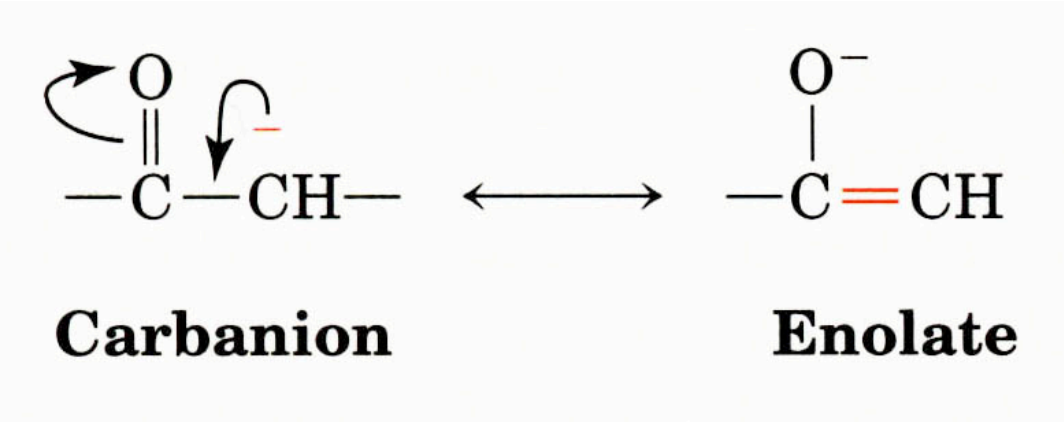
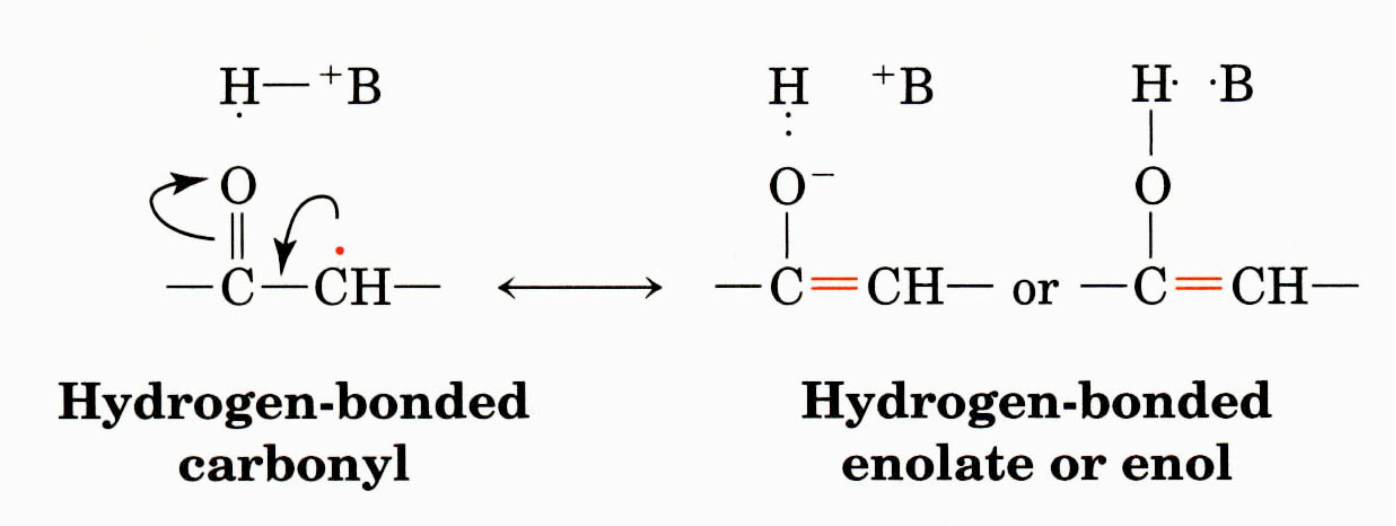
Stabilization of carbanions

Carbanions adjacent to carbonyl groups are stabilized by the formation of enolates.



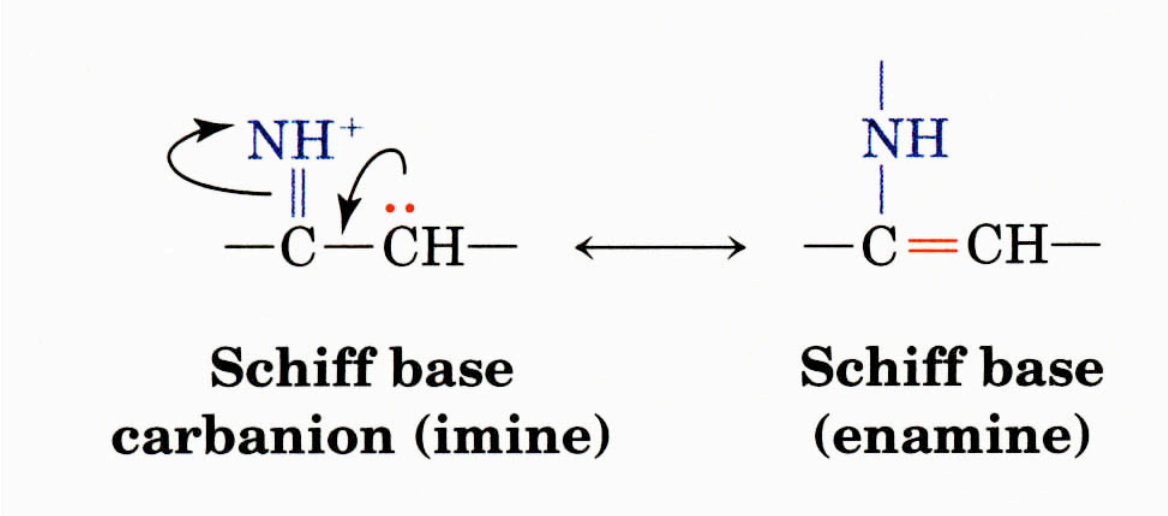
Stabilization of carbanions

Carbanions adjacent to carbonyl groups hydrogen-bonded to general acids are stabilized electrostatically or by charge neutralization.



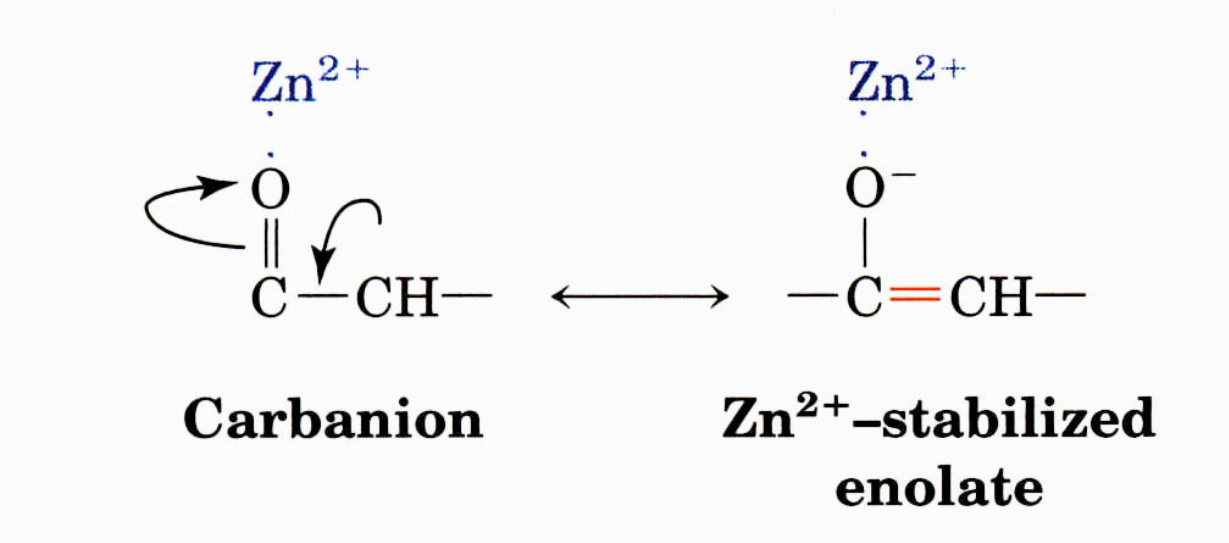
Stabilization of carbanions

Carbanions adjacent to protonated imines (Schiff bases) are stabilized by the formation of enamines.



Stabilization of carbanions

Metal ions stabilize carbanions adjacent to carbonyl groups by the electrostatic stabilization of the enolate.



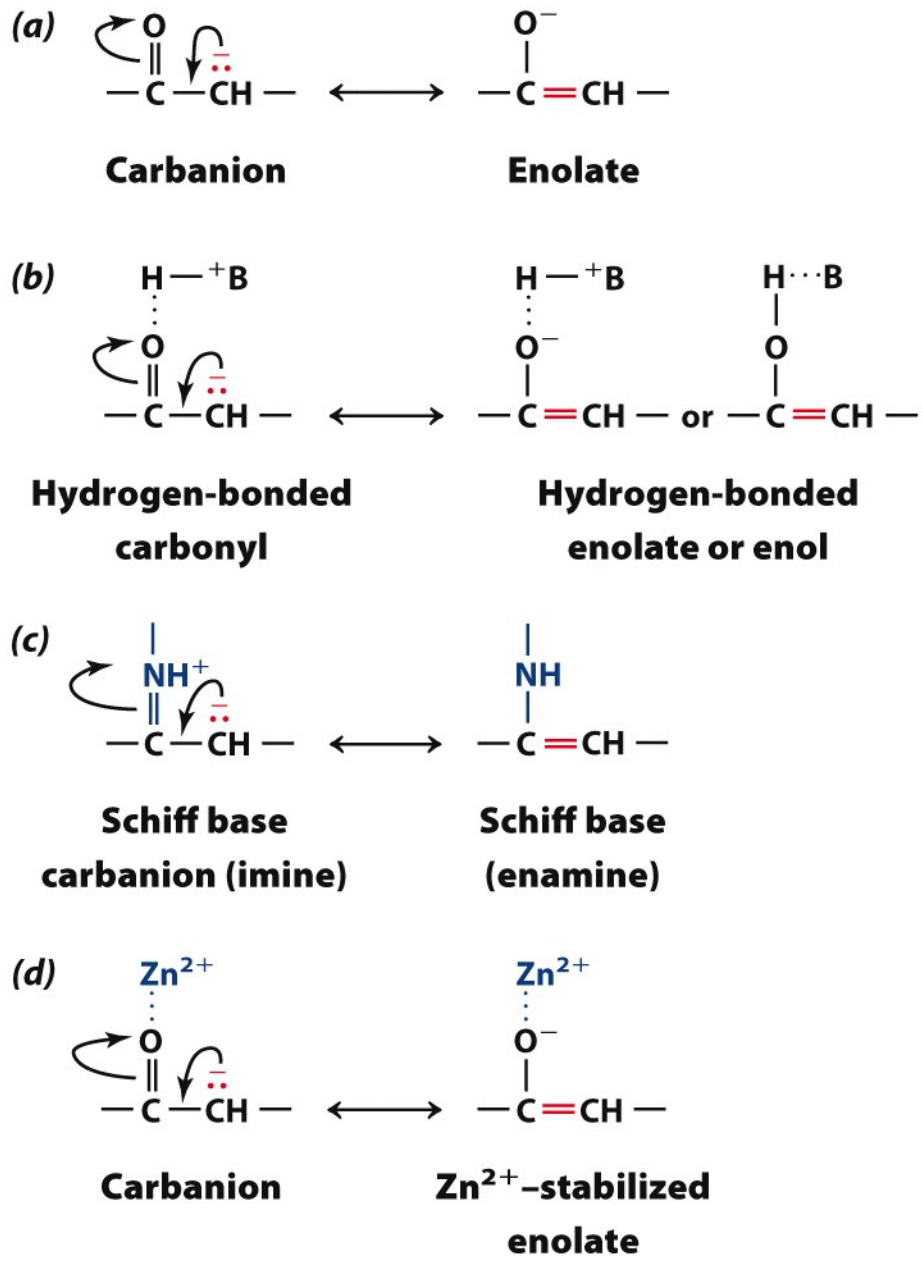


Figure 16-12

Summary of the different ways to stabilize carbanions

Common coenzymes

Coenzyme	Reaction Mediated	Section Discussed
Biotin	Carboxylation	23-1A
Cobalamin (B_{12}) coenzymes	Alkylation	25-2E
Coenzyme A	Acyl transfer	21-2A
Flavin coenzymes	Oxidation–reduction	16-5C
Lipoic acid	Acyl transfer	21-2A
Nicotinamide coenzymes	Oxidation–reduction	13-2A
Pyridoxal phosphate	Amino group transfer	26-1A
Tetrahydrofolate	One-carbon group transfer	26-4D
Thiamine pyrophosphate	Aldehyde transfer	17-3B

Definitions

cofactor: an inorganic or organic species or compound required for the activity of a particular enzyme

coenzyme: an organic cofactor

apoenzyme: the enzyme devoid of all of the cofactors required for activity (protein only)

holoenzyme: the enzyme with all of its cofactors required for catalytic activity

prosthetic group: a tightly bound cofactor (does not freely dissociate from the protein/enzyme

Vitamins are coenzyme precursors.

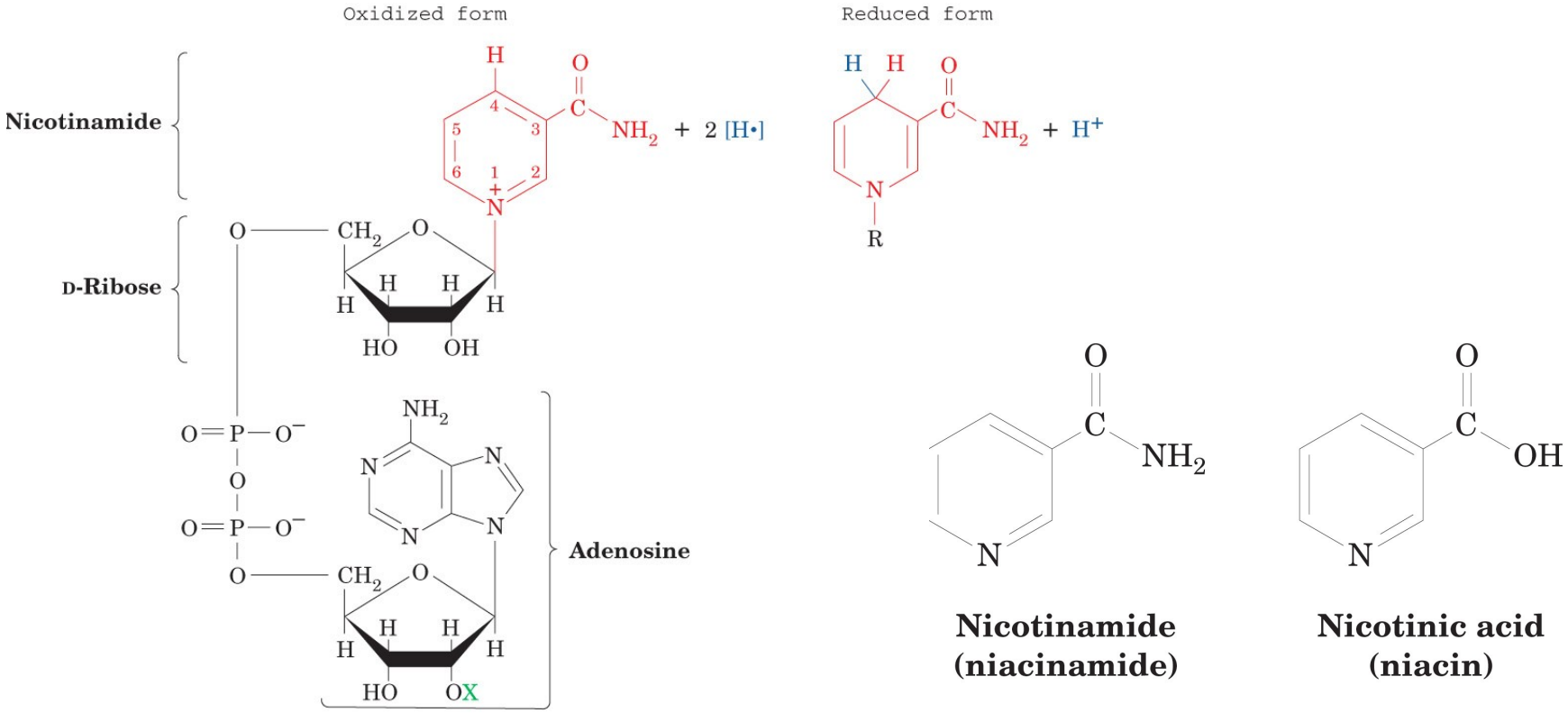
Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	*a*
Cobalamin (B_{12})	Cobalamin (B_{12}) coenzymes	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellagra
Pantothenate	Coenzyme A	*a*
Pyridoxine (B_6)	Pyridoxal phosphate	*a*
Riboflavin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

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

Why does a protein/enzyme require a cofactor for activity?

Nicotinamide and flavin coenzymes: Coenzymes of redox processes

Structures and reactivity of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$)



Nicotinamide (niacinamide)

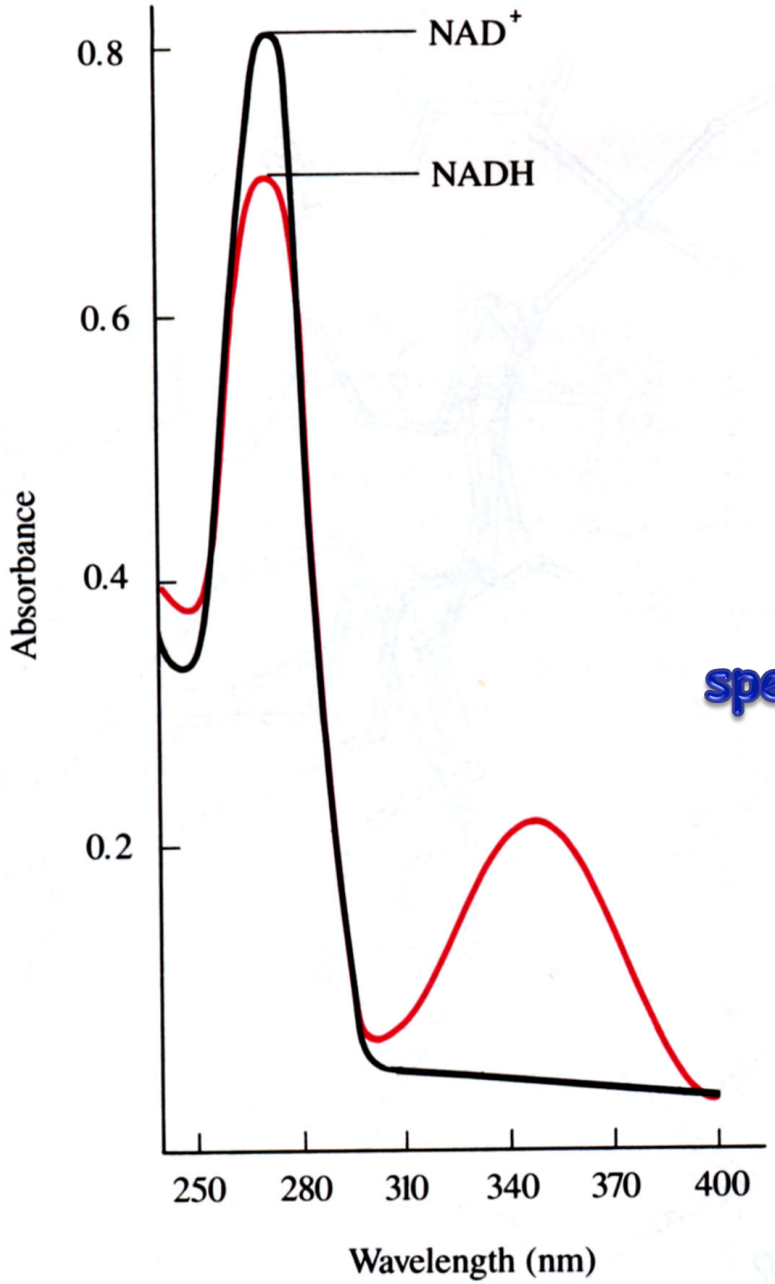
Nicotinic acid (niacin)

X = H **Nicotinamide adenine dinucleotide (NAD^+)**
X = PO_3^{2-} **Nicotinamide adenine dinucleotide phosphate ($NADP^+$)**

Vitamins are coenzyme precursors.

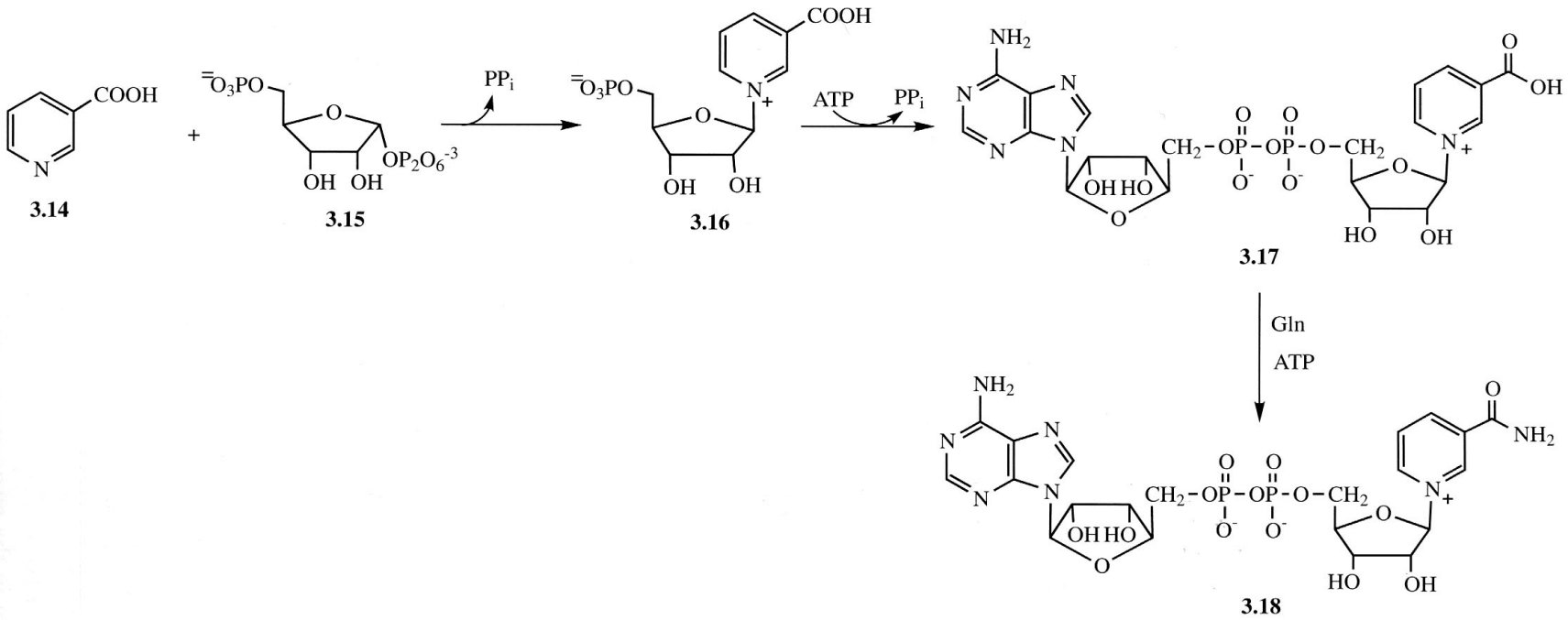
Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	*a*
Cobalamin (B_{12})	Cobalamin (B_{12}) coenzymes	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellagra
Pantothenate	Coenzyme A	*a*
Pyridoxine (B_6)	Pyridoxal phosphate	*a*
Riboflavin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

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



Effect of reduction of the aromatic NAD^+ to the non-aromatic NADH on UV-VIS spectrophotometric properties

Biosynthesis of NAD+



SCHEME 3.11 Biosynthesis of nicotinamide adenine dinucleotide.

Biosynthetic pathways involved in the production of NAD^+ and $NADP^+$ *in vivo*

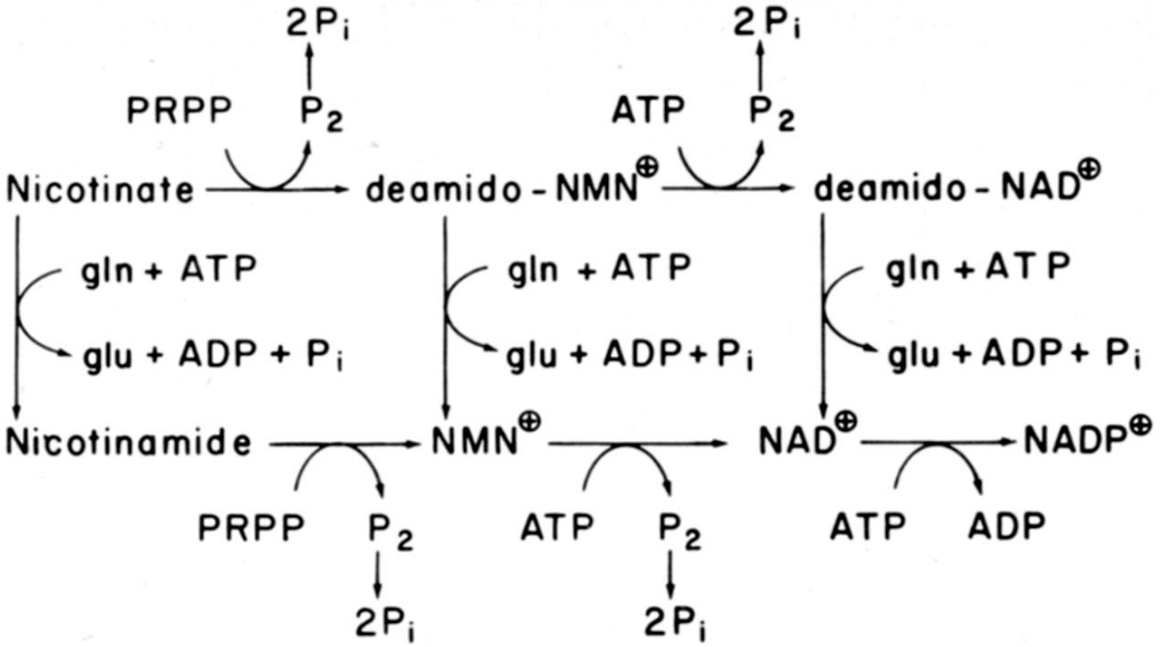


Figure 4.9 Biosynthetic pathways for the syntheses of NAD^+ and $NADP^+$ from nicotinate and nicotinamide. The vertical arrows indicate the amidation reactions while horizontal arrows illustrate all other biosynthetic interconversions. Abbreviations include: PRPP, phosphoribosyl pyrophosphate; P_2, pyrophosphate; NMN, nicotinamide mononucleotide; gln, glutamine; glu, glutamic acid.

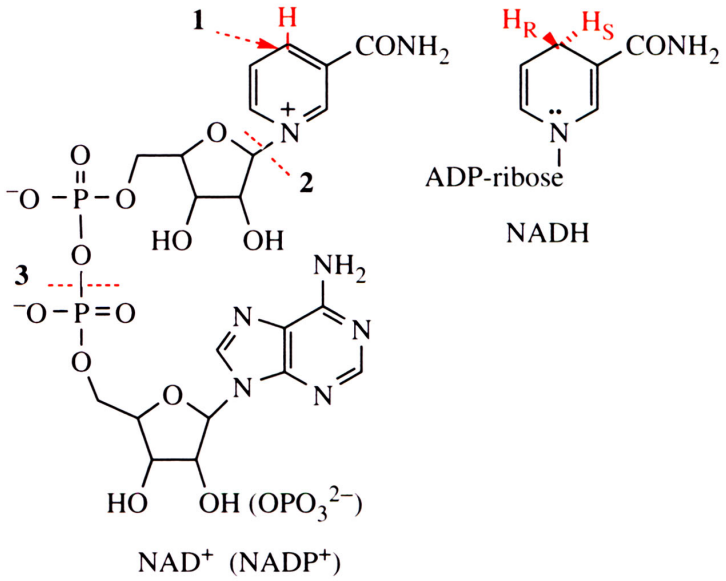


Fig. 3-1. Structures and metabolic cleavage points of nicotinamide coenzymes. The structure of NAD^+ and $NADP^+$ are shown with markings to indicate which bonds undergo chemical changes in various enzymatic reactions. Notice the designations H_R and H_S in NADH, which refer to the stereospecificities of various dehydrogenases for transferring hydrogen to and from nicotinamide coenzyme. 4-Pro-*R* and 4-pro-*S* specificities of some dehydrogenases are listed in table 3-1.

1: redox reactions (***coenzyme***) (oxidoreductases, dehydrogenases) (H^+ and $2e^-$ acceptor)

2: ADP-ribosylation (***metabolite***)

3: phosphoanhydride cleavage: DNA ligase activation of the 5'-P of DNA fragments (DNA replication and nick repair) (***metabolite***)

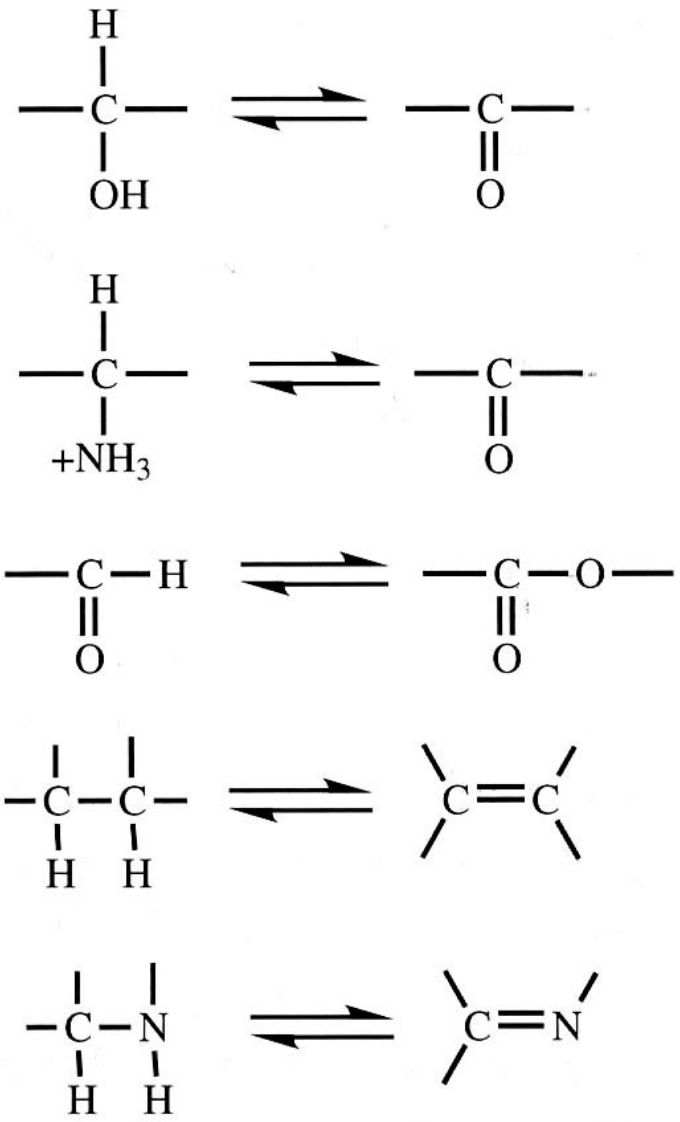
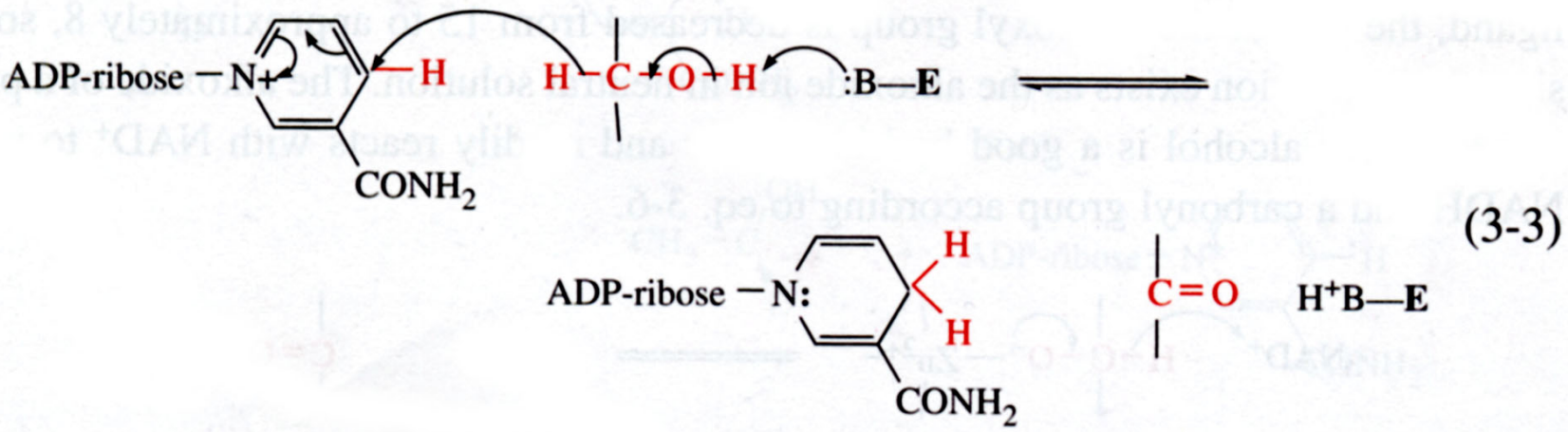


FIGURE 3.1 Reactions catalyzed by pyridine nucleotide-containing enzymes.

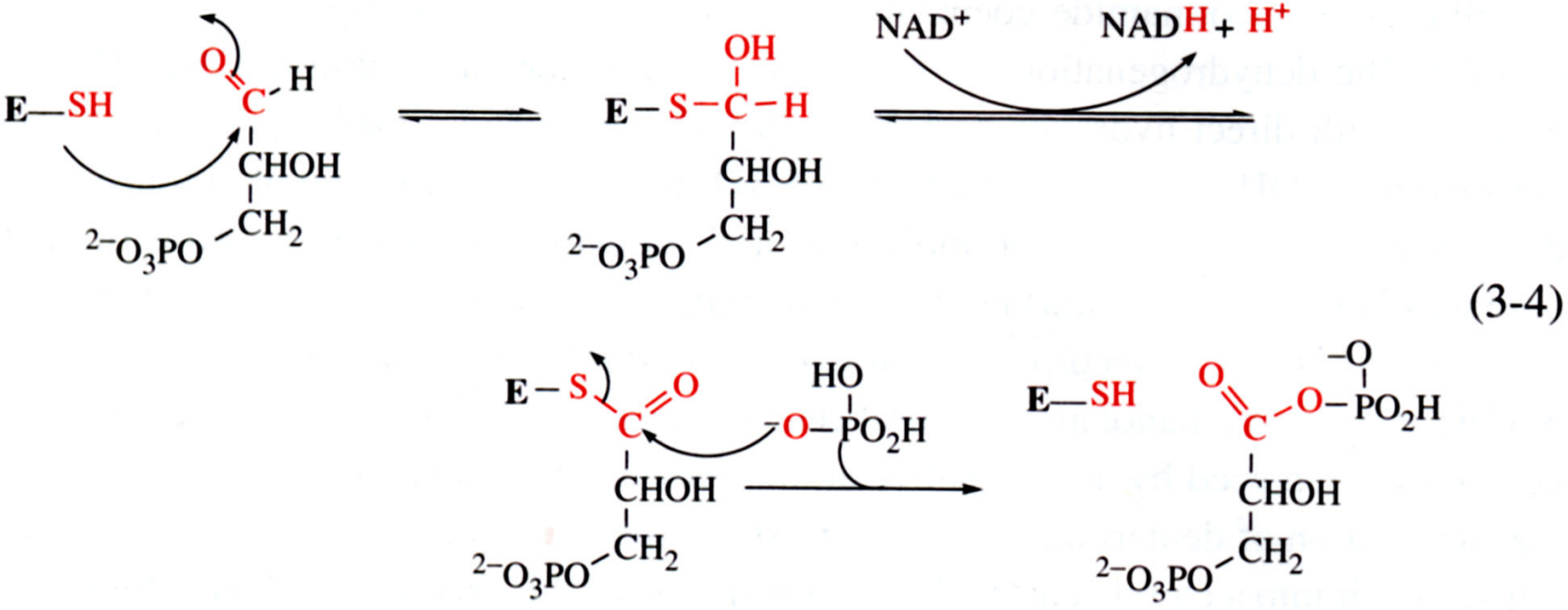
NAD^+ serving as a hydride ($H^- = H^+ + 2e^-$) acceptor, with an alcohol serving as the hydride donor



(3-3)

The hydride is accommodated at C4 of the nicotinamide ring; note that C4 of NADH is prochiral.

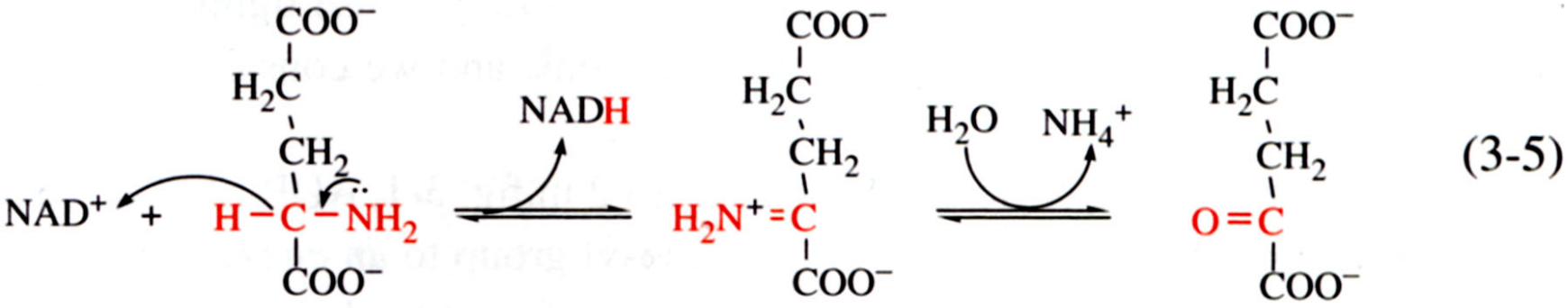
The redox reaction catalyzed by D-glyceraldehyde 3P Dehydrogenase (EC 1.2.1.12) to form the high-energy phosphate metabolite, 1,3-bisphospho-D-glycerate (1,3-BPG)



(3-4)

An amino acid dehydrogenase: L-glutamate dehydrogenase (EC 1.4.1.3)

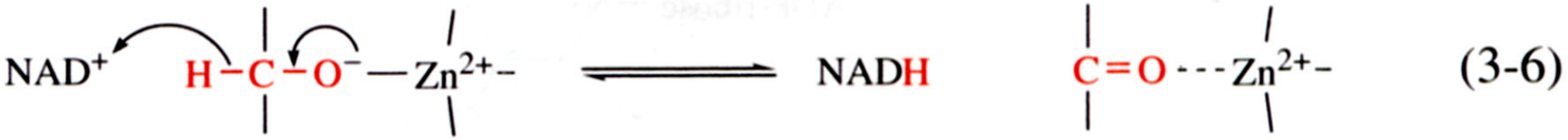
(conversion of an α-amino acid to an α-ketoacid: α-ketoglutarate)



Note the initial formation of an iminium group (protonated imine), which undergoes subsequent hydrolysis on the enzyme to form the α-ketoacid.

This reaction is an example of oxidative deamination.

Some alcohol dehydrogenases use Zn^{2+} ion to facilitate proton abstraction on the alcohol substrate through metal complexation.

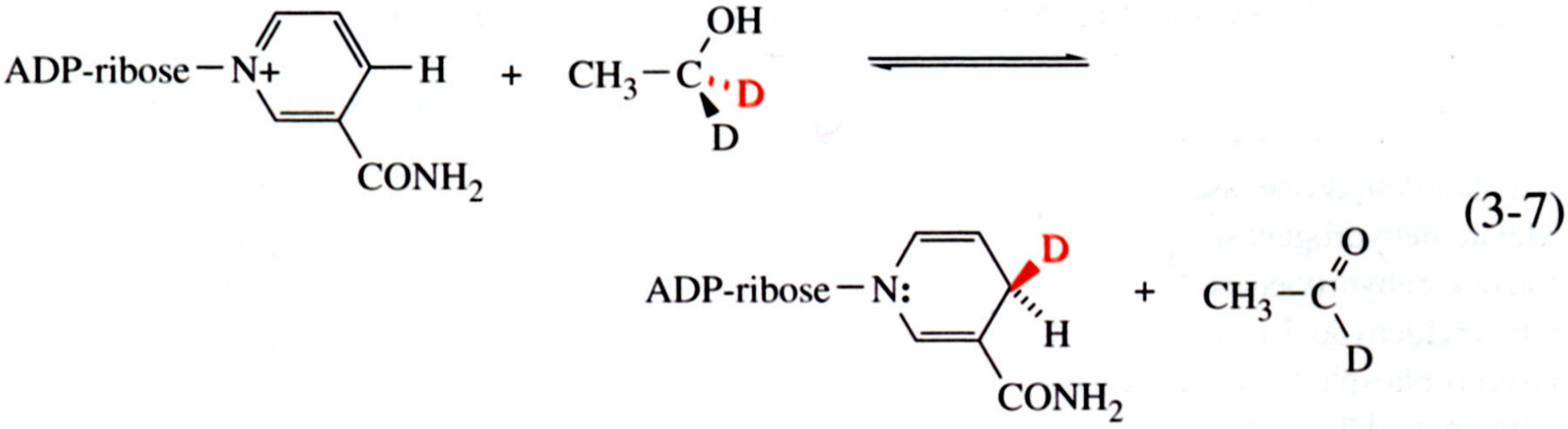


(3-6)

Metal complexation lowers the pK_a of the hydroxyl proton from ~15 to ~8 so that a significant fraction of the alcohol is in the ionized (alkoxide) form at physiological pH.

Stereospecific reduction of NAD^+: Using deuterium as a tracer

Prochiral recognition by alcohol dehydrogenase (ADH; EC 1.1.1.1)



(3-7)

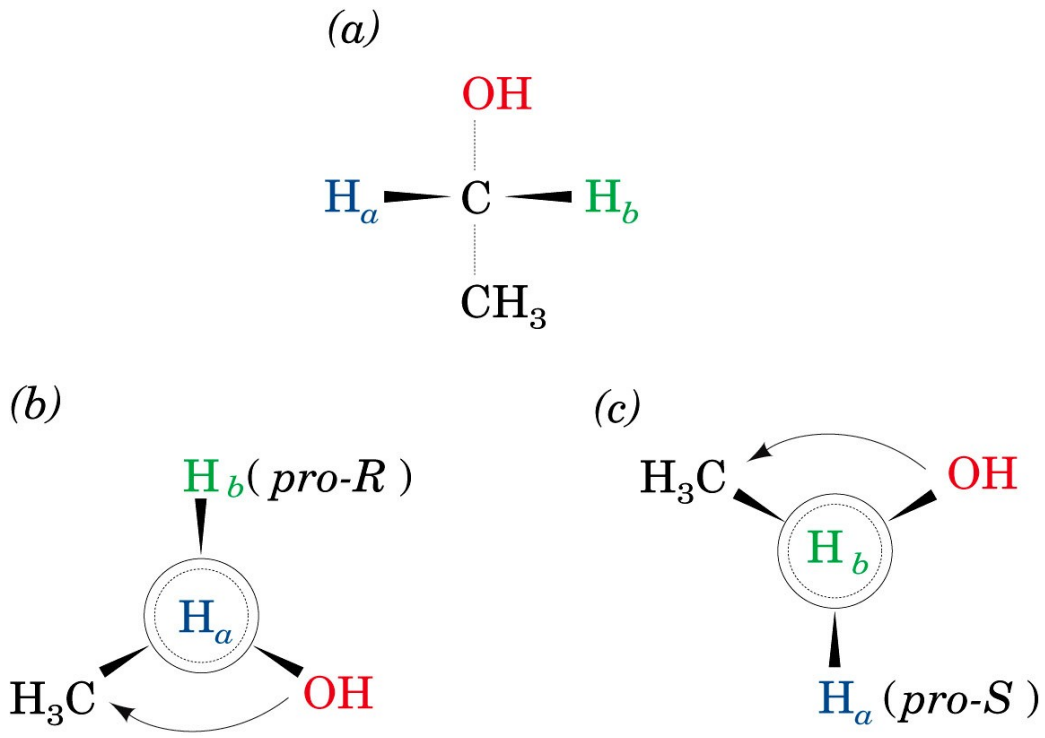
The reduction of NAD^+ with 1,1-dideuteroethanol as the substrate gives 4*R*-[nicotinamide-4-$^2H_2$]NADH as product; the ^2H is transferred specifically to the 4-pro-*R* site of NAD^+.

Enzyme classification according to reaction type

Classification	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization
6. Ligases	Bond formation coupled with ATP hydrolysis

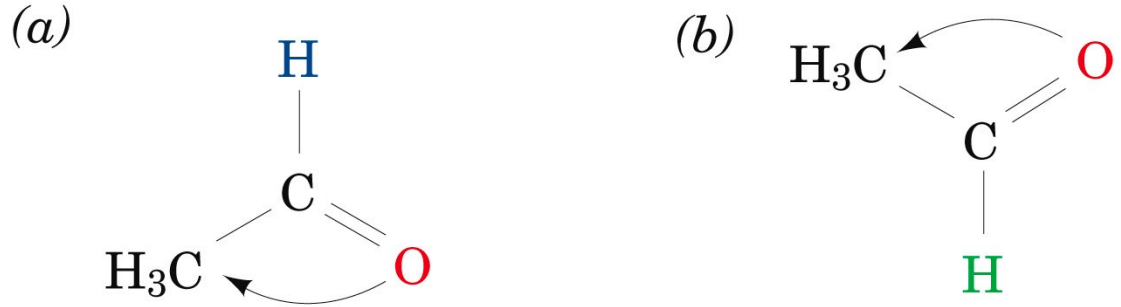
Example of EC Classification: Carboxypeptidase A EC 3.4.17.1

3: enzyme major class = hydrolase
4: subclass of hydrolase = peptide hydrolase
17: sub-subclass = metallocarboxypeptidase (carboxypeptidase A has a Zn^{2+} ion bound in its active site
1: arbitrarily assigned serial number in its sub-subclass



Ethanol is a prochiral molecule. Prochiral sites can be distinguished in the chiral active sites of enzymes. Glycine and citric acid are also prochiral molecules.

Planar carbonyl groups are also prochiral.



Views of acetaldehyde: *re* (a) and *si* (b) faces

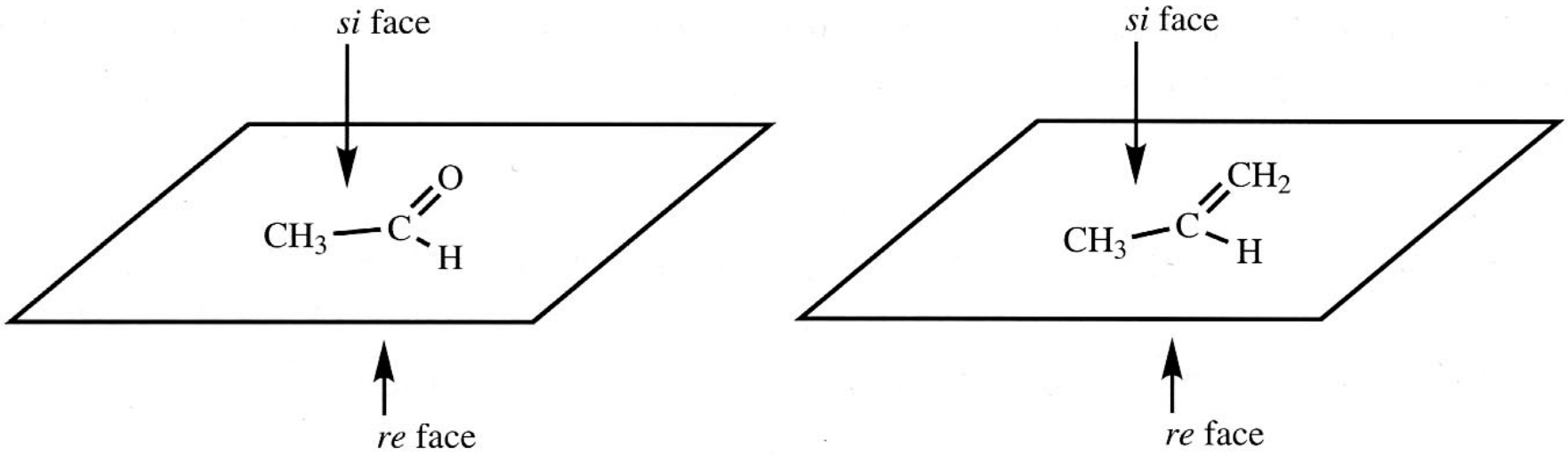
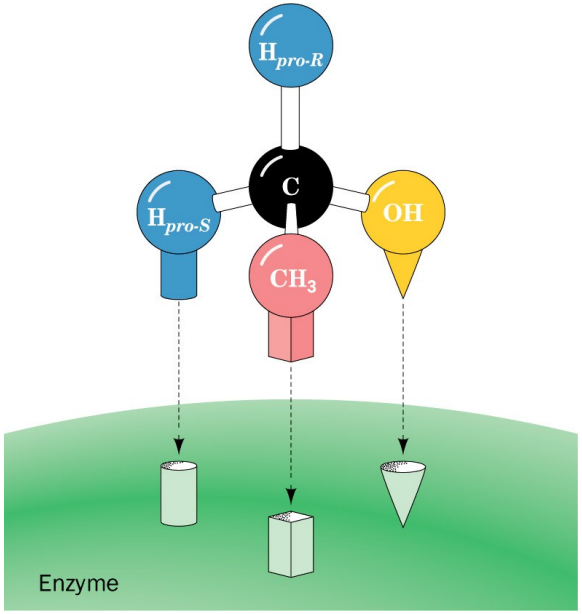


FIGURE 3.4 Determination of carbonyl and alkene chirality.

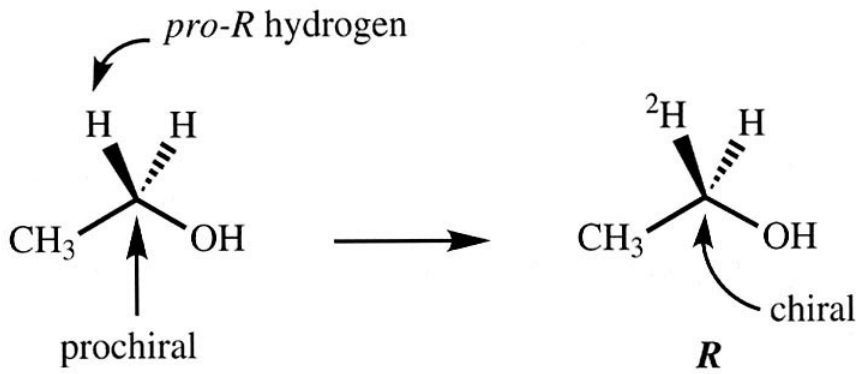
Dealing with prochiral sites in substrates: ethanol, citric acid



Prochiral differentiation in a chiral protein binding site: Distinguishing between the pro-*R* and pro-*S* hydrogens in achiral ethanol

C_{aacd} ⟶ C_{abcd}

prochiral **chiral**



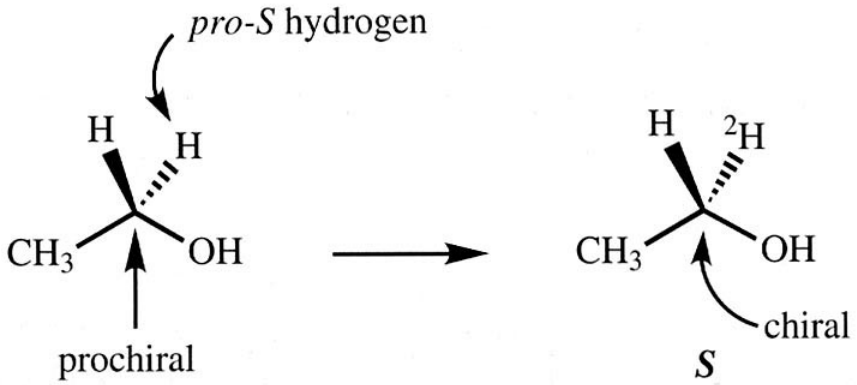
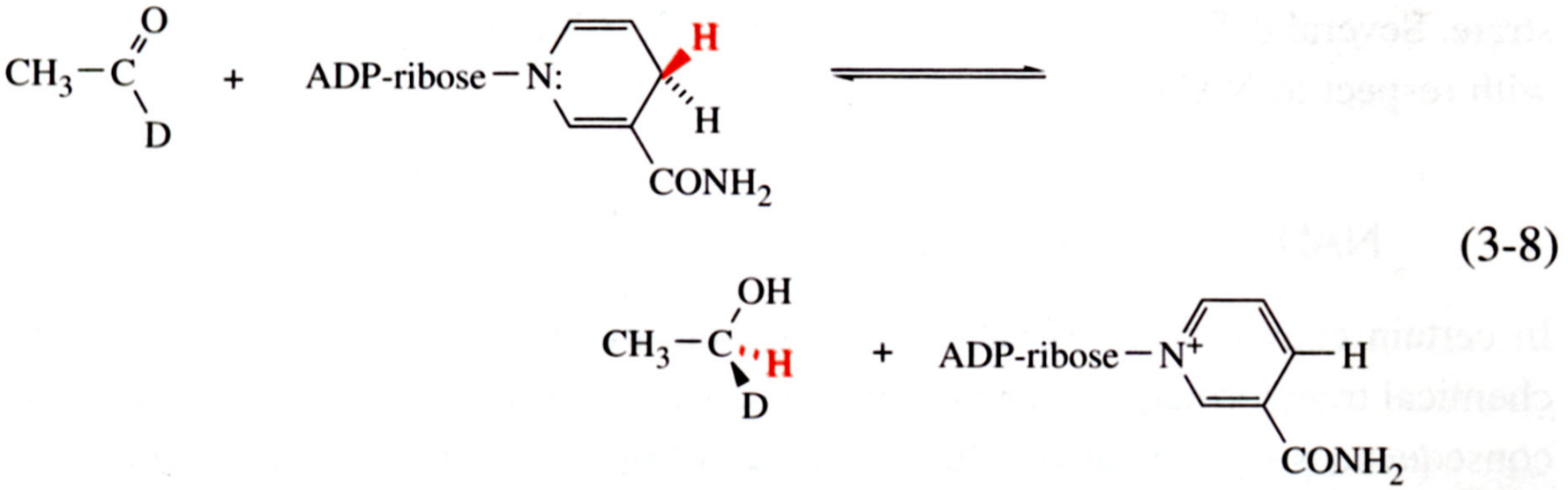


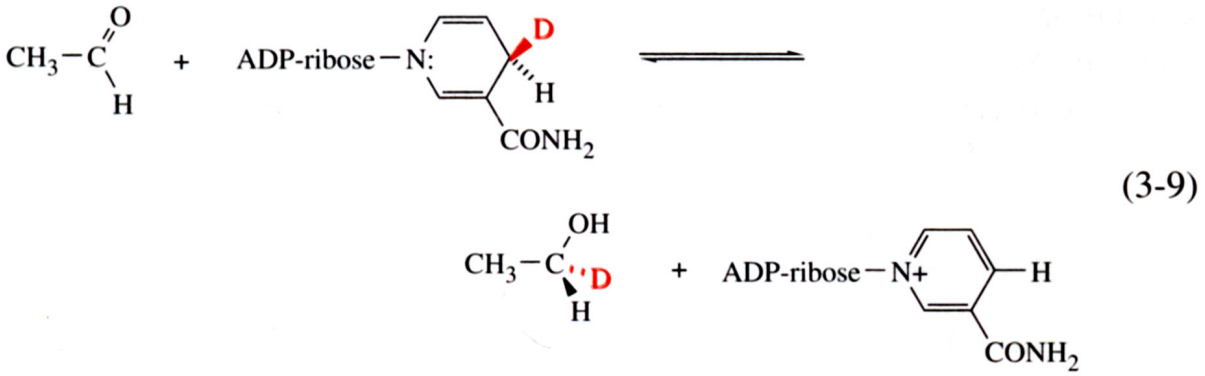
FIGURE 3.3 Determination of prochirality.

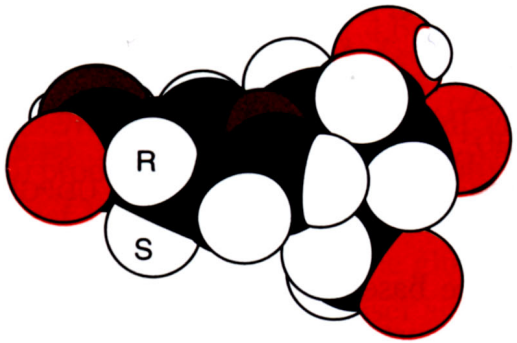
Assigning the pro-R and pro-S hydrogens in ethanol

The ADH Reaction



The reduction of deuteroacetaldehyde by NADH gives NAD^+ and *S*-[1-^{2}H]ethanol; the hydrogen in the 1-pro-*S* position of ethanol is transferred to NAD^+ (reverse reaction).





1,4-Dihydronicotinamide riboside

Positions of the pro-*R* and pro-*S* H4 hydrogens in NADH

Fig. 3-2. The stereochemical relationship between (*R*)-4H and (*S*)-4H in NADH.

The reduction of acetaldehyde by 4*R*-[nicotinamide-4-^{2}H]NADH gives *R*-[1-^{2}H]ethanol (*R*-[1-^{2}H]ethanol and *S*-[1-^{2}H]ethanol can be distinguished by the signs of their specific optical rotations).

Each NADH-dependent dehydrogenase exhibits a characteristic stereospecificity with respect to whether NAD^+ accepts hydrogen into the 4-pro-*R* or 4-pro-*S* position and with respect to the dehydrogenation of its co-substrate.

Table 3-1. Pro-*R* and Pro-*S* Stereospecificities for NAD(P)H of Dehydrogenases

Enzyme	NAD(P)H Stereospecificity
Alcohol dehydrogenase	pro-*R*
Malate dehydrogenase	pro-*R*
Lactate dehydrogenase	pro-*R*
Glyceraldehyde-3-P dehydrogenase	pro-*S*
Glycerophosphate dehydrogenase	pro-*S*
Glutamate dehydrogenase	pro-*S*

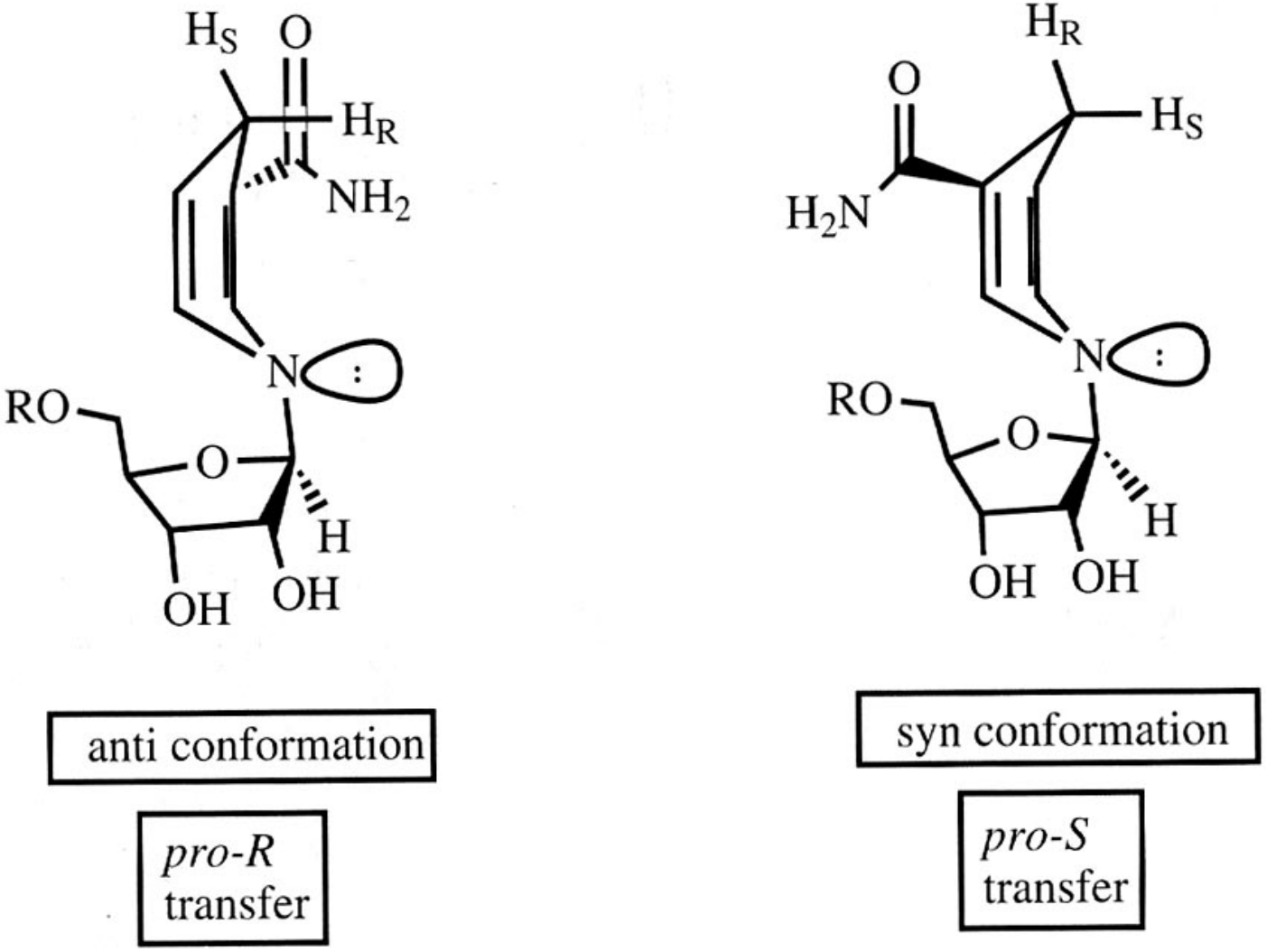


FIGURE 3.5 *Anti*- and *syn*-conformations of NADH.

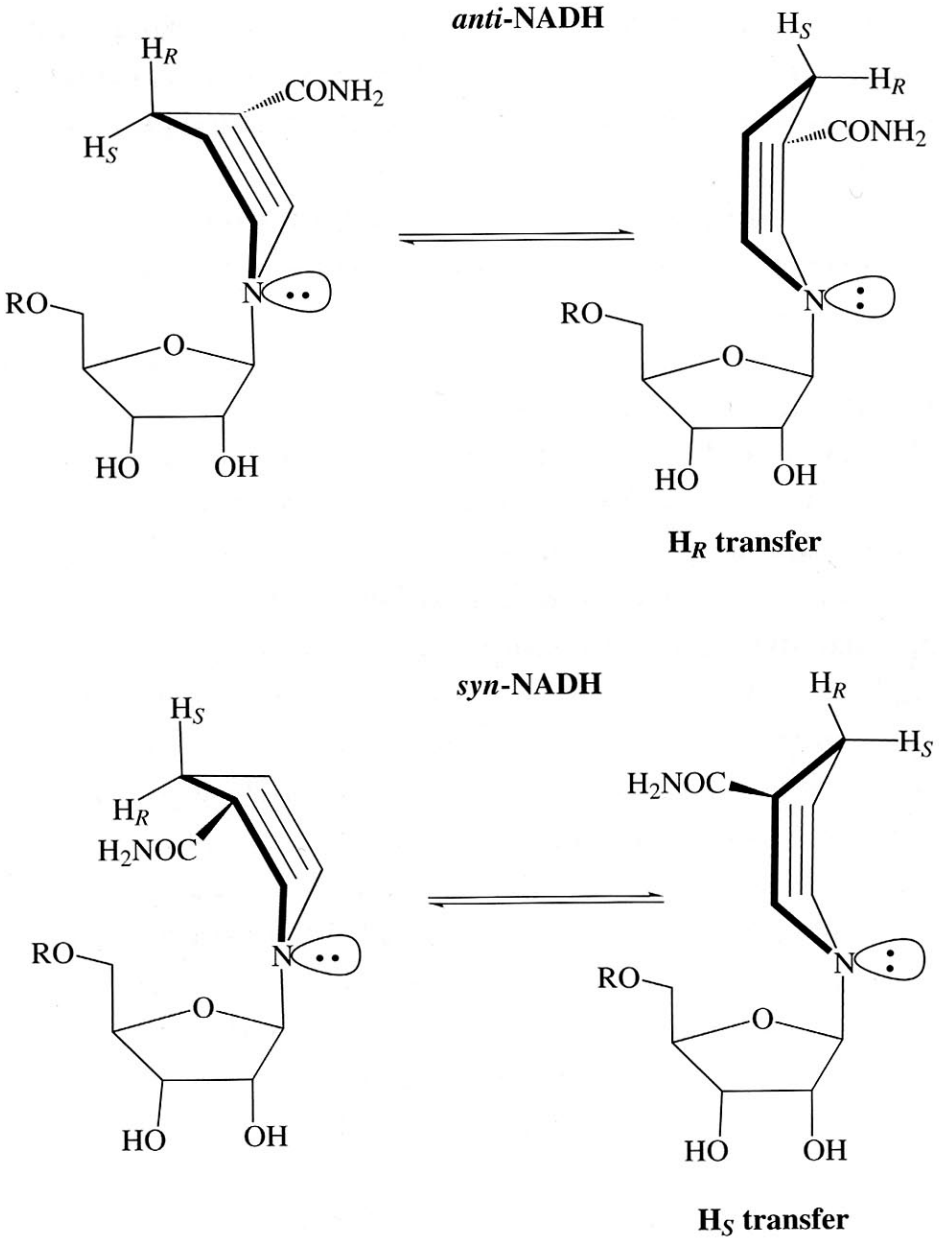


FIGURE 3.6 Boat-boat equilibria of NADH.

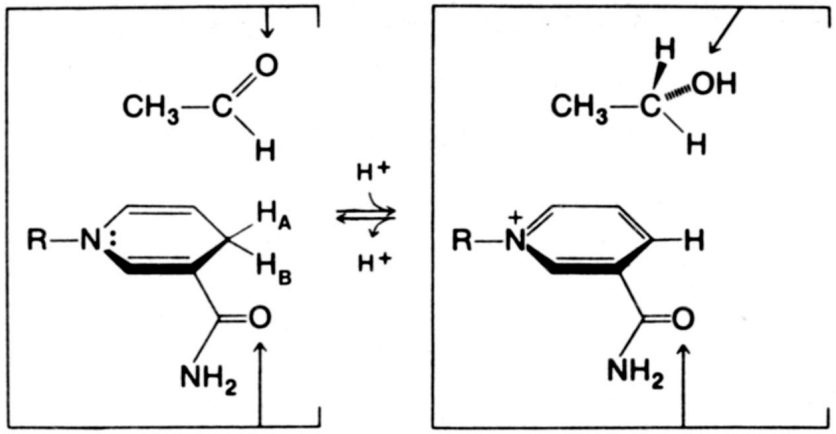
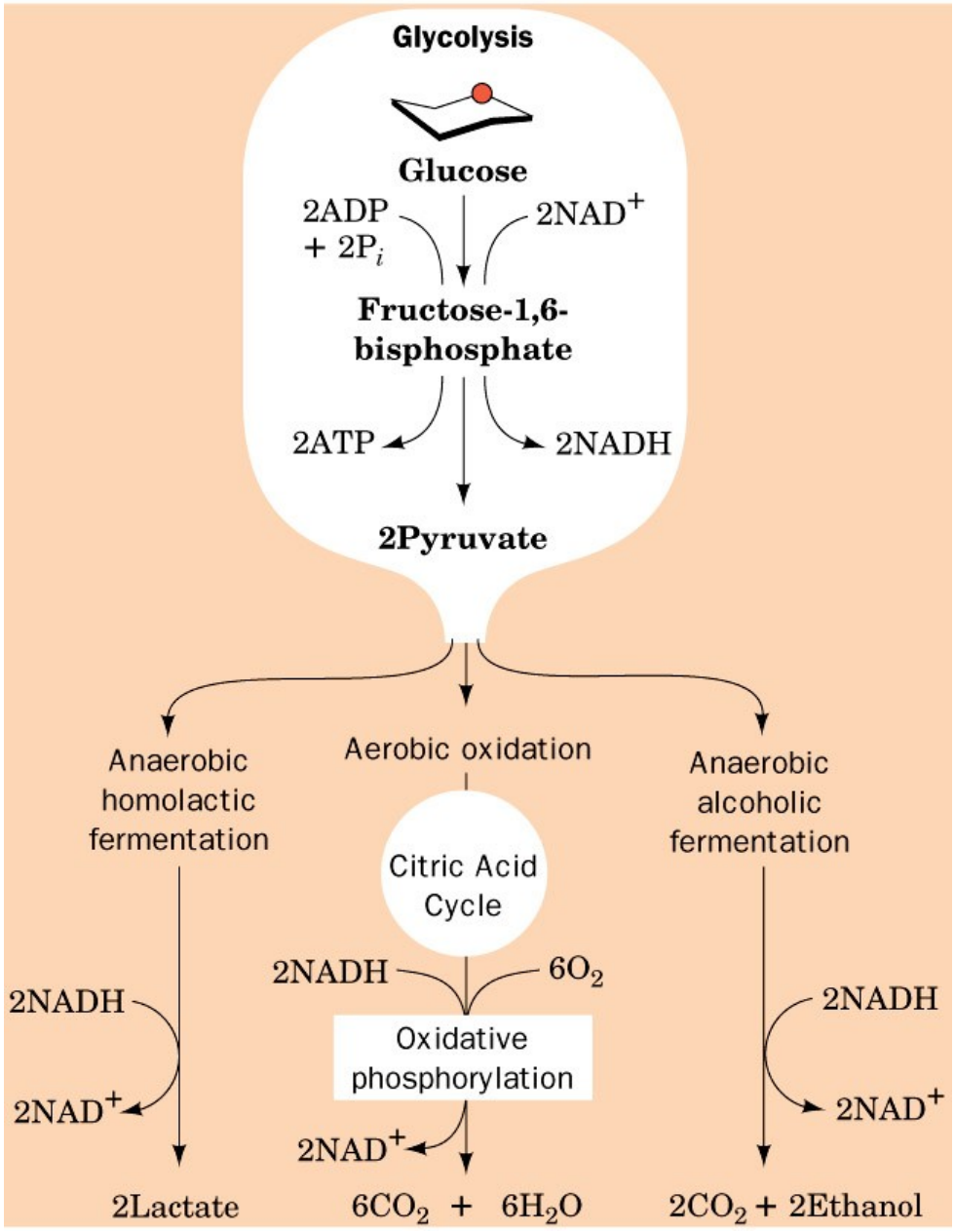


Figure 4.10 Schematic illustration of the enzyme-substrate and enzyme-product complexes for alcohol dehydrogenase with the two-carbon substrate acetaldehyde (left) or ethanol (right) above the coenzyme, NADH (left) or NAD^+ (right). This figure illustrates the stereospecific transfer of H^- from one of two possible positions at C-4 of the pyridine ring of the reduced coenzyme to a specific position in acetaldehyde, to give ethanol. If the atom transferred is deuterium, the monodeuterated ethanol produced is optically active by virtue of the new chiral center at C-1. Similarly, enzymatically produced monodeuterated NADH is optically active by virtue of the chiral center at C-4 of the pyridine ring of NADH. The subscripted stereochemical symbols A and B are explained in the text and are the same as the R and S designations, respectively. The arrows represent enzyme binding interactions. (Reproduced with permission from P. A. Frey, "Structure and Function of Coenzymes," in *Biochemistry,* G. Zubay, ed., Addison-Wesley, Reading, MA, 1983.)

Another view of the stereochemical additions that occur at C4 of the nicotinamide ring of NAD^+ and at C1 of acetaldehyde in the ADH active site



L-Lactate dehydrogenase (LDH) EC 1.1.1.24

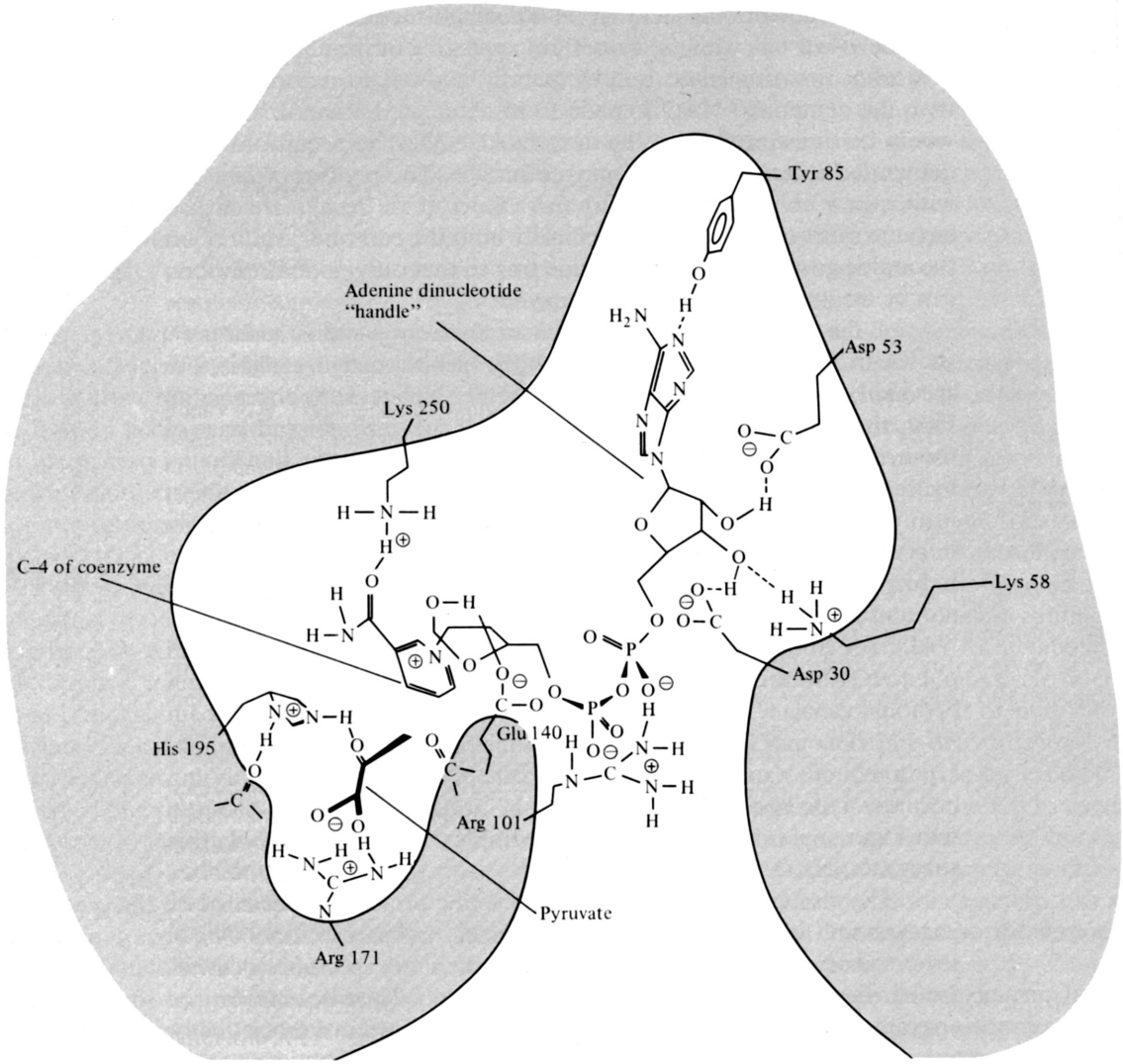


Figure 4.11 Schematic depiction of the ternary "abortive" complex between L-lactate dehydrogenase, pyruvate (oxidized lactate), and NAD^+. The interactions of pyruvate and NAD^+ with some of the amino acyl residues in the protein involved in substrate binding and/or catalysis are shown. (Adapted from J. J. Holbrook, A. Liljas, S. J. Steindel, and M. G. Rossmann, in *The Enzymes,* P. D. Boyer, ed., Third Edition, vol. 11, p. 240, Academic, New York, 1975.)

The lactate dehydrogenase active site; reduction of pyruvate to L-lactate with NADH as a cofactor

Generalized examples of bond cleavage potentiated by NAD⁺-dependent substrate oxidation: C-H and C-C bond breaking

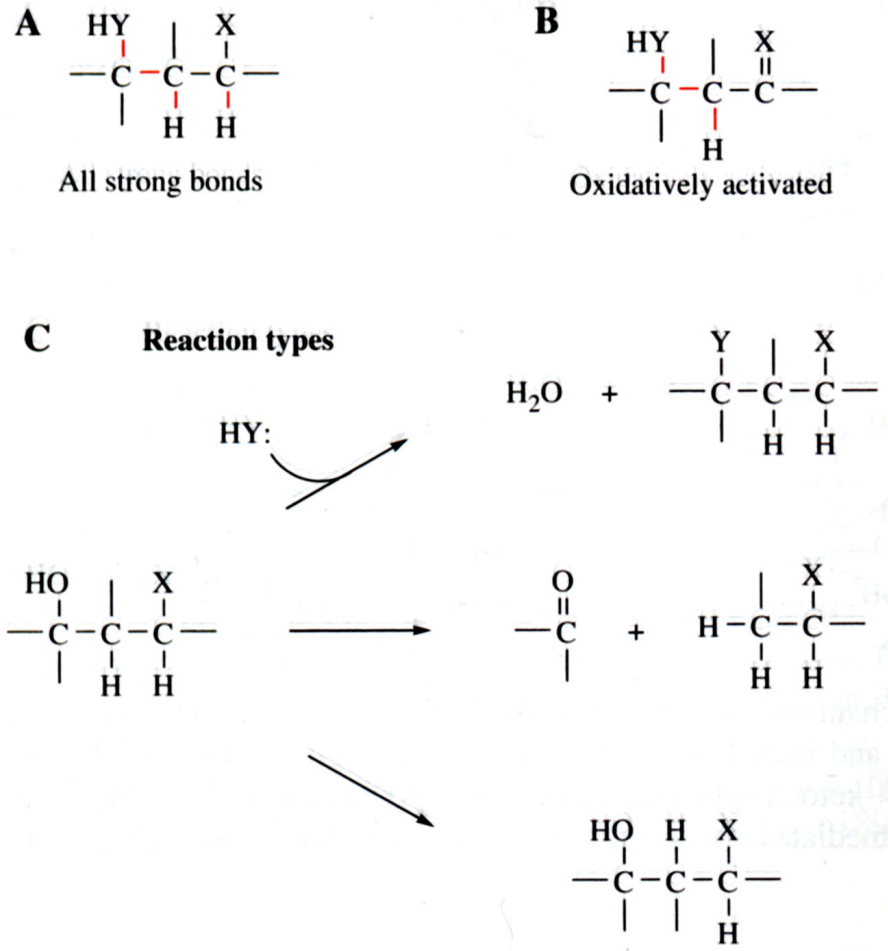
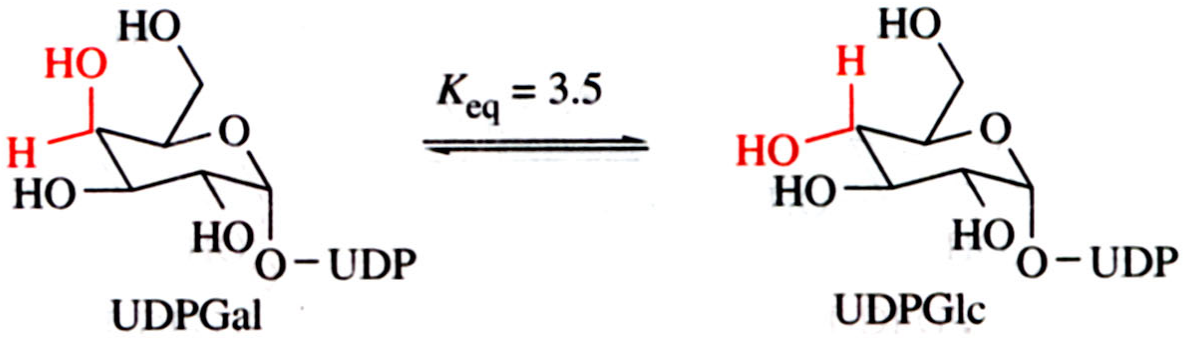


Fig. 3-3. Bond cleavages potentiated by NAD⁺-dependent reversible oxidation of a substrate. (A) The bonds highlighted in red are subject to cleavage after oxidative activation as shown in (B). (C) Several different reaction types may be enhanced by oxidative activation.

UDP-Galactose 4-epimerase (EC 5.1.3.2)

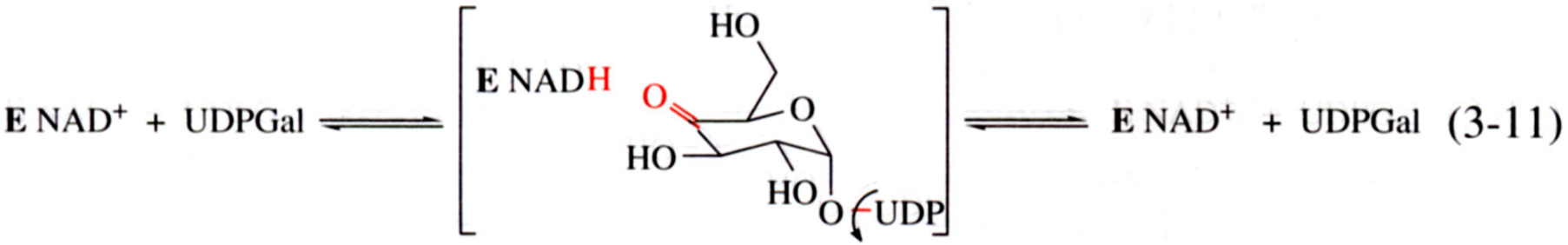


(3-10)

The NAD^+/NADH coenzyme is tightly bound by the enzyme (prosthetic group).

UDP-Galactose 4-epimerase

Rotation about the O-UDP bond allows either face of the C4 carbonyl to accept a hydride from NADH.



UDP-4-ketoglucopyranose intermediate

The enzyme displays stereospecific NAD^+ reduction (pro-*S* face) but is non-specific with respect to substrate (accepts either UDP-Gal or UDP-Glc).