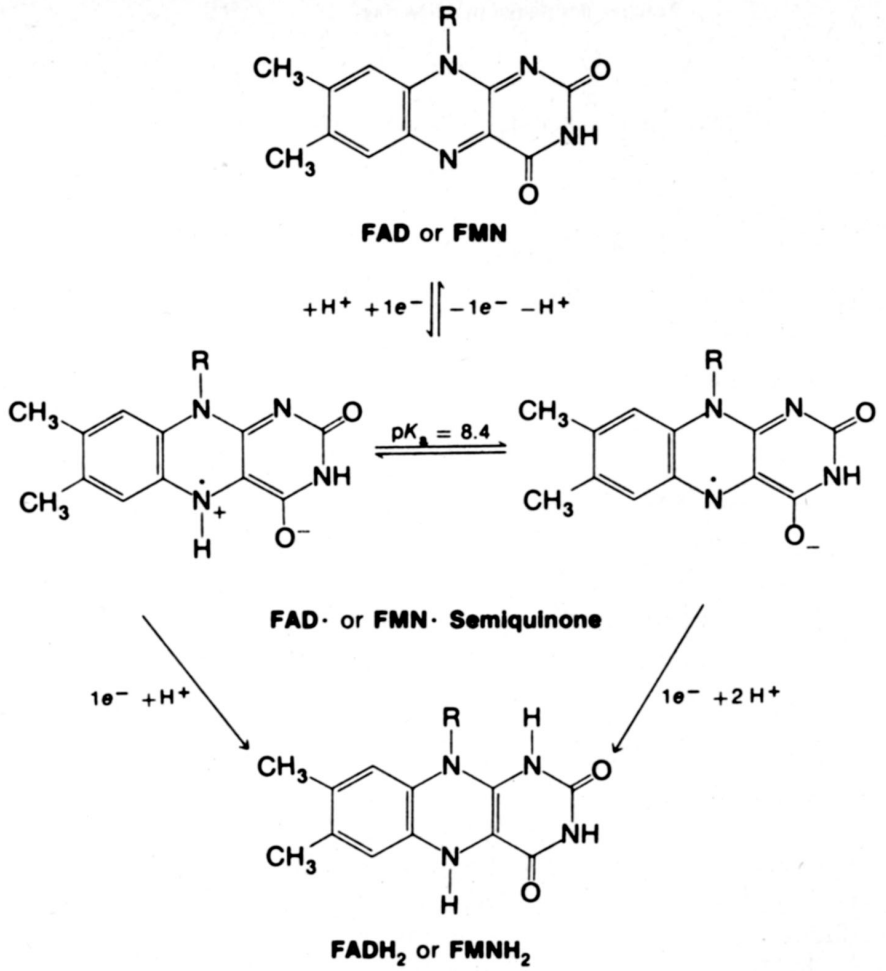


Figure 4.7 Illustration of the free radical mechanism for the reduction of FAD or FMN (top) to $FADH_2$ or $FMNH_2$ (bottom). The figure shows the two free radical forms of each coenzyme which result from the transfer of a single electron to the oxidized coenzyme: middle left, the zwitterionic semiquinone radical which predominates below pH 8.4, and middle right, the free radical anion which predominates above pH 8.4. (Reproduced with permission from P. A. Frey, "Structure and Function of Coenzymes" in *Biochemistry,* G. Zubay, ed., Addison-Wesley, Reading, MA, 1983.)

FMN and FAD can be reduced by either 1-electron or 2-electrons (interface between 2e^- redox chemistry in cytoplasmic metabolism and 1e^- redox chemistry of membrane-bound electron transport in mitochondria).

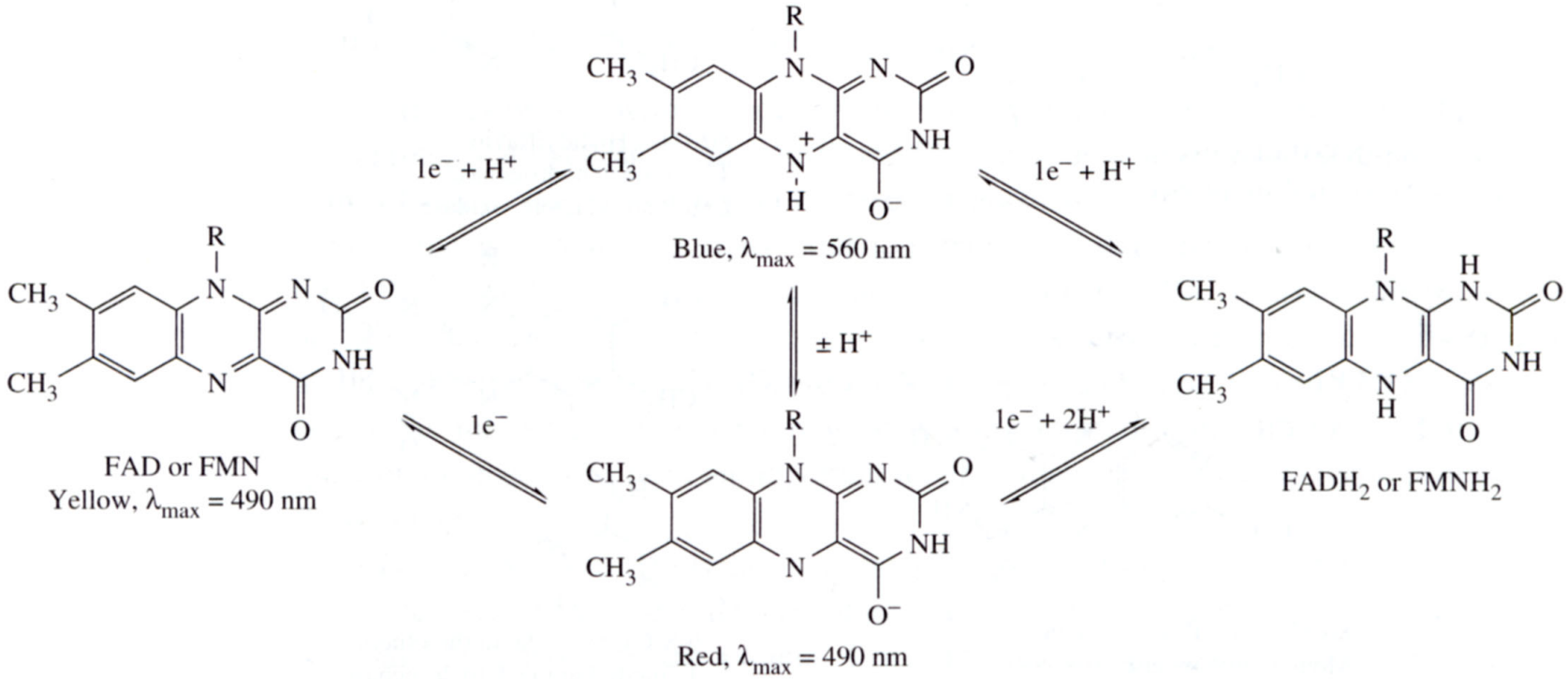


Fig. 3-29. Structures of one-electron, reduced semiquinone forms of flavins. One-electron redox reactions of flavins allow the central forms shown to participate in the redox biochemistry of flavoproteins. The dihydroflavins ($FADH_2$ and $FMNH_2$) are intermediates in most enzymatic reactions of flavin coenzymes.

Flavoprotein oxidases

$$\mathbf{E}\ \mathrm{FAD} + \mathrm{Glucose} \rightarrow \mathbf{E}\ \mathrm{FADH_2} + \mathrm{Gluconolactone} \qquad (3\text{-}25a)$$

$$\mathbf{E}\ \mathrm{FADH_2} + \mathrm{O_2} \rightarrow \mathbf{E}\ \mathrm{FAD} + \mathrm{H_2O_2} \qquad (3\text{-}25b)$$

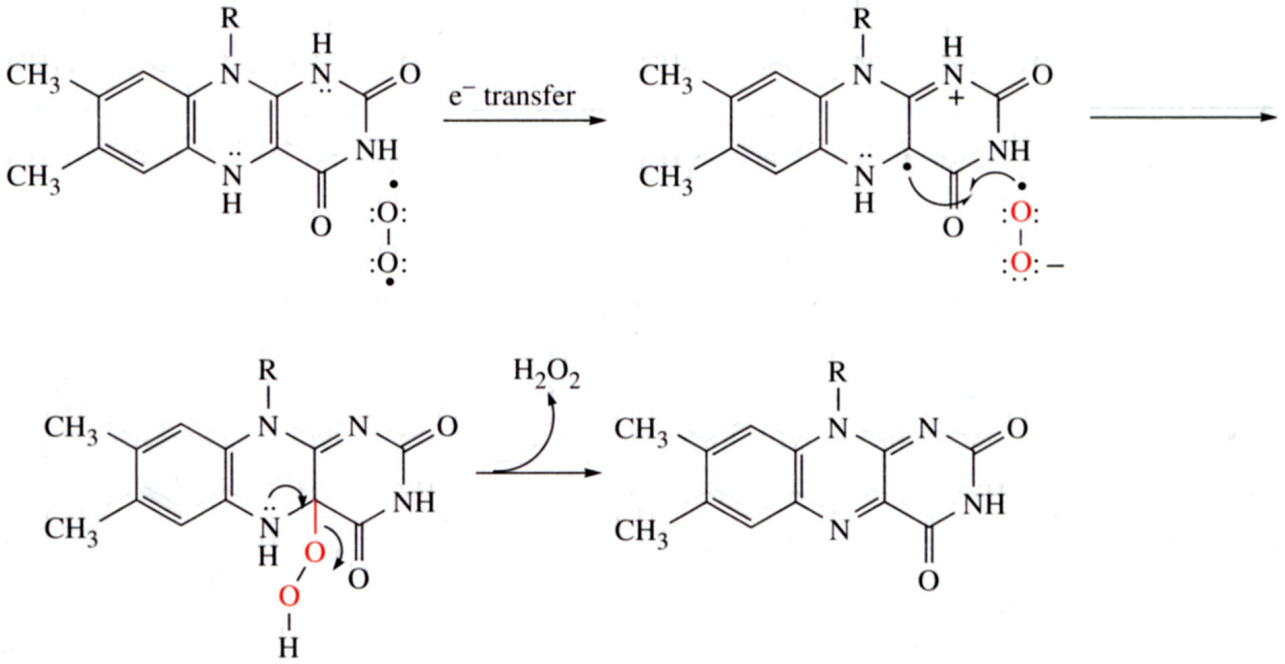


Fig. 3-30. Flavin 4a-hydroperoxide as an intermediate in the oxidation of a dihydroflavin by O_2. The formation of a flavin 4a-hydroperoxide is likely to begin with a one-electron transfer from the dihydroflavin to oxygen to form the flavin semiquinone, shown in one resonance form, and superoxide radical anion. These two species react by electron pairing at C4a to form the 4a-hydroperoxide, which subsequently eliminates hydrogen peroxide to form the oxidized flavin.

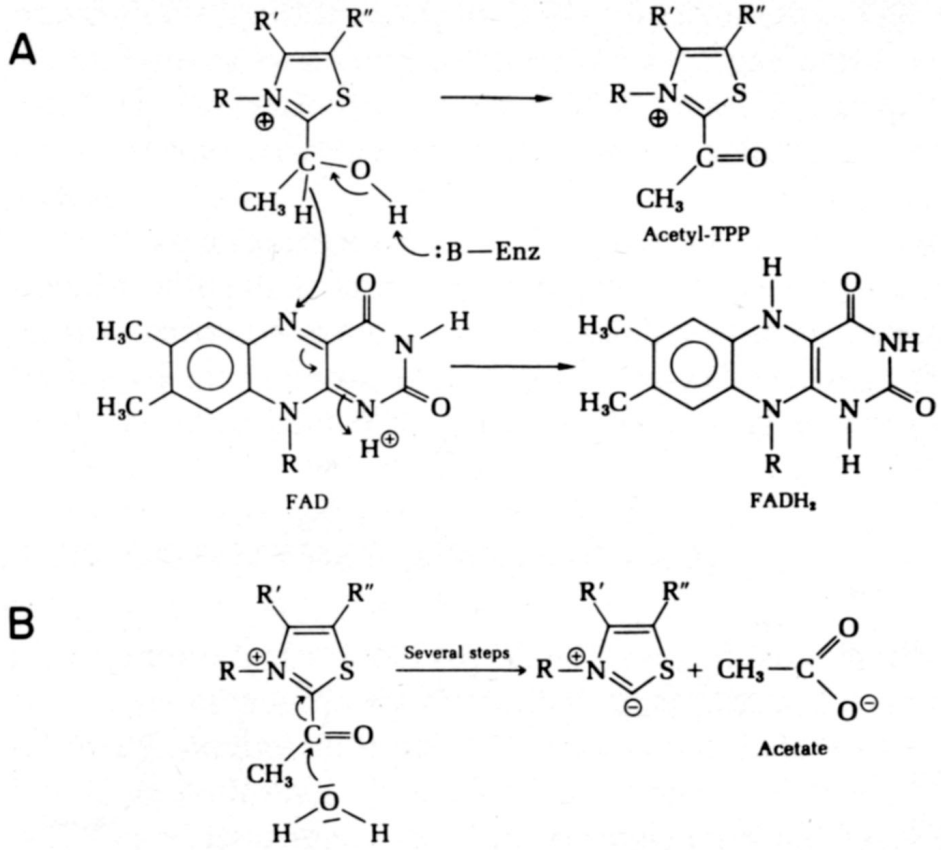


Figure 4.6 Illustration of the reduction of FAD to $FADH_2$ by a hydride shift-type mechanism. This figure shows the participation of FAD in the oxidation of hydroxyethyl-TPP to acetyl-TPP (A). Subsequently, acetyl-TPP hydrolyzes to give free acetate and TPP (B). The reaction is catalyzed by pyruvate oxidase from *E. coli*. While the mechanism shown involves hydride shift, the details of this process have not been elucidated. A carbanion addition reaction may occur.

Pyruvate oxidase (also requires TPP)