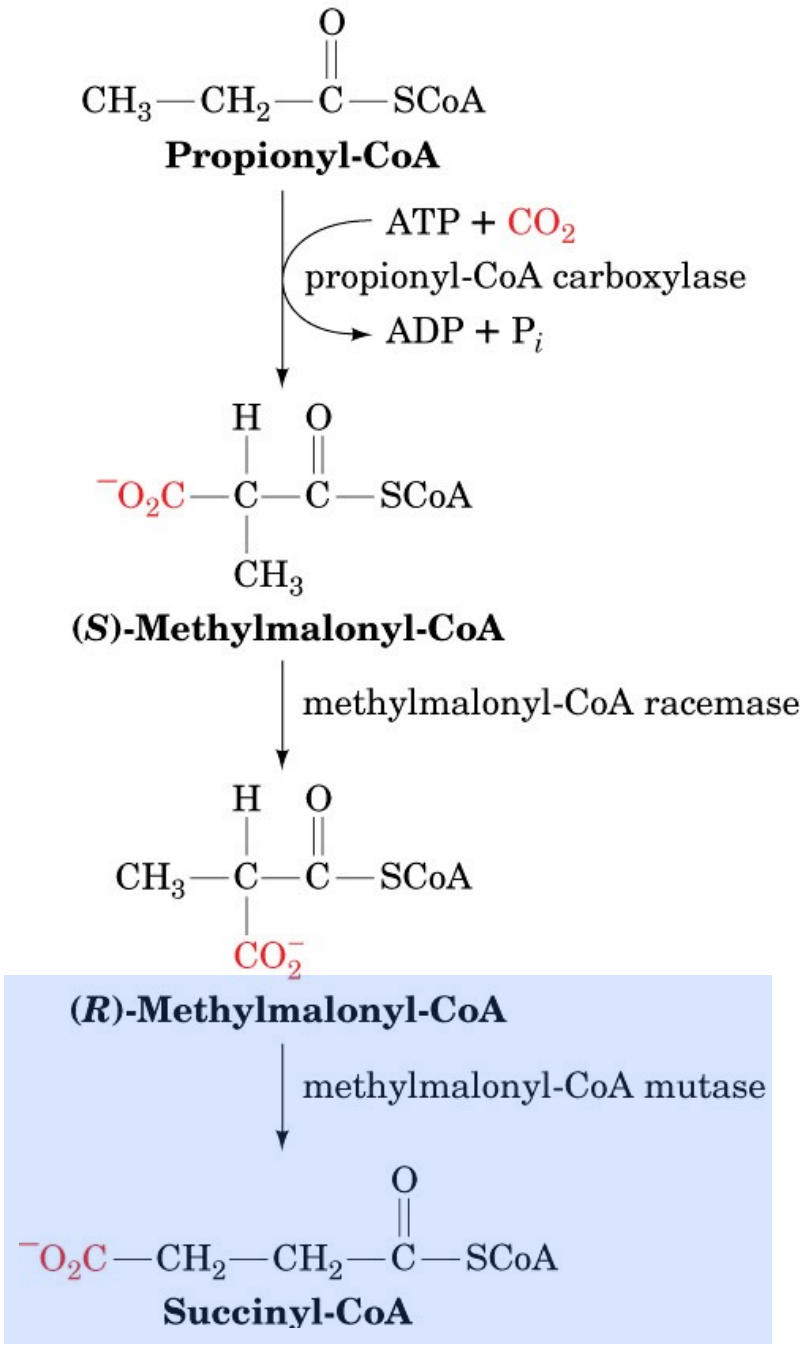
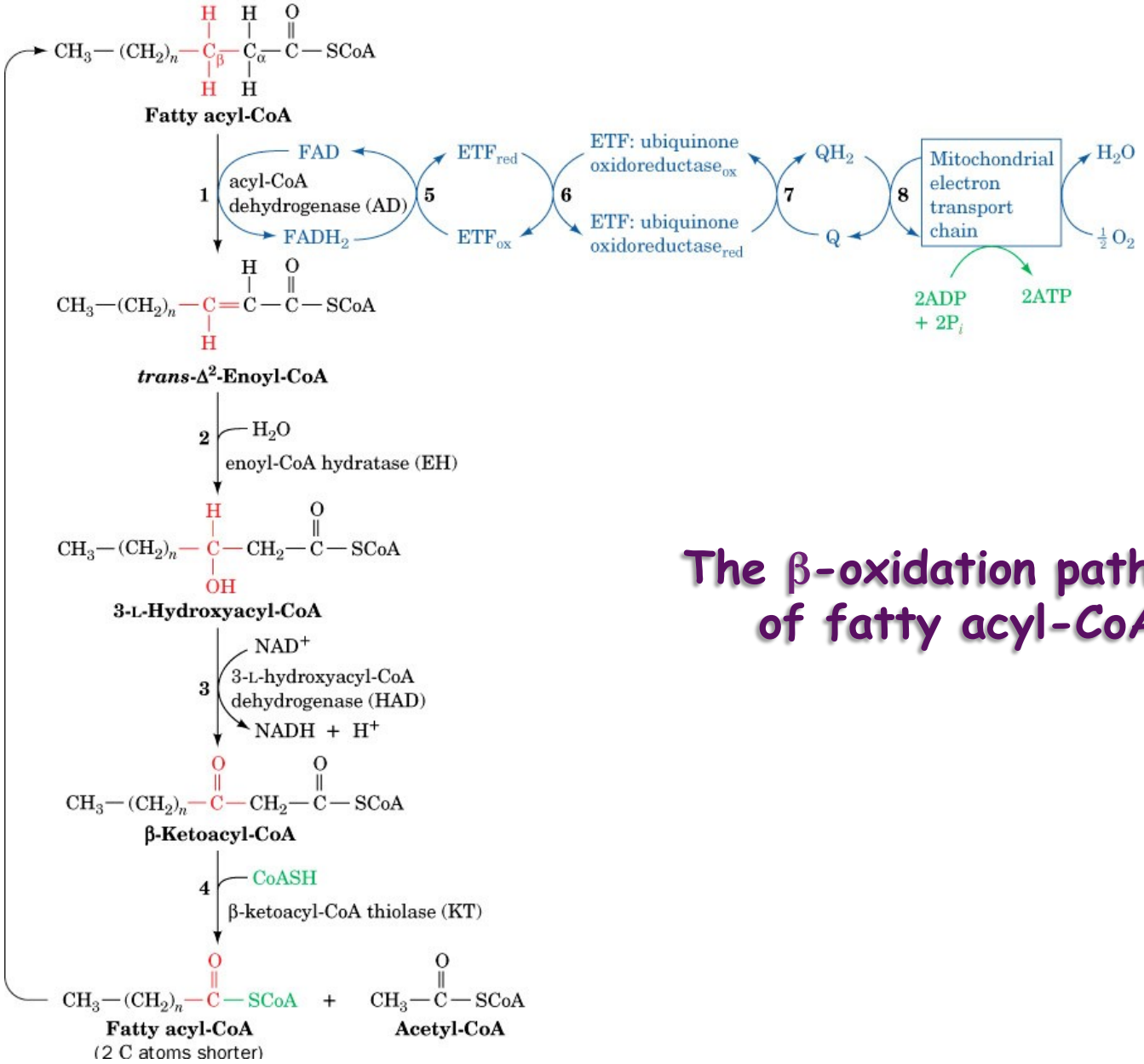
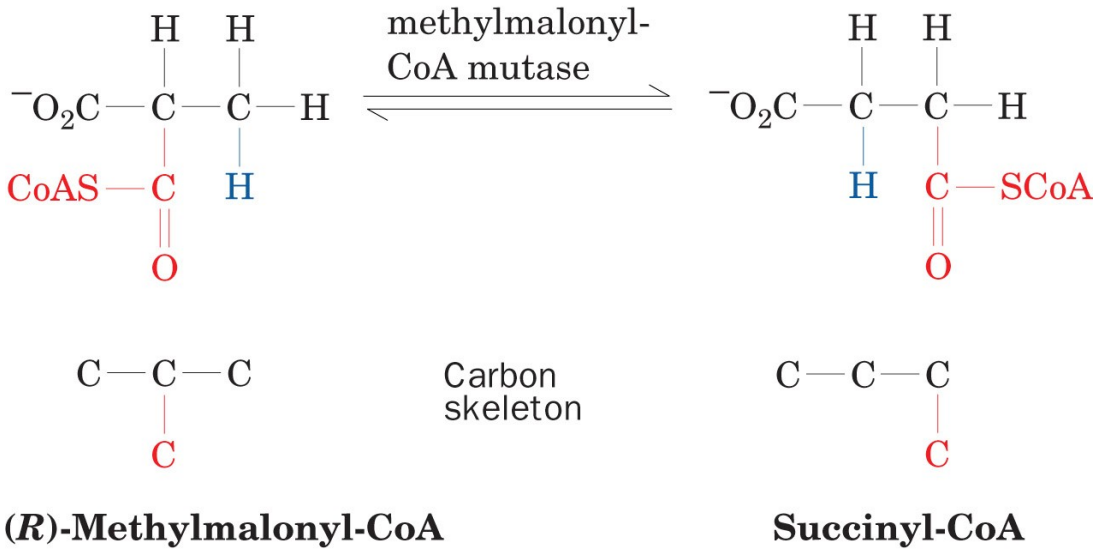


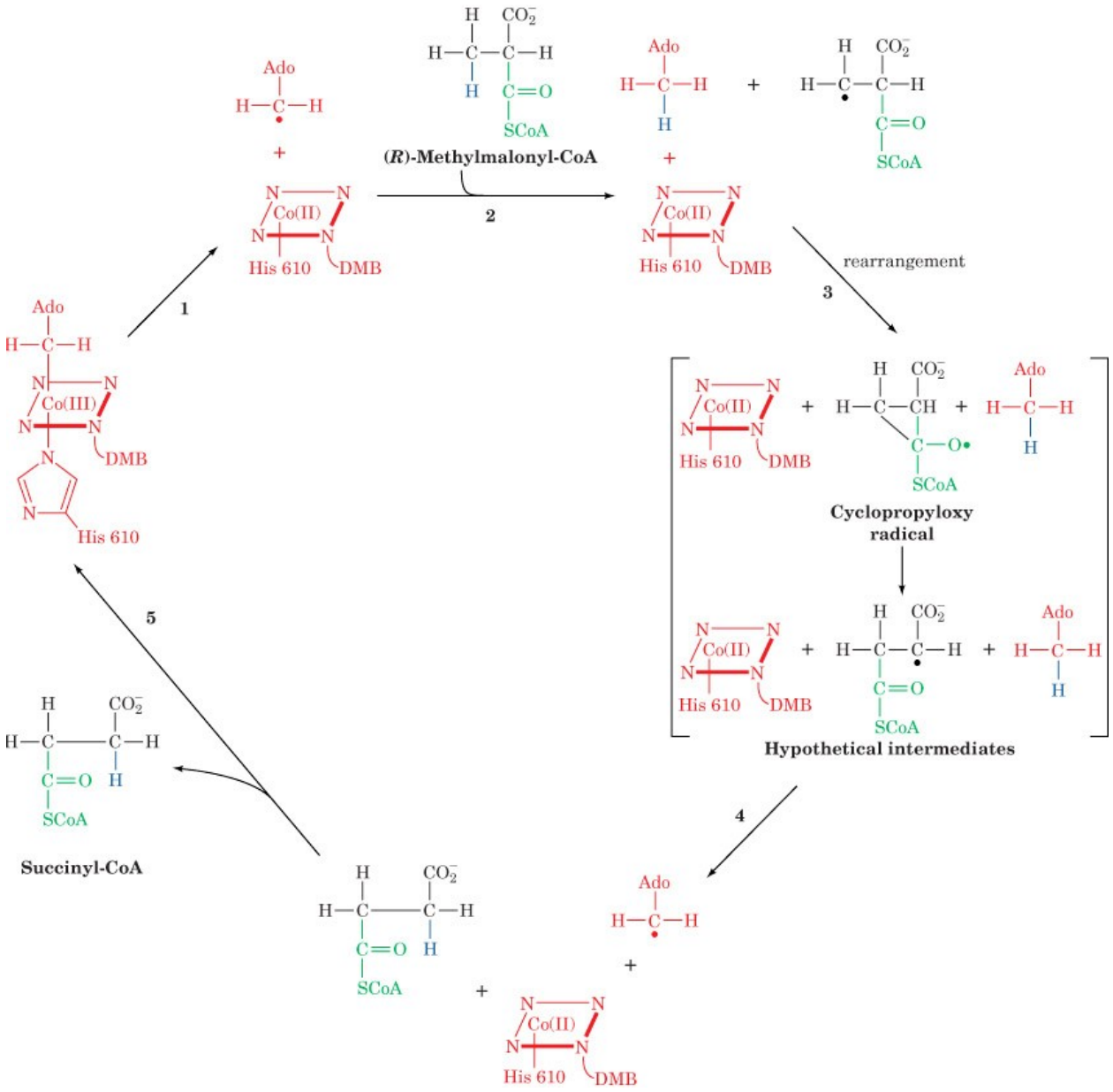
Methylmalonyl-CoA mutase:
Conversion of (*R*)-methylmalonyl-CoA to succinyl-CoA (degradation of odd-carbon fatty acids)



The β-oxidation pathway of fatty acyl-CoA

The reaction catalyzed by methylmalonyl-CoA mutase: requires coenzyme B_{12}

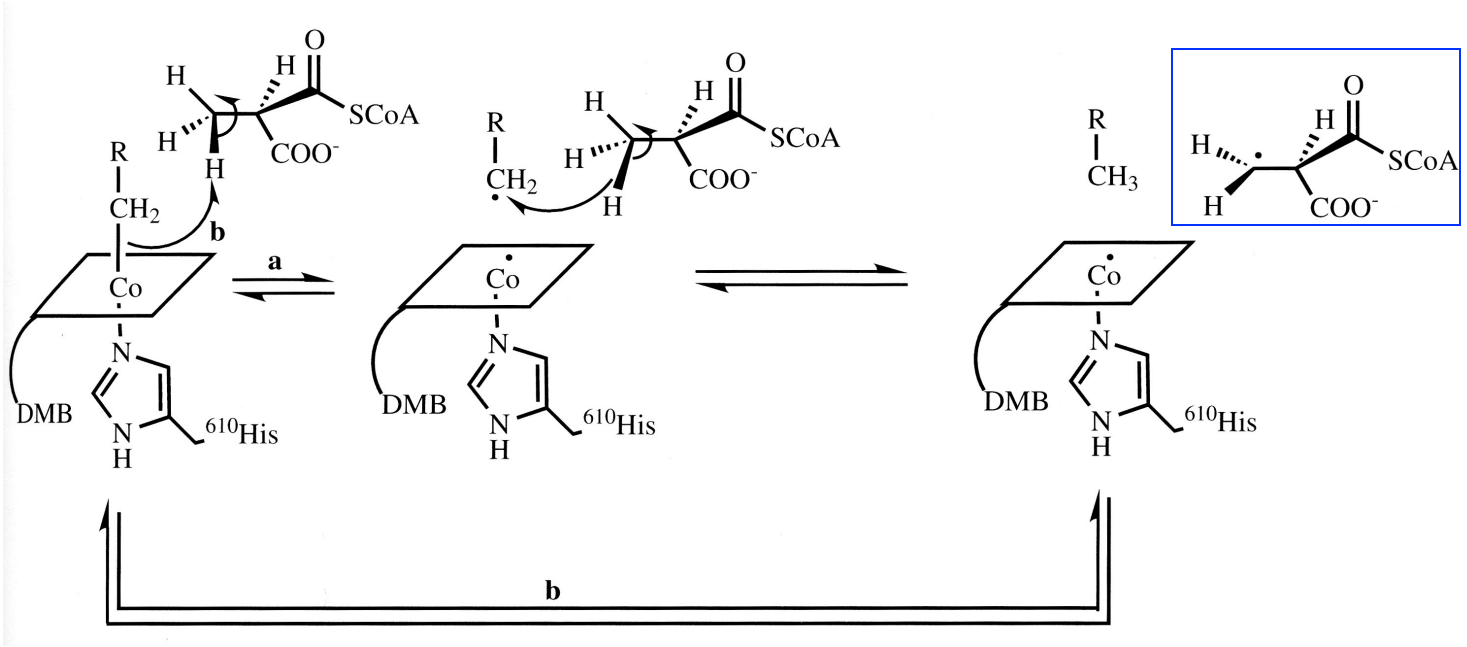


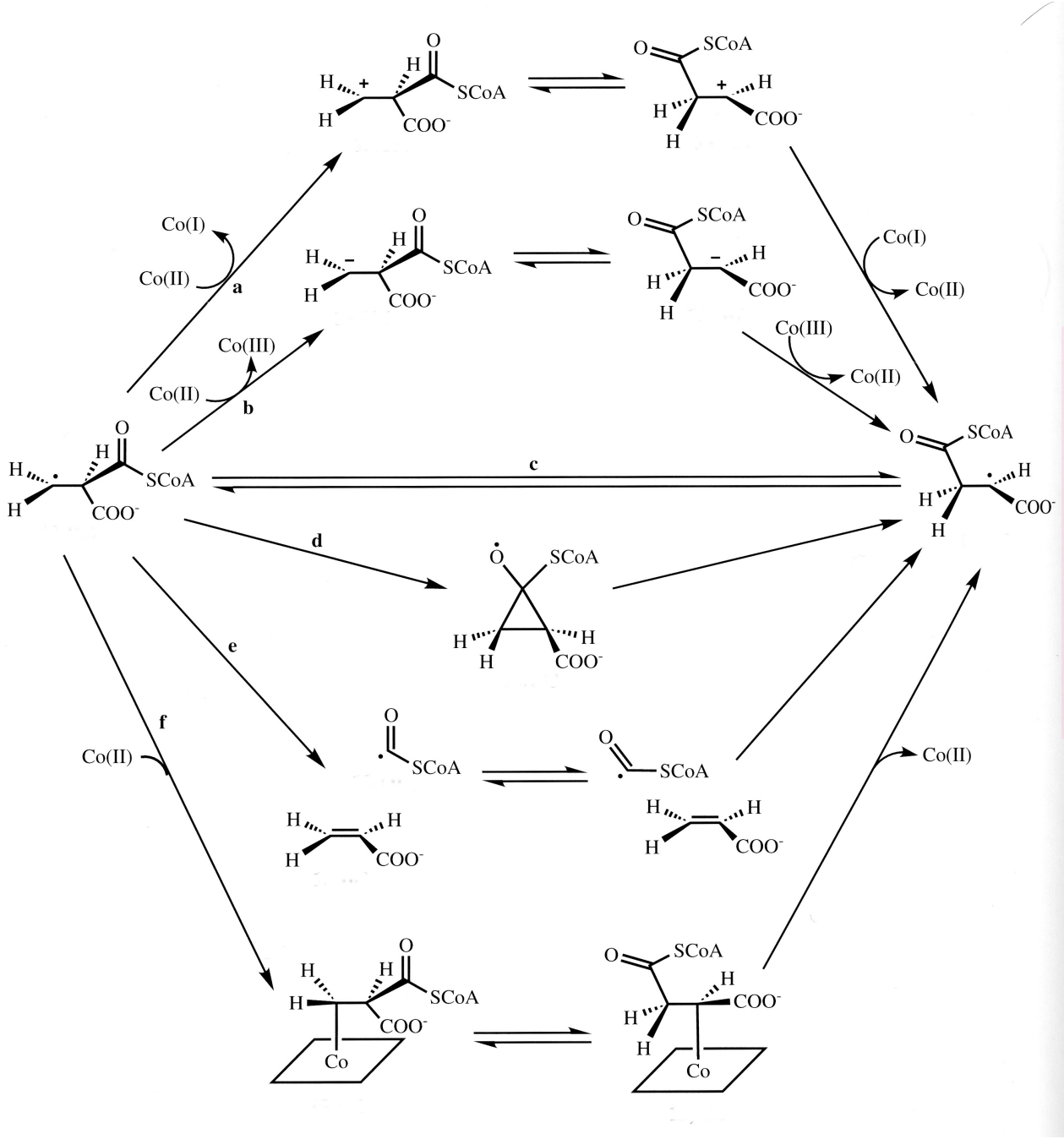


Proposed mechanism of methylmalonyl-CoA mutase

Co^{+3} is the "resting" oxidation state of the B_{12} coenzyme; during catalysis, Co^{+3} is converted to the Co^{+2} oxidation state.

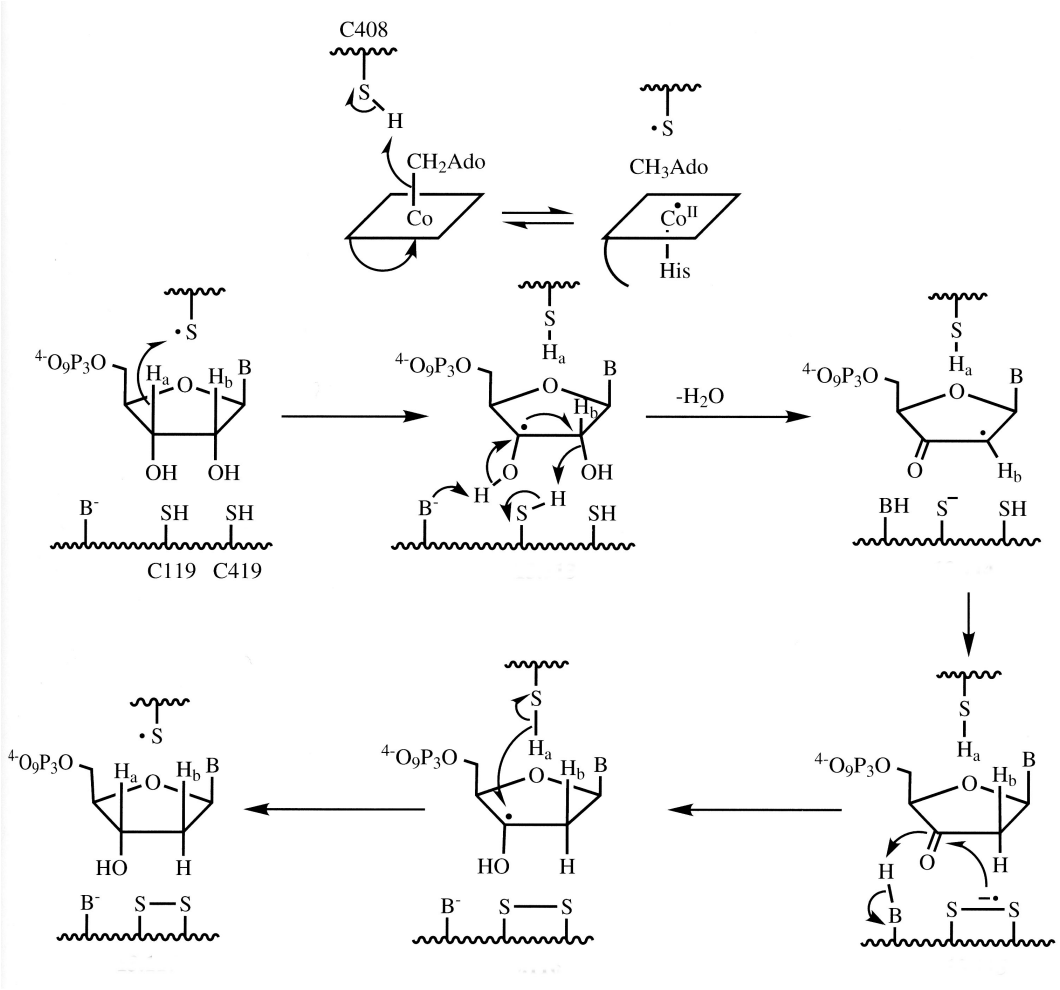
Stepwise (a) versus concerted (b) mechanisms for the methylmalonyl-CoA mutase-catalyzed generation of 5'-deoxyadenosine, cob(II)alamin, and substrate radical



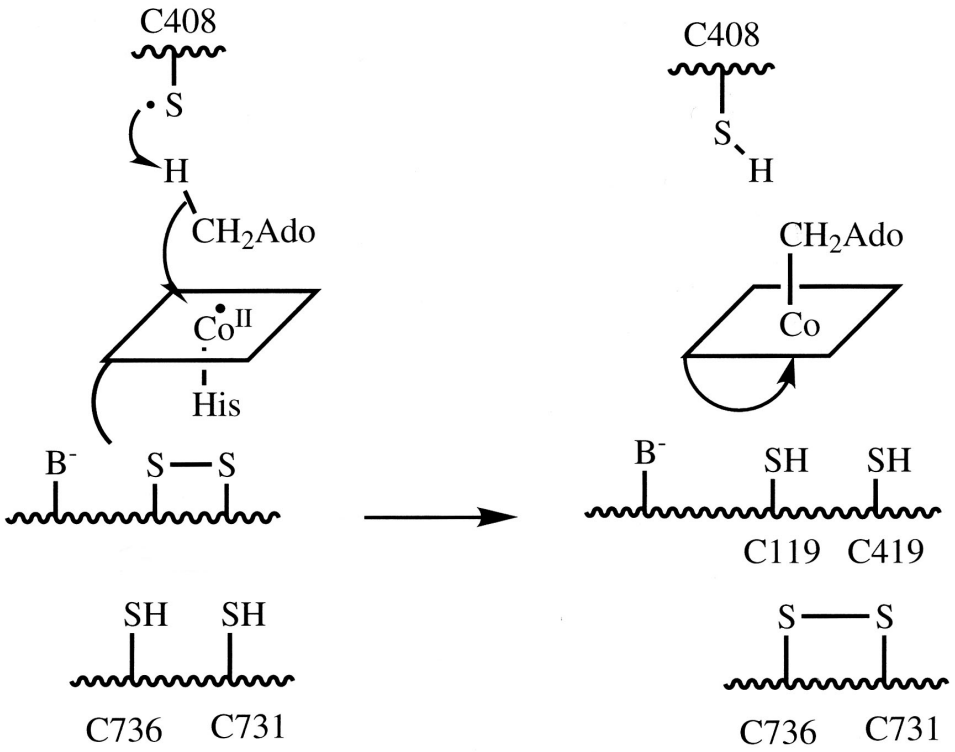


Six possible mechanisms for the conversion of methylmalonyl-CoA radical to succinyl-CoA radical catalyzed by methylmalonyl-CoA mutase

Mechanism proposed for coenzyme B_{12}-dependent ribonucleotide reductase



Mechanism proposed for reducing and reestablishing the active site of coenzyme B_{12}-dependent ribonucleotide reductase

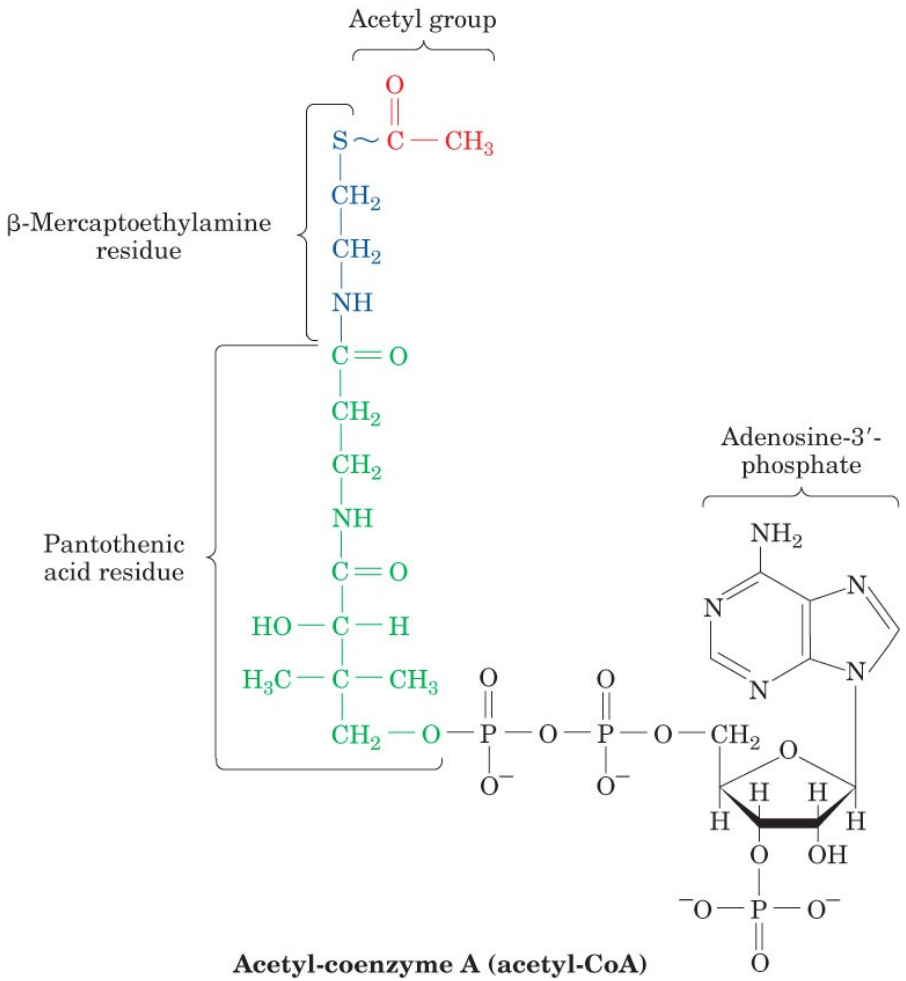


Acetyl coenzyme A

(a biologically-activated acetyl group;
a thioester that serves more as a substrate
than as a coenzyme)

Chemical structure of acetyl-CoA

A pantothenic acid-containing coenzyme



Acetyl-coenzyme A (acetyl-CoA)

Phosphopantothenic acid coenzymes

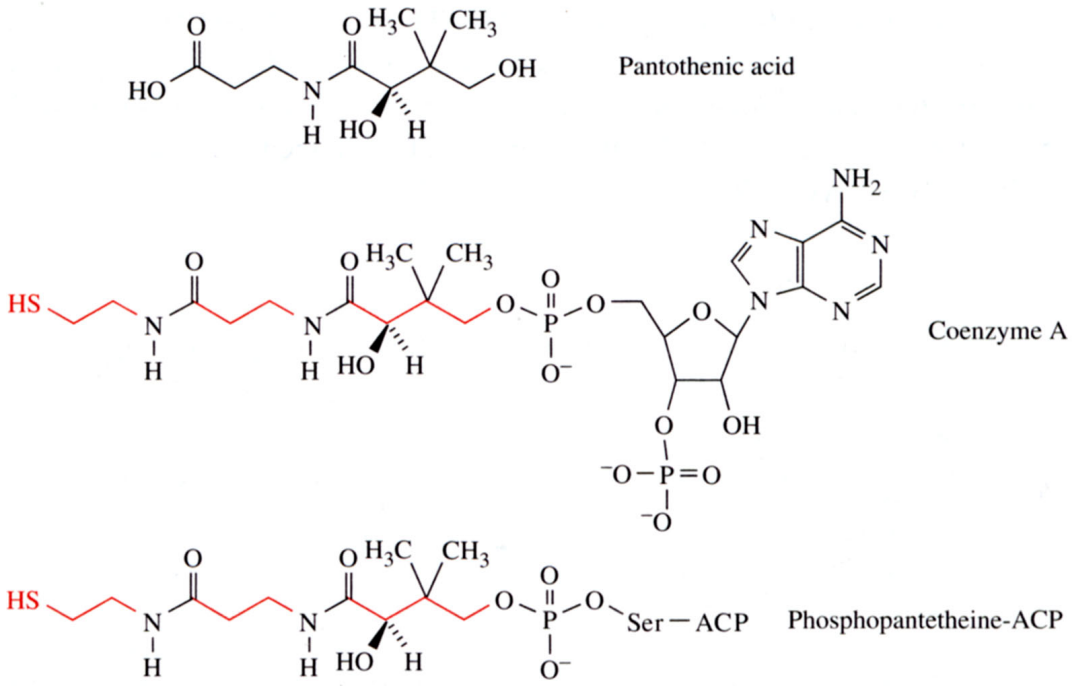
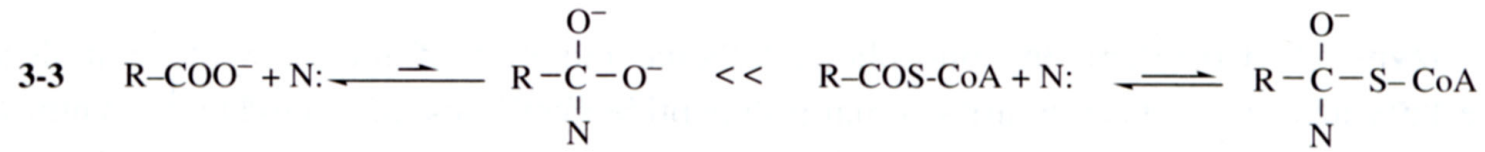


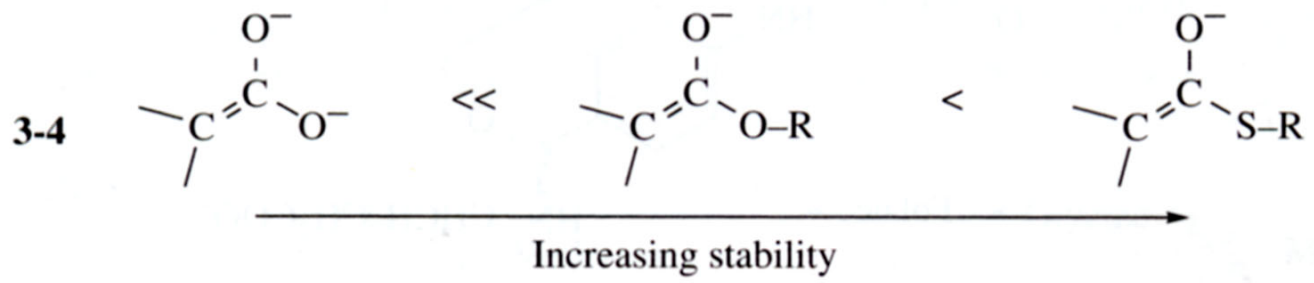
Fig. 3-32. Structures of pantothenic acid and phosphopantetheine coenzymes. The structures of coenzyme A (CoA) and phosphopantetheine prosthetic groups of proteins are based on the vitamin pantothenic acid. The —SH group covalently binds acyl groups and chemically activates them. The phosphopantetheine moiety may be tightly bound and immobilized through protein interactions, especially in reactions of CoA, or it may be relatively freely mobile in the reactions of acyl carrier protein (ACP). The bonds that allow rotation are highlighted.

Biological activation of a carboxyl group



During nucleophilic attack on a carboxylic acid, (-) charge accumulates in the tetrahedral intermediate, and breakdown of the latter involves the loss of an oxide^{-2} anion (not favorable). In thioester substrates, (-) charge accumulation is reduced, and a good leaving group is present. Thioesters are more ketone-like (and thus more electrophilic) than oxyesters due to less overlap and less delocalization of non-bonding electrons from sulfur onto the carbonyl oxygen than in the oxyester.

Enolization of carboxylic acids, oxyesters and thioesters



The rate of base-catalyzed enolization is fastest for the species that contains the most electrophilic carbonyl and produces the most stable enolate ion.

Acetyl CoA plays a key role in C-C bond formation *in vivo*

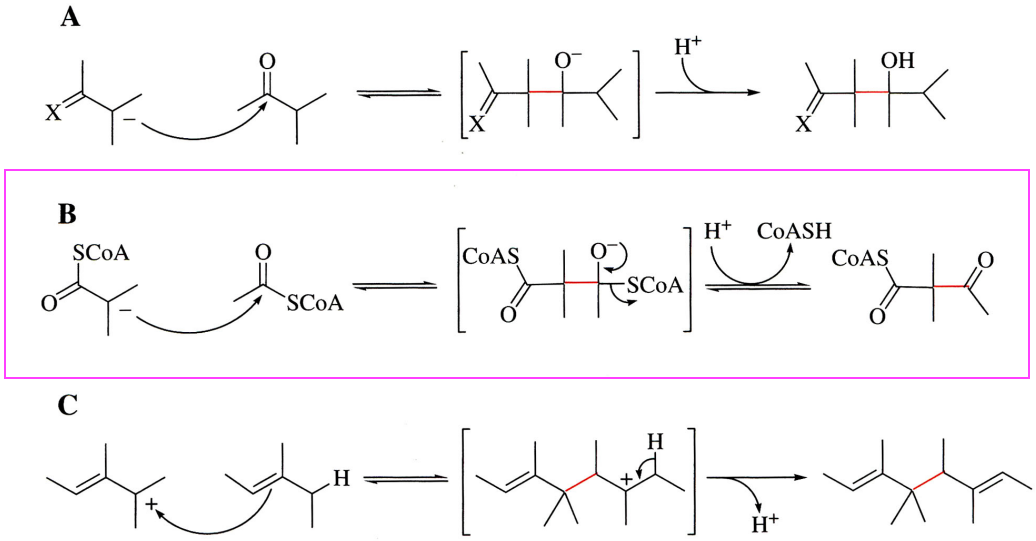
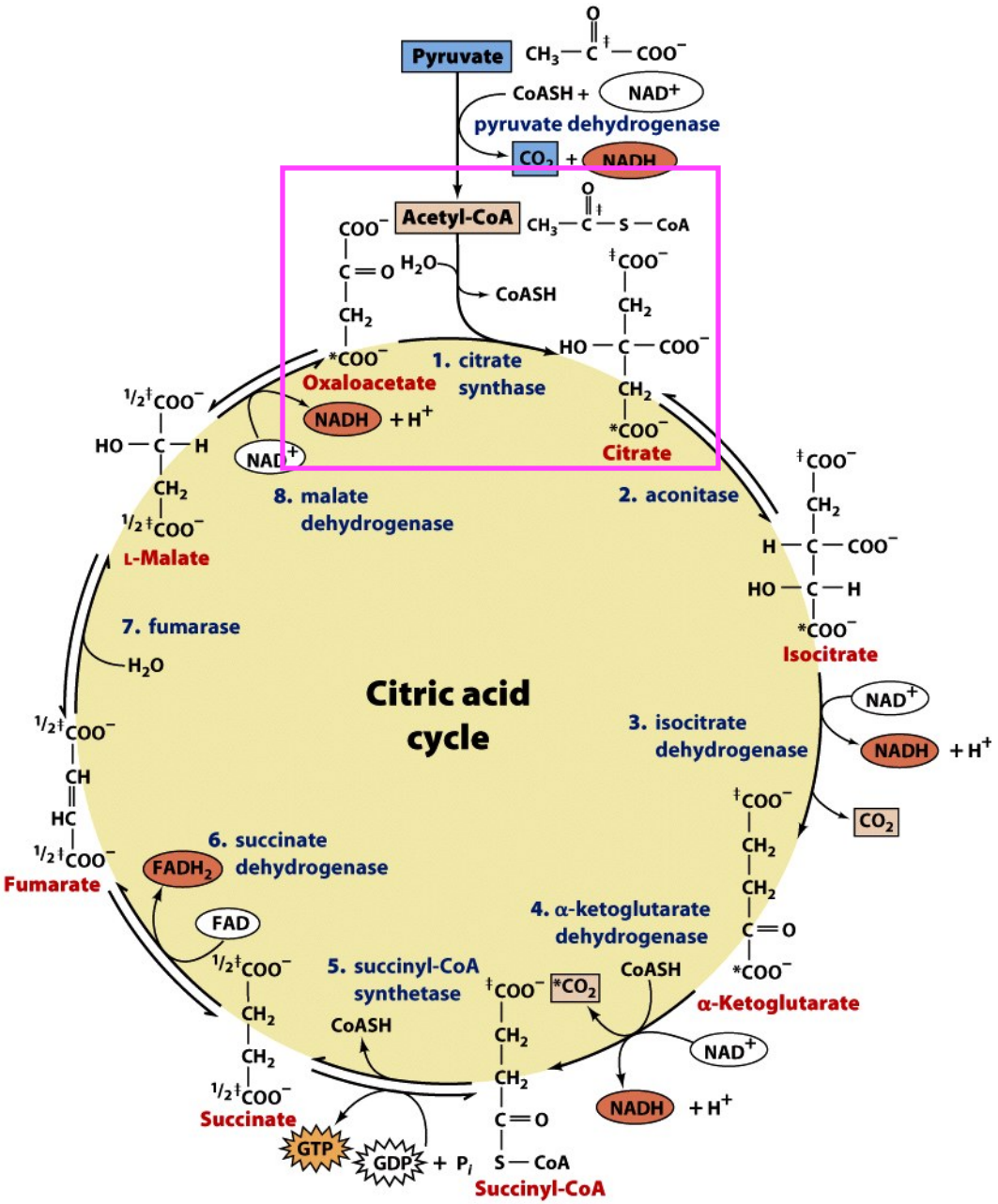


Fig. 14-1. Chemical patterns in biological carbon-carbon bond formation. (A) In many enzymatic reactions linking two molecules with new carbon-carbon bonds, a carbanion is added to the carbonyl group of an aldehyde or ketone in a two-step process through a tetrahedral intermediate, which is quenched by protonation. The requisite carbanions are stabilized by the group C=X, where X can be the carbonyl oxygen of an aldehyde or ketone in an aldolase reaction, the acyl-carbonyl group of a CoA-ester in the action of citrate synthase, the thiazole ring of thiamine pyrophosphate (TPP) in the action of transketolase, or the pyridoxamine ring in the reaction of serine hydroxymethyltransferase. (B) In reactions such as that of β-ketothiolase, a carbanionic CoA ester can add to another CoA ester and then eliminate CoASH to form a β-ketothioester. (C) In many reactions of terpene biosynthesis, allylic carbenium ions form carbon bonds by addition to carbon-carbon double bonds, and elimination of protons from the resulting carbenium ion intermediates stabilizes the new carbon-carbon bonds.



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Citrate synthase: An ordered bi-bi reaction involving a ternary complex (sequential, single displacement)

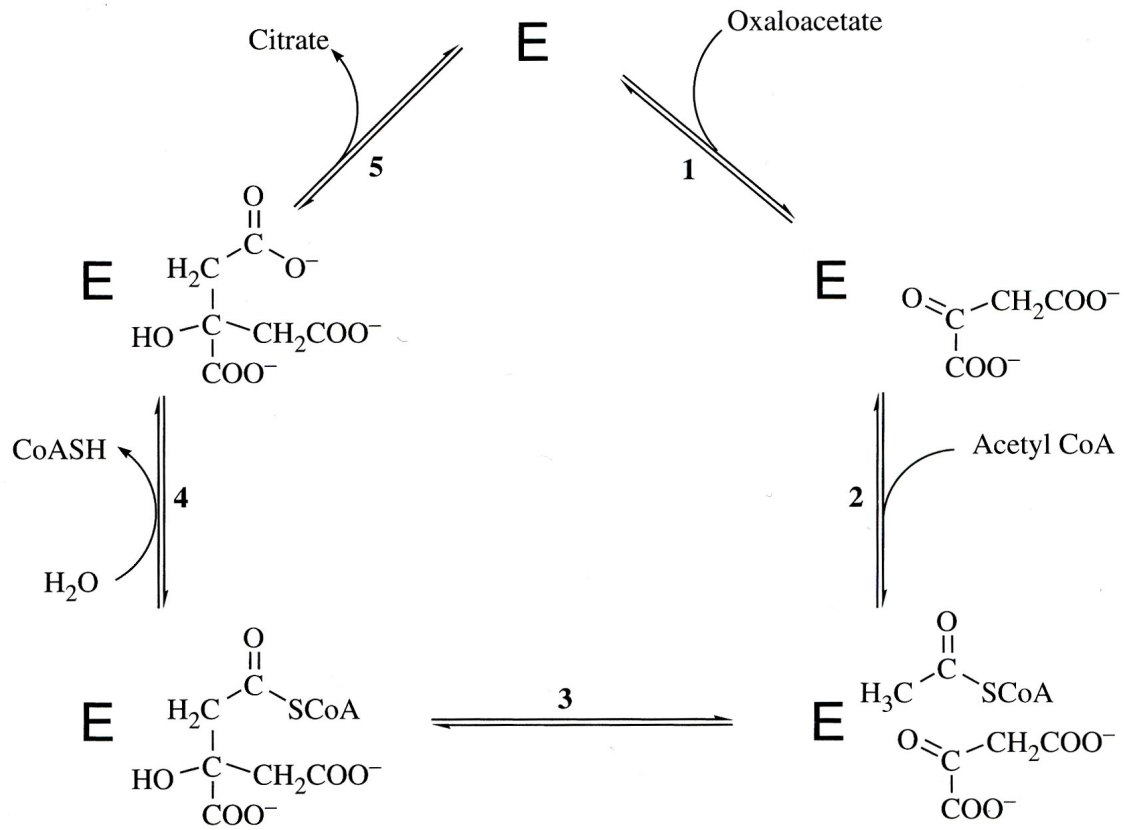
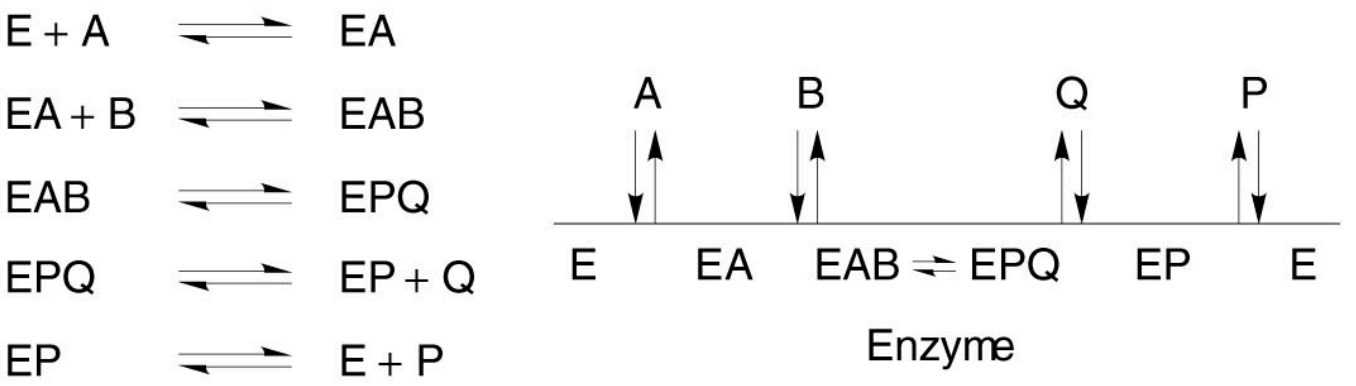
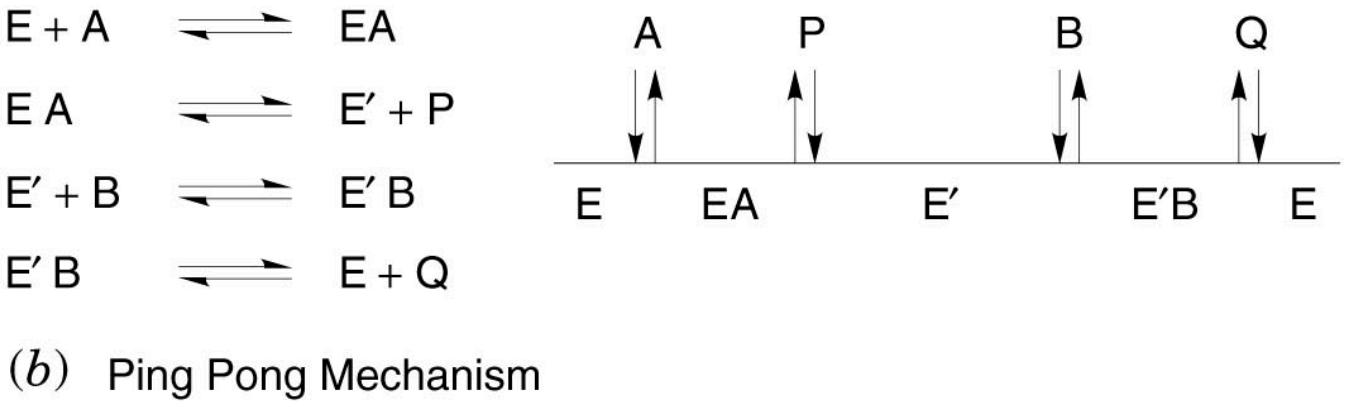


Fig. 14-4. The mechanism for the overall action of citrate synthase shows the condensation of acetyl CoA with oxaloacetate in five stages: binding of oxaloacetate, binding of acetyl CoA, reaction of the ternary complex to form citryl CoA, hydrolysis of citryl CoA to release CoASH, and dissociation of citrate.

Cleland notation



(a) Sequential Mechanism



(b) Ping Pong Mechanism

Figure 10.53. Mechanisms of interaction for two substrate reactions.

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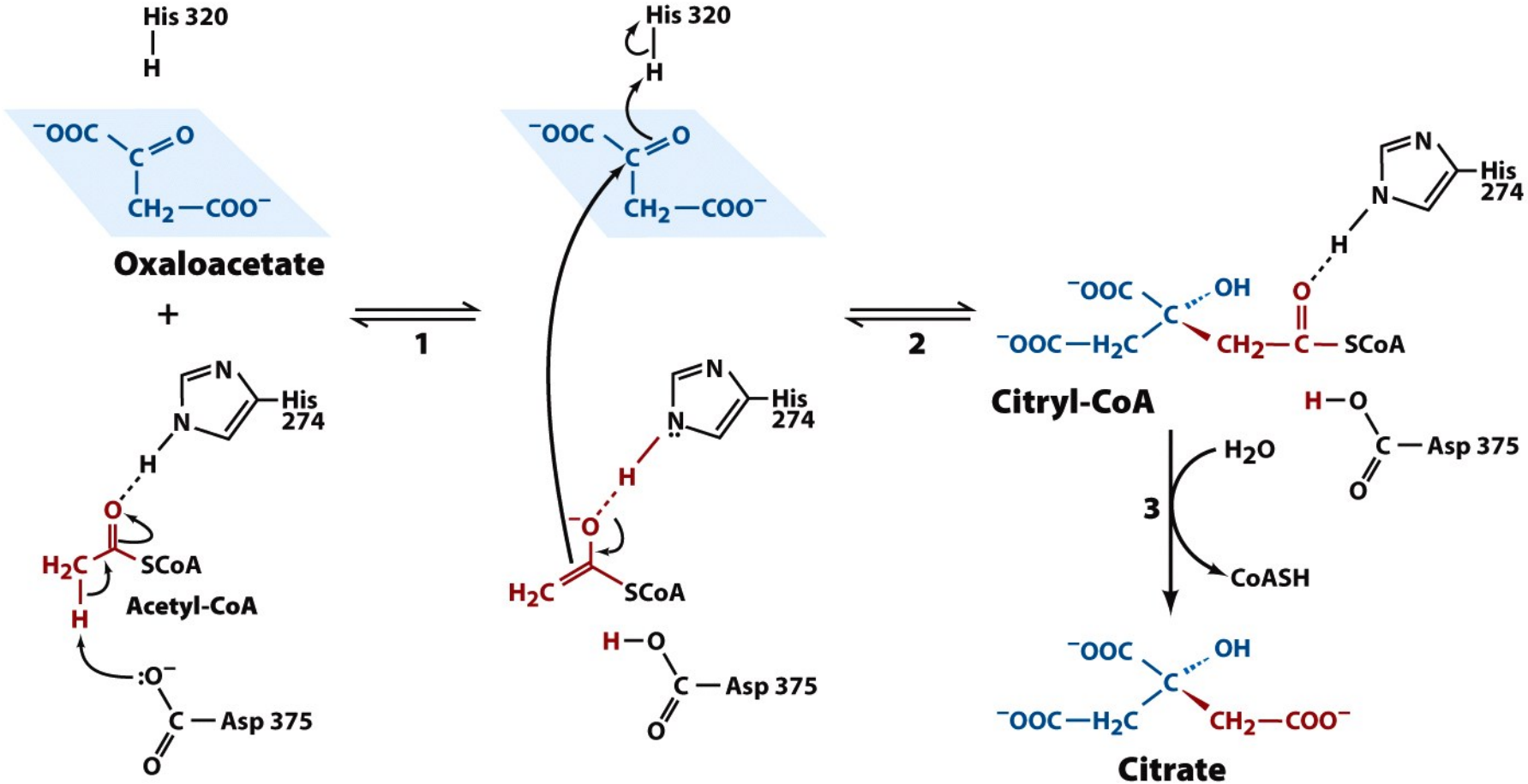
The mechanism of citrate synthase



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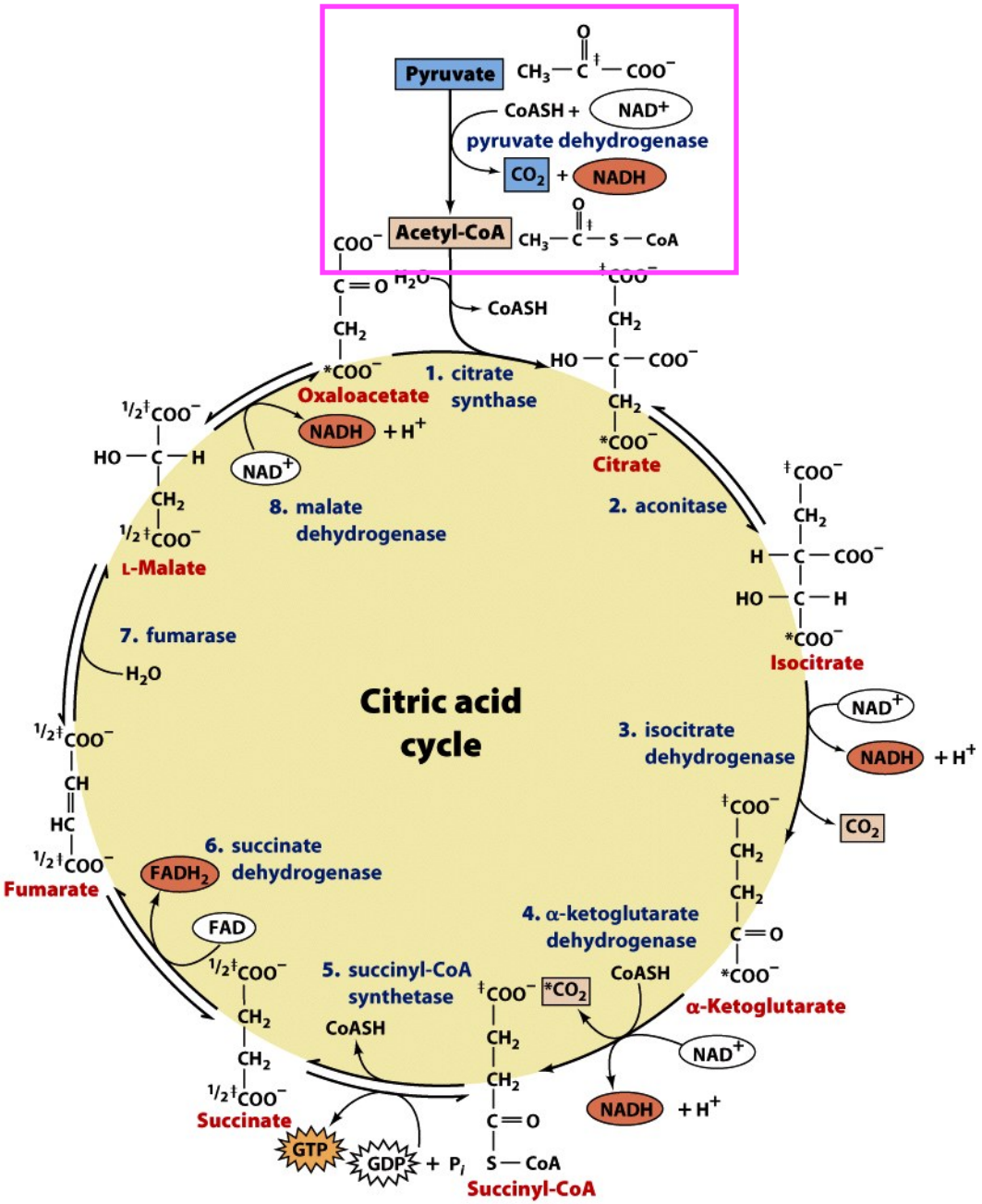

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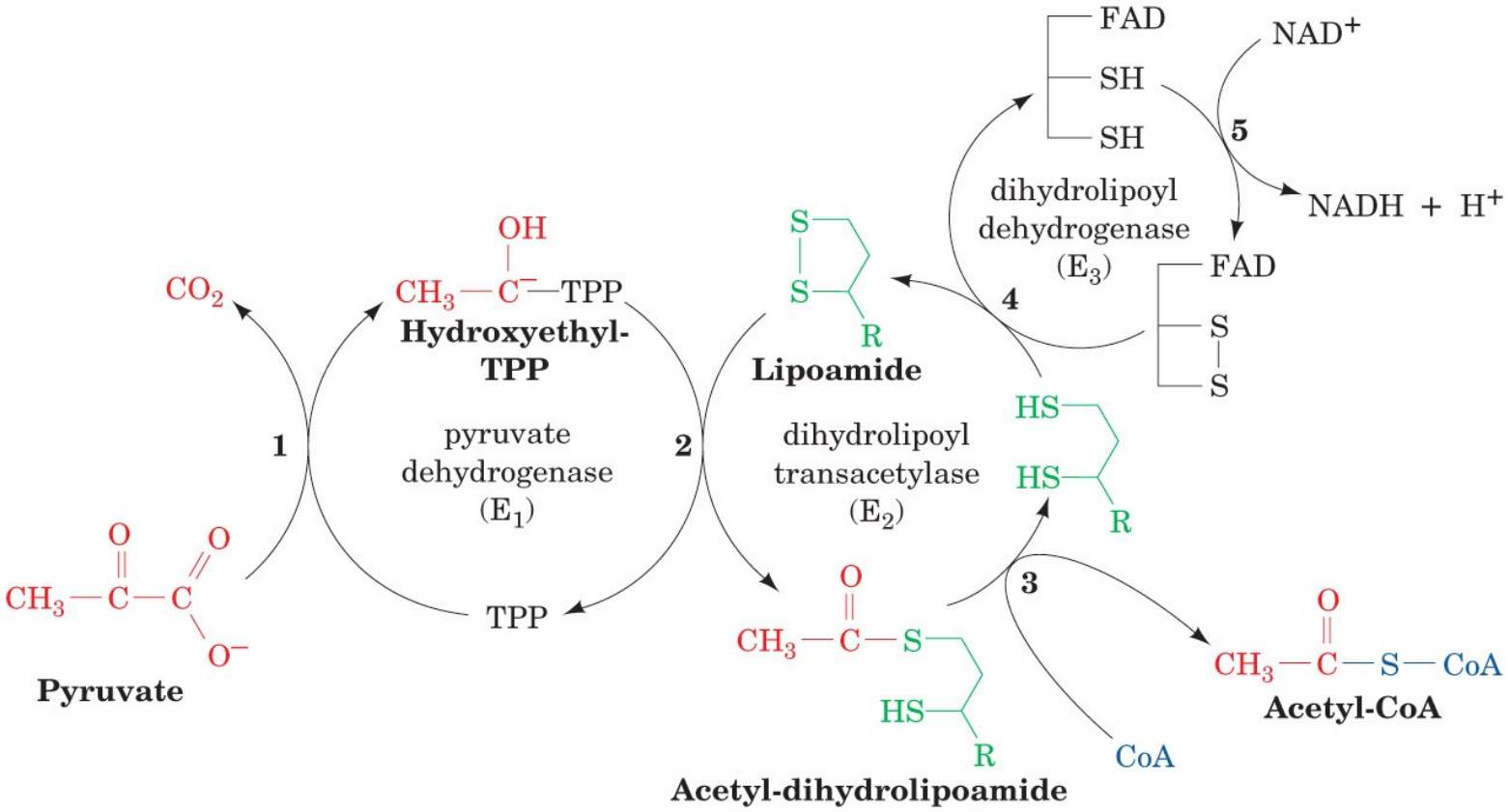
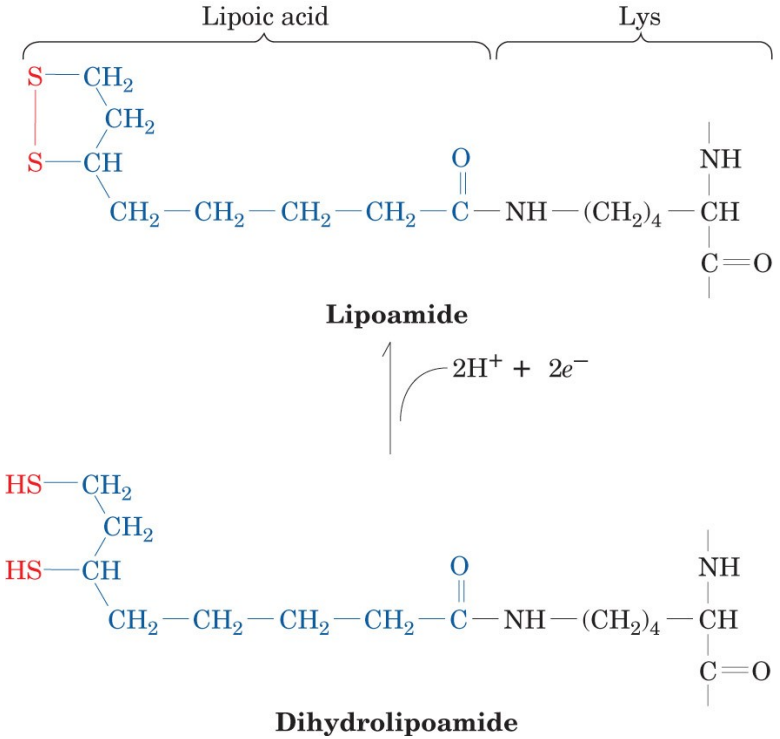
The five reactions of the pyruvate dehydrogenase complex (PDC)

(a multi-enzyme complex; involves five coenzymes and three enzymes)



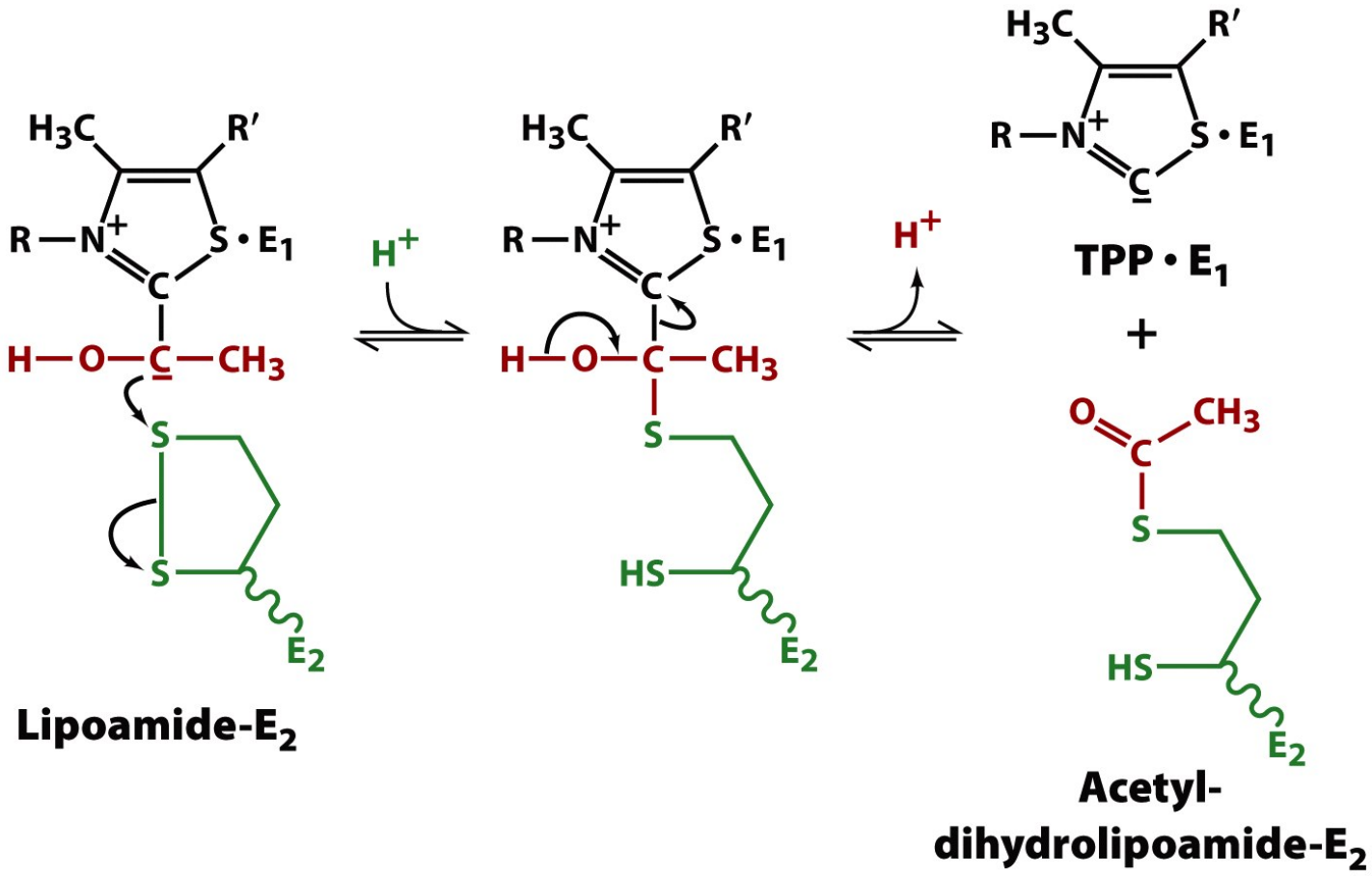
Lipoic acid (acyl group carrier)

Interconversion of lipoamide and dihydrolipoamide



Linked to ε-amino groups of Lys in lipoyl-bearing domains of the dihydrolipoyl acyltransferase components of α-ketoacid dehydrogenase complexes

Couples electron transfer and acyl group transfer reactions in α-ketoacid dehydrogenase multienzyme complexes; are conformationally flexible



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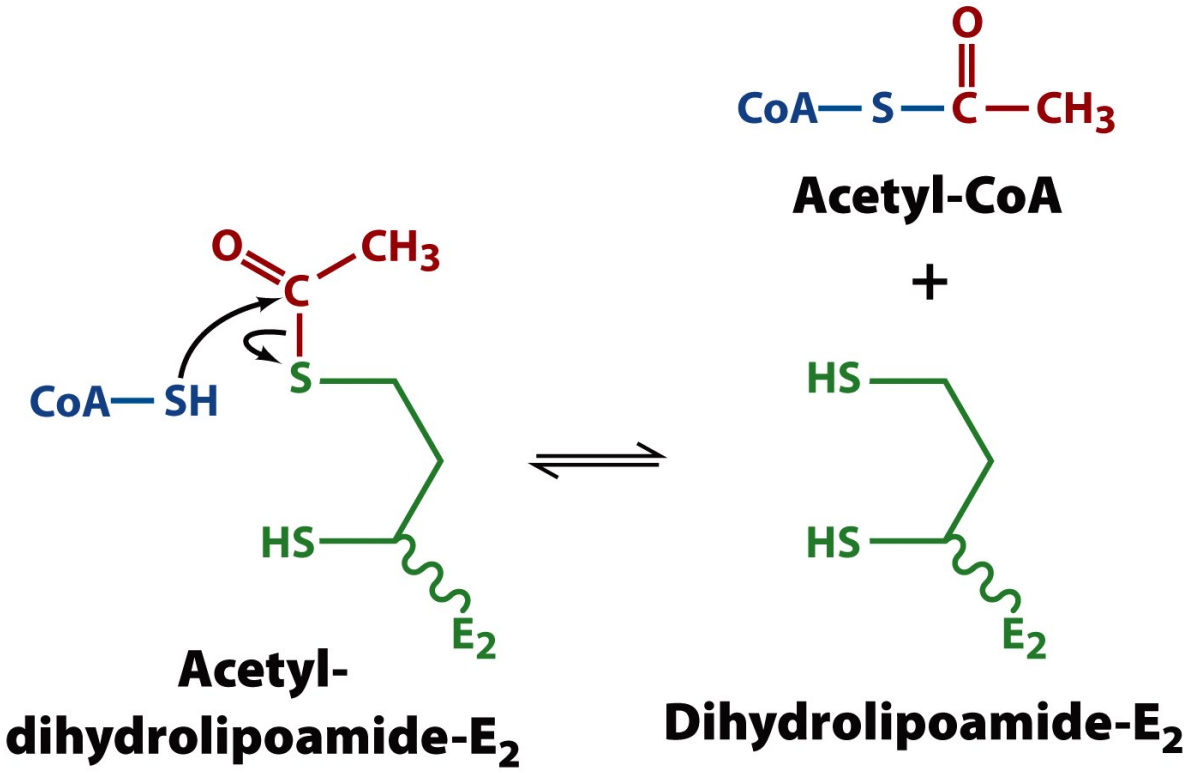

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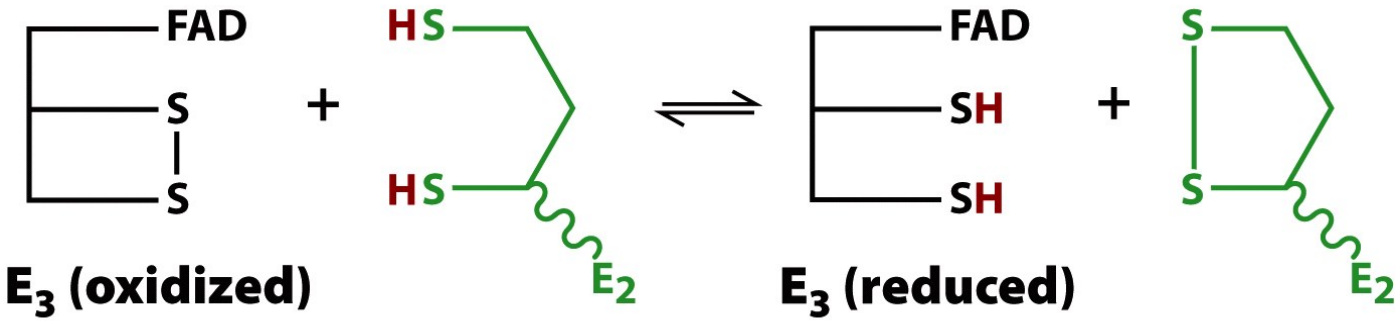

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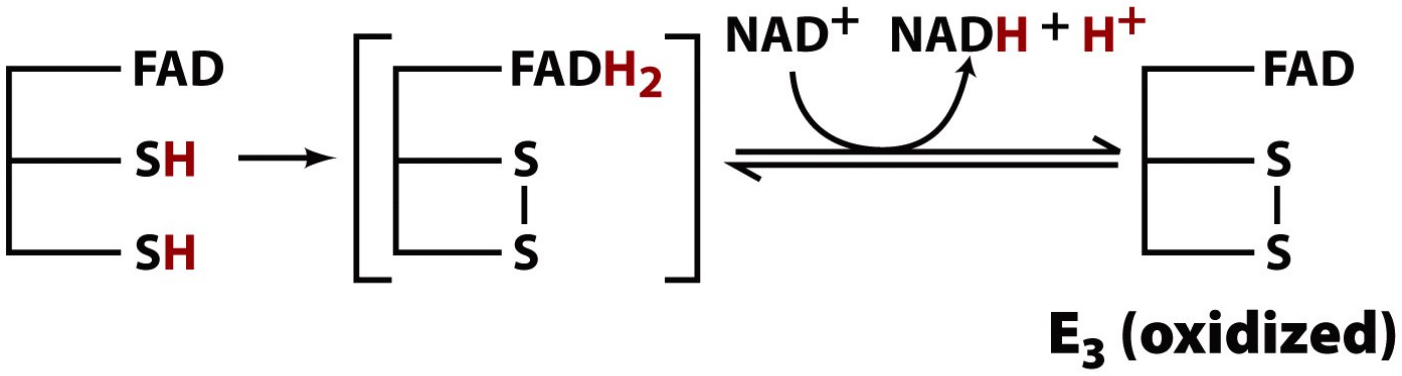

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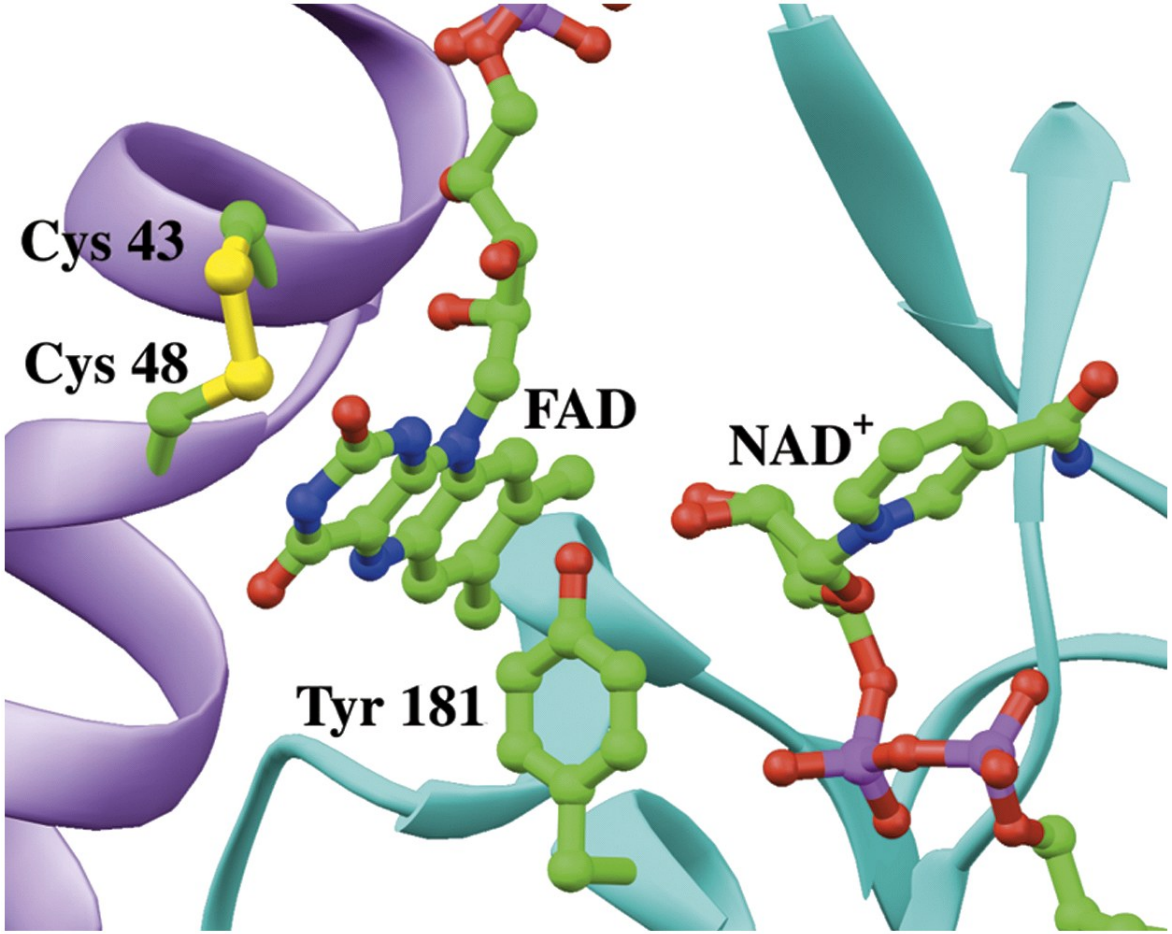

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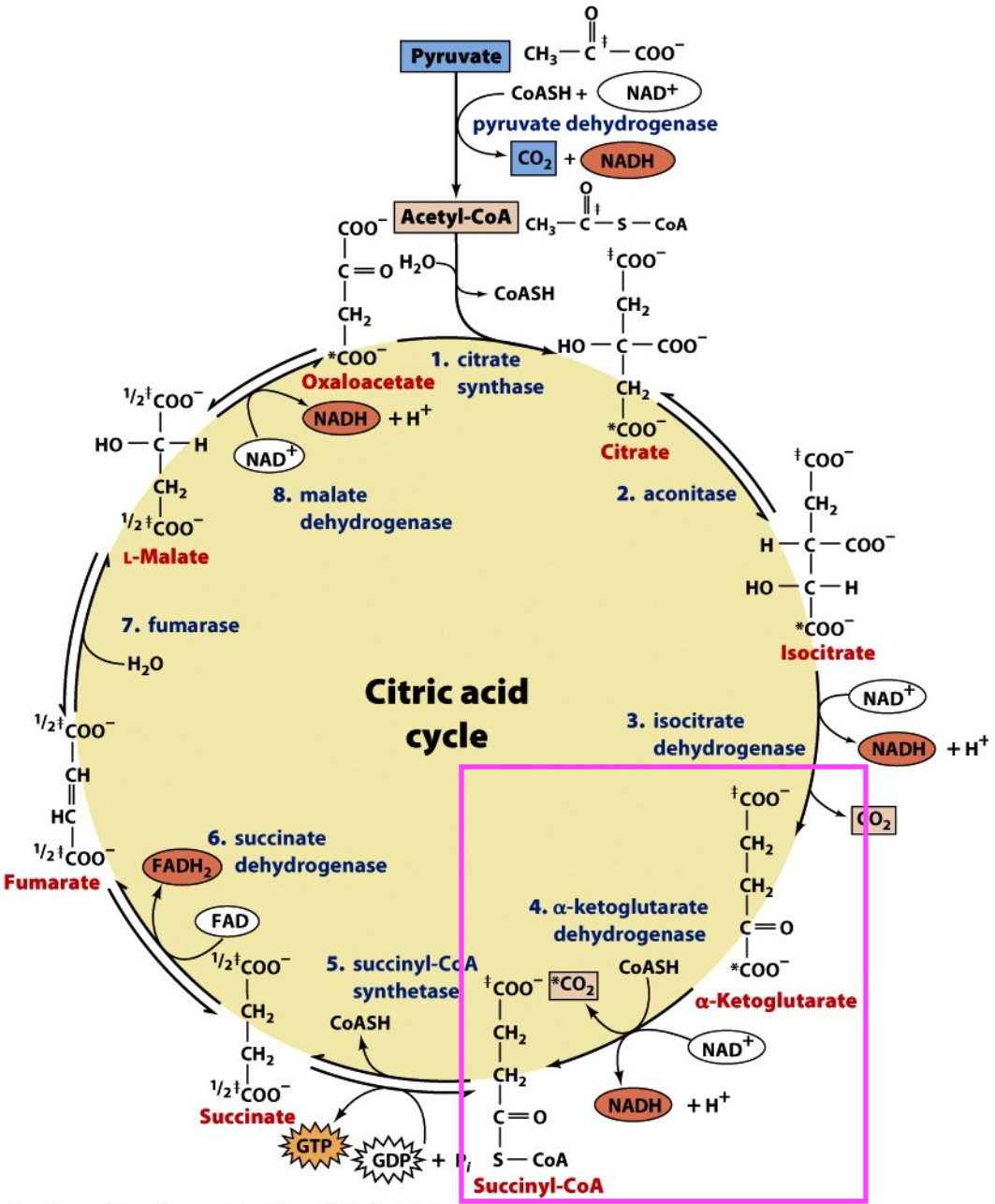
The coenzymes and prosthetic groups of the pyruvate dehydrogenase complex (PDC)

Cofactor	Location	Function
Thiamine pyrophosphate (TPP)	Bound to E_1	Decarboxylates pyruvate, yielding a hydroxyethyl-TPP carbanion
Lipoic acid	Covalently linked to a Lys on E_2 (lipoamide)	Accepts the hydroxyethyl carbanion from TPP as an acetyl group
Coenzyme A (CoA)	Substrate for E_2	Accepts the acetyl group from acetyl-dihydrolipoamide
Flavin adenine dinucleotide (FAD)	Bound to E_3	Reduced by dihydrolipoamide
Nicotinamide adenine dinucleotide (NAD^+)	Substrate for E_3	Reduced by $FADH_2$

α-Ketoacid dehydrogenase multi-enzyme complexes catalyze the reactions of α-ketoacids with NAD^+ and CoA to produce acyl CoA, NADH and CO_2 (oxidative decarboxylation of α-ketoacids)



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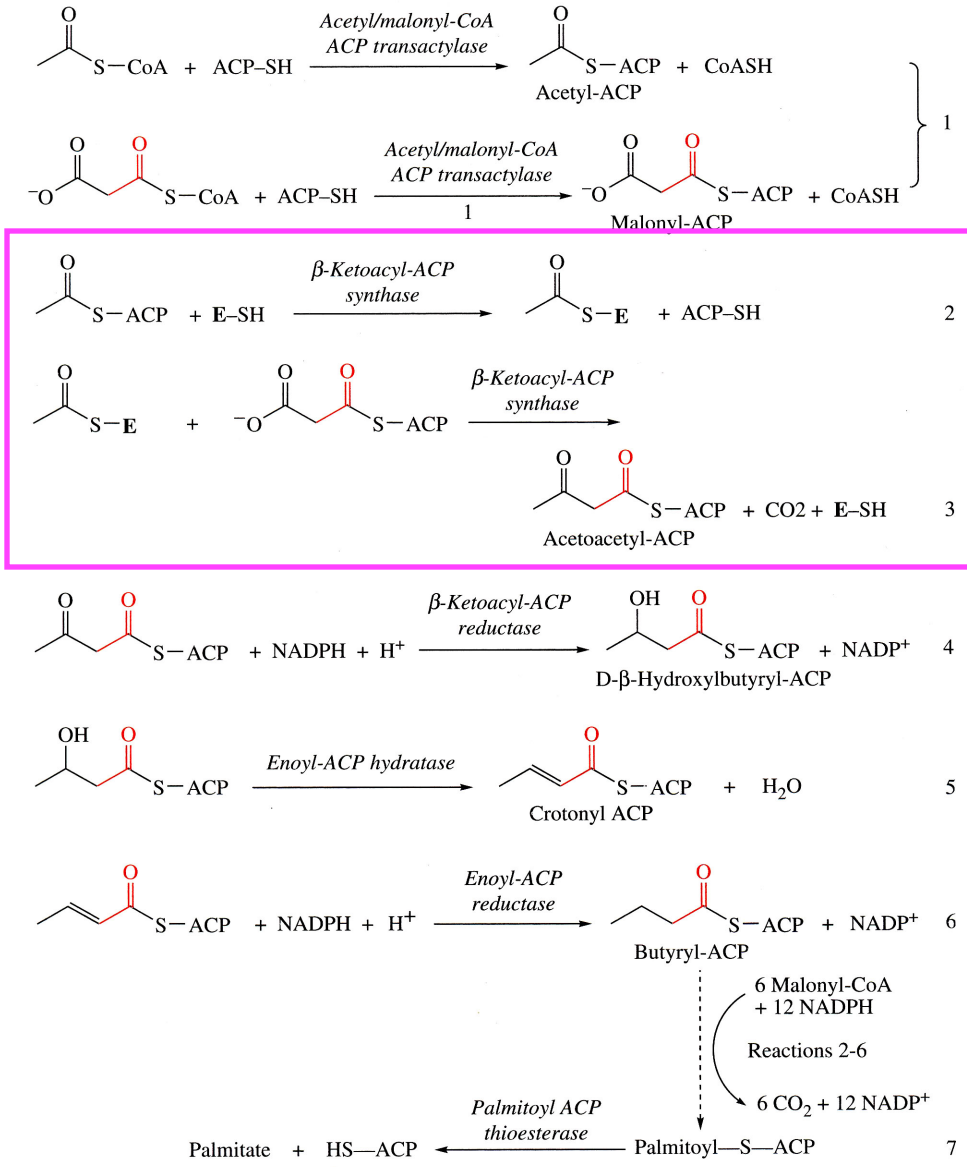
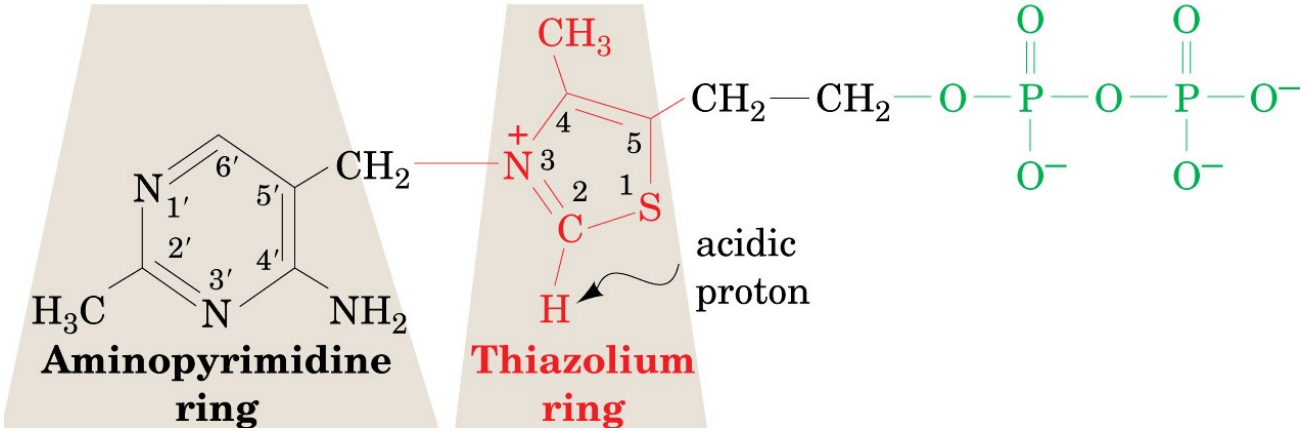


Fig. 18-9. Reactions catalyzed by the fatty acid synthase complex in the biosynthesis of palmitic acid.

Thiamine pyrophosphate

The structure of thiamine pyrophosphate (TPP)



The thiazolium ring is the reactive part of the molecule. TPP functions to delocalize the (-) charge on acylium ions during the decarboxylation of α-ketoacids.

Biosynthesis of TPP from vitamin B_1

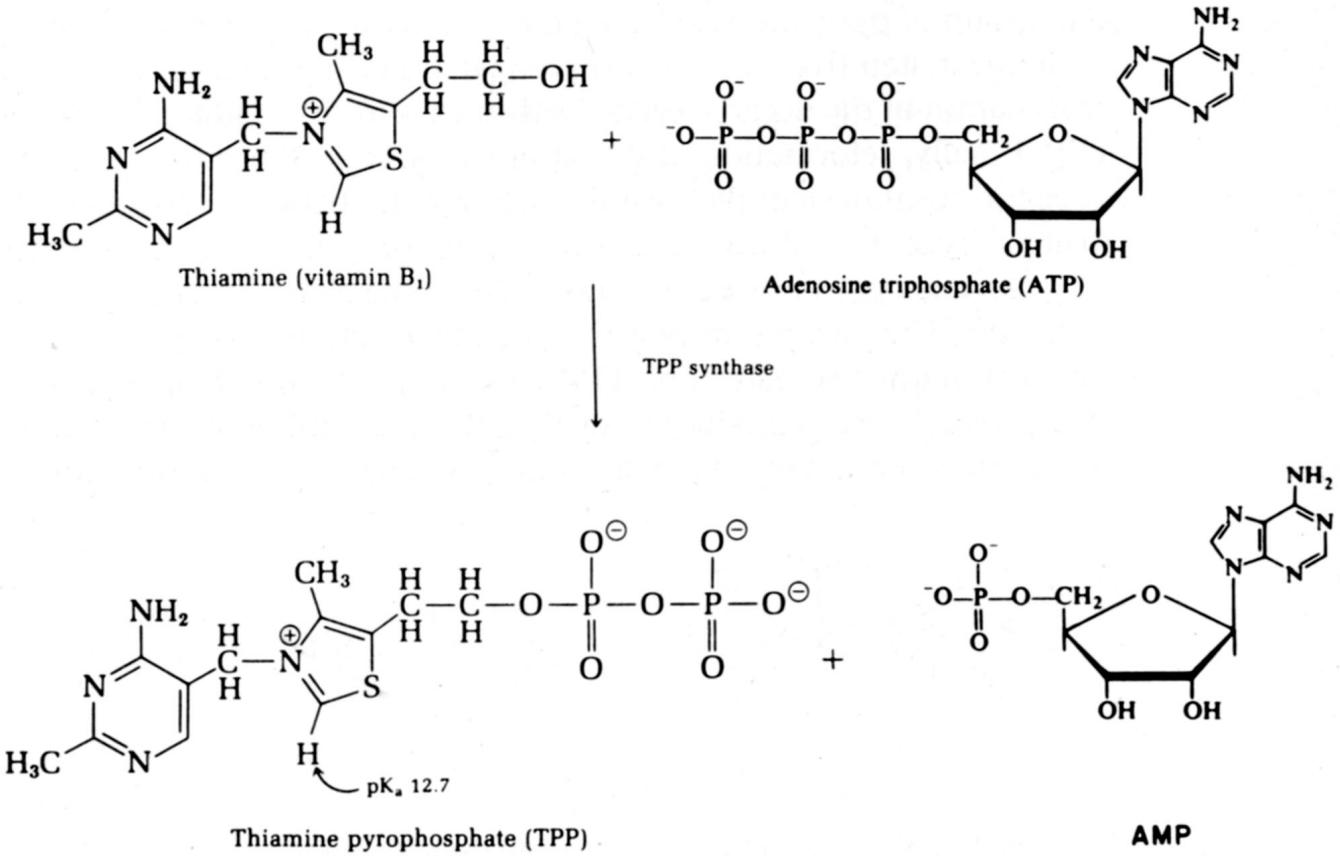


Figure 4.2 Biosynthesis of thiamine pyrophosphate from adenosine triphosphate (ATP) and thiamine (vitamin B_1). The structures of ATP, the vitamin, and the active coenzyme are illustrated. A single reaction converts thiamine to thiamine pyrophosphate as shown. The byproduct of the reaction is adenosine monophosphate (AMP). The enzyme catalyzing this reaction is called thiamine pyrophosphate synthase.

TPP is involved in enzyme-catalyzed reactions involving C-C bond formation or cleavage in carbonyl or carbonyl-like substrates.

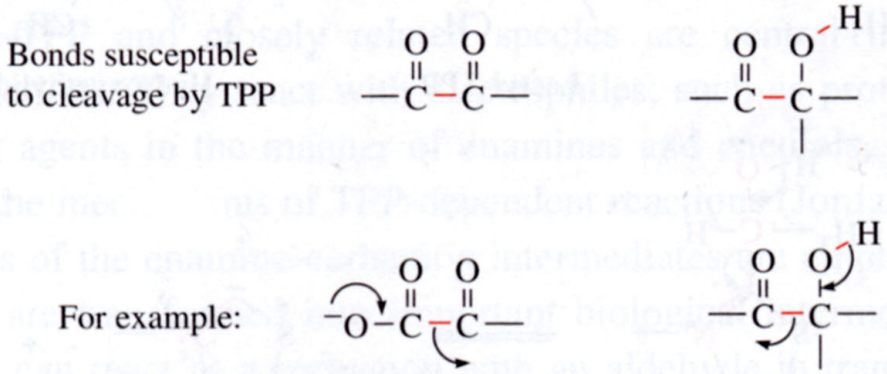


Fig. 3-10. Types of covalent bonds cleaved by action of thiamine pyrophosphate (TPP). Bonds of the type shown in red appear in α-ketoacids, vicinal diketones, and α-hydroxyketones and are cleaved by TPP-dependent enzymes. The nature of the cleavage reactions is illustrated in the lower part of the figure. The chemical properties of the thiazolium ring of TPP in forming adducts with substrates obviate the necessity to produce the unacceptably high-energy acylium ions implied by the electron flow.

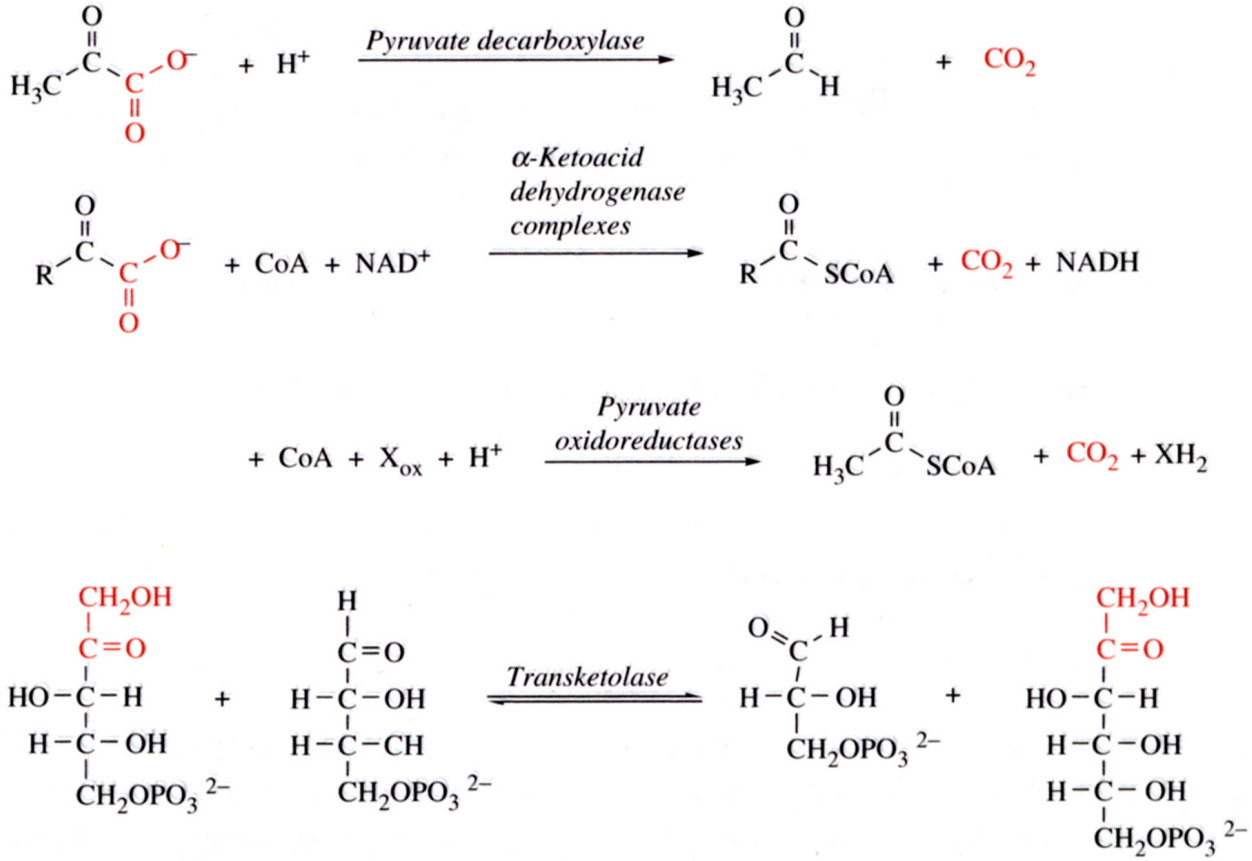
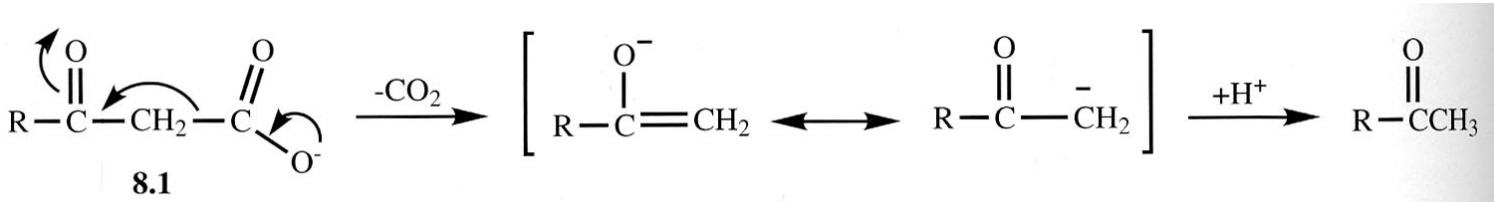


Fig. 3-11. Typical reactions of thiamine pyrophosphate (TPP)–dependent enzymes. Pyruvate decarboxylase and transketolase are TPP-dependent enzymes that do not require other coenzymes or cofactors. Pyruvate oxidoreductases couple the decarboxylation of pyruvate, with its further oxidation to the acetate level; they require other cofactors, including coenzyme A and an electron acceptor such as $NADP^+$, a quinone, or dioxygen. Electron transfer is also mediated by iron-sulfur clusters or flavin coenzymes, or both. The α-ketoacid dehydrogenase complexes consist of at least three proteins and require coenzyme A, NAD^+, lipoic acid, and FAD to support the acetyl group transfer and electron transfer, in addition to TPP for decarboxylation.

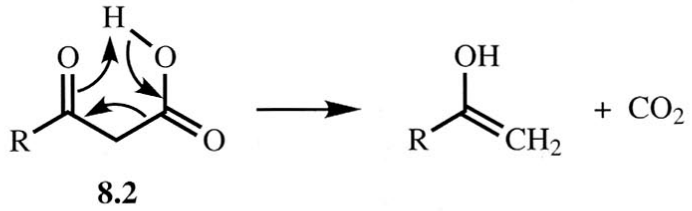
Examples of enzyme reactions involving TPP as a coenzyme

Decarboxylation of β-ketoacids



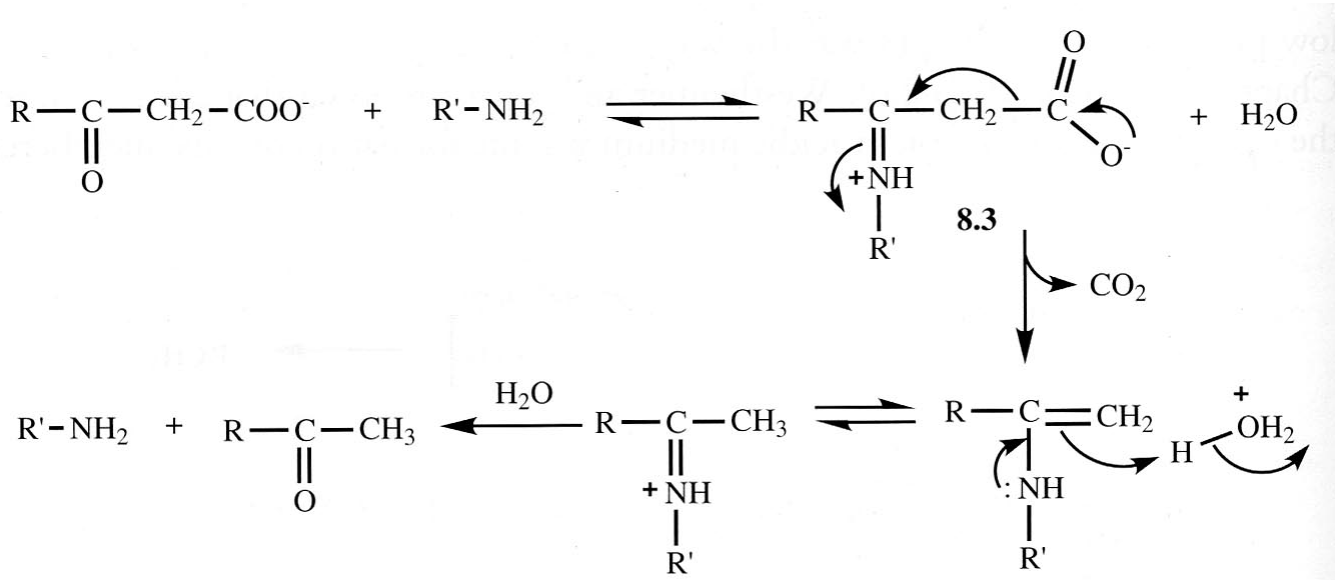
8.1

SCHEME 8.2 Decarboxylation of β-keto acids.



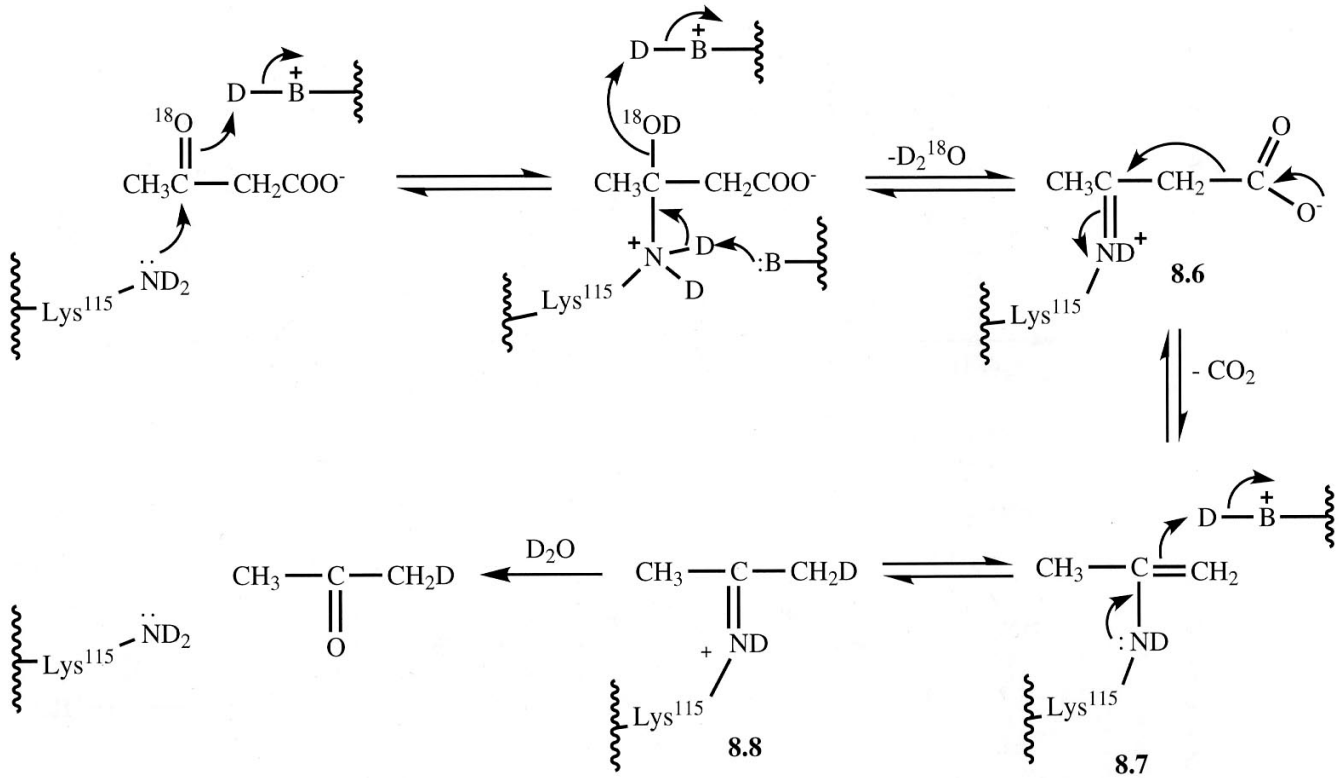
8.2

SCHEME 8.3 Cyclic transition state for decarboxylation of β-keto acids.

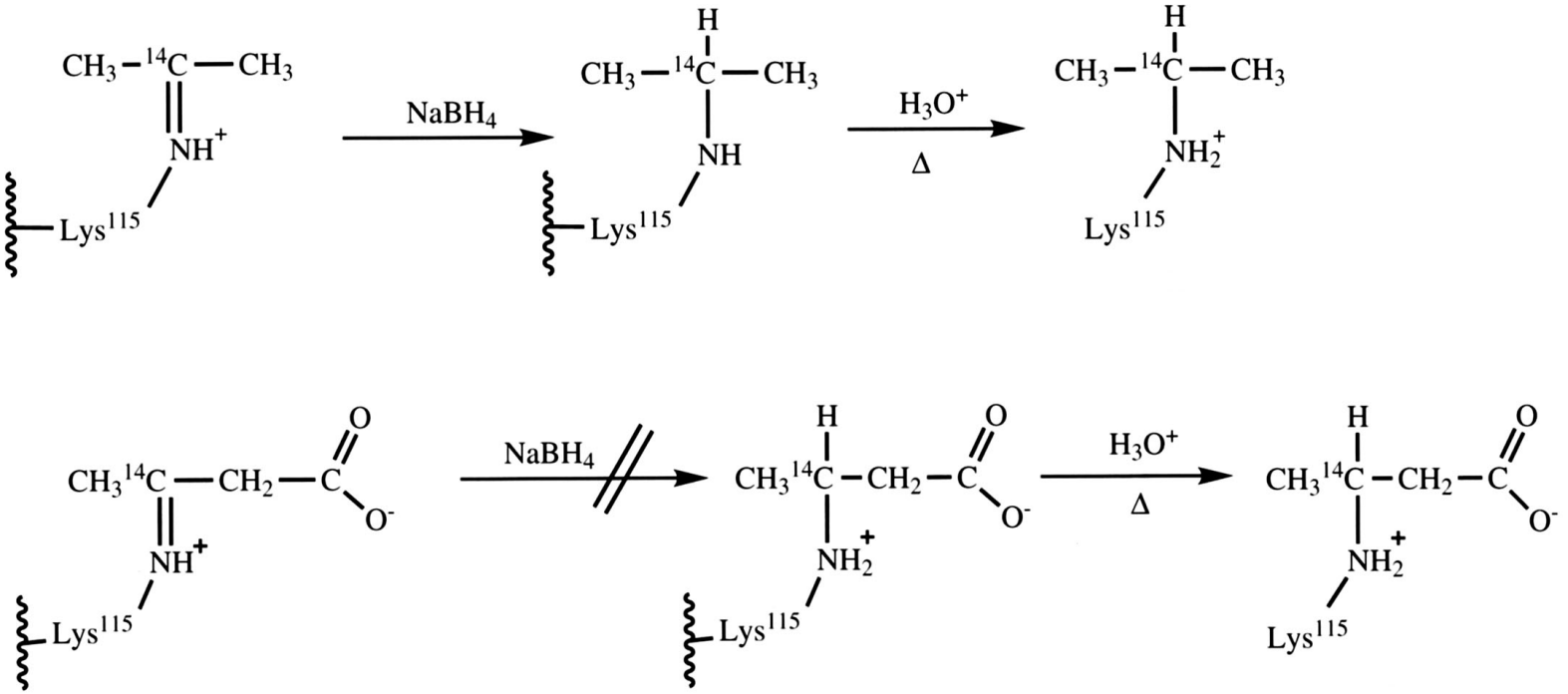


SCHEME 8.4 Amine-catalyzed decarboxylation of β-keto acids.

Applicable to 1° and 2° amines only

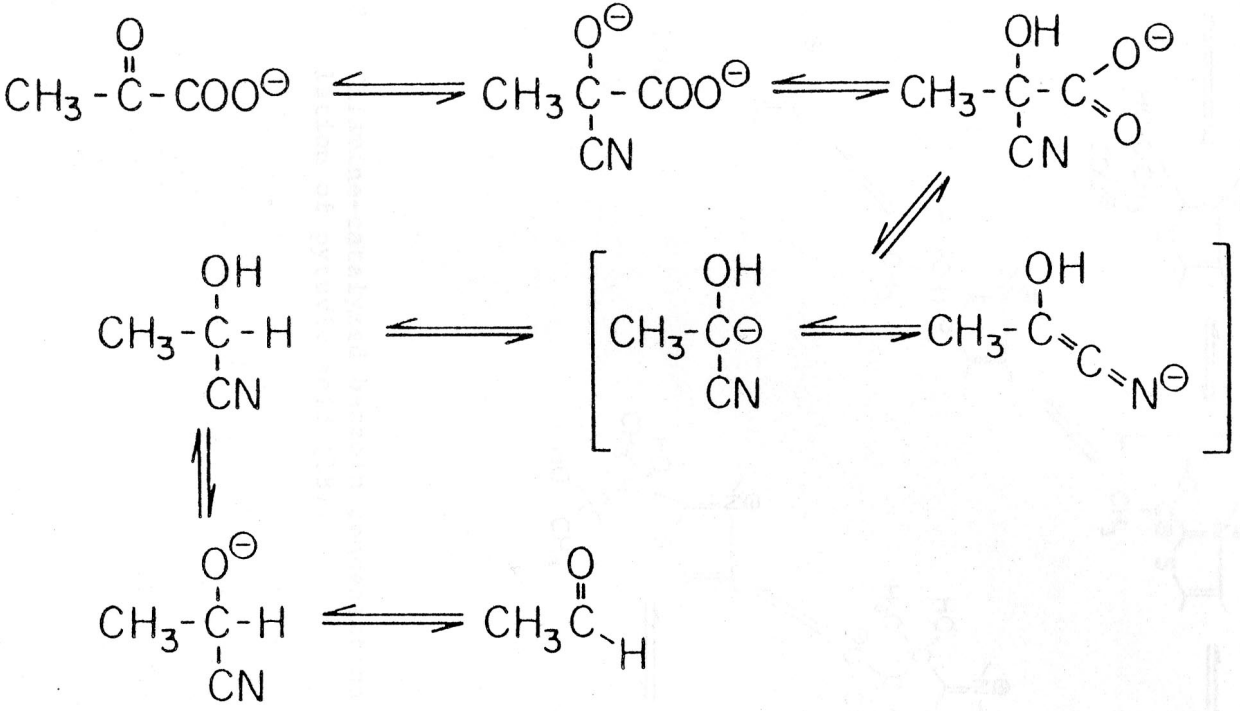


SCHEME 8.8 Proposed mechanism for acetoacetate decarboxylase.



SCHEME 8.9 Fate of $NaBH_4$ reduction during the reaction catalyzed by acetoacetate decarboxylase.

CN^--catalyzed decarboxylation of α-ketoacids



How TPP functions to stabilize an acylium ion during the decarboxylation of pyruvate

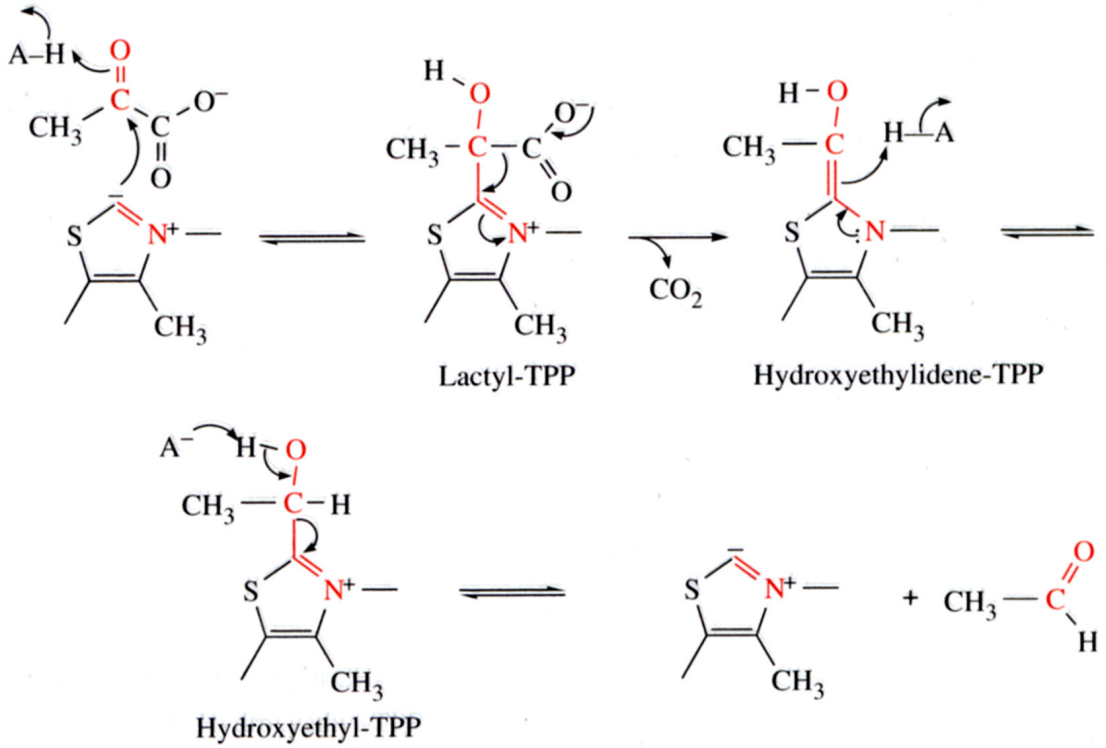
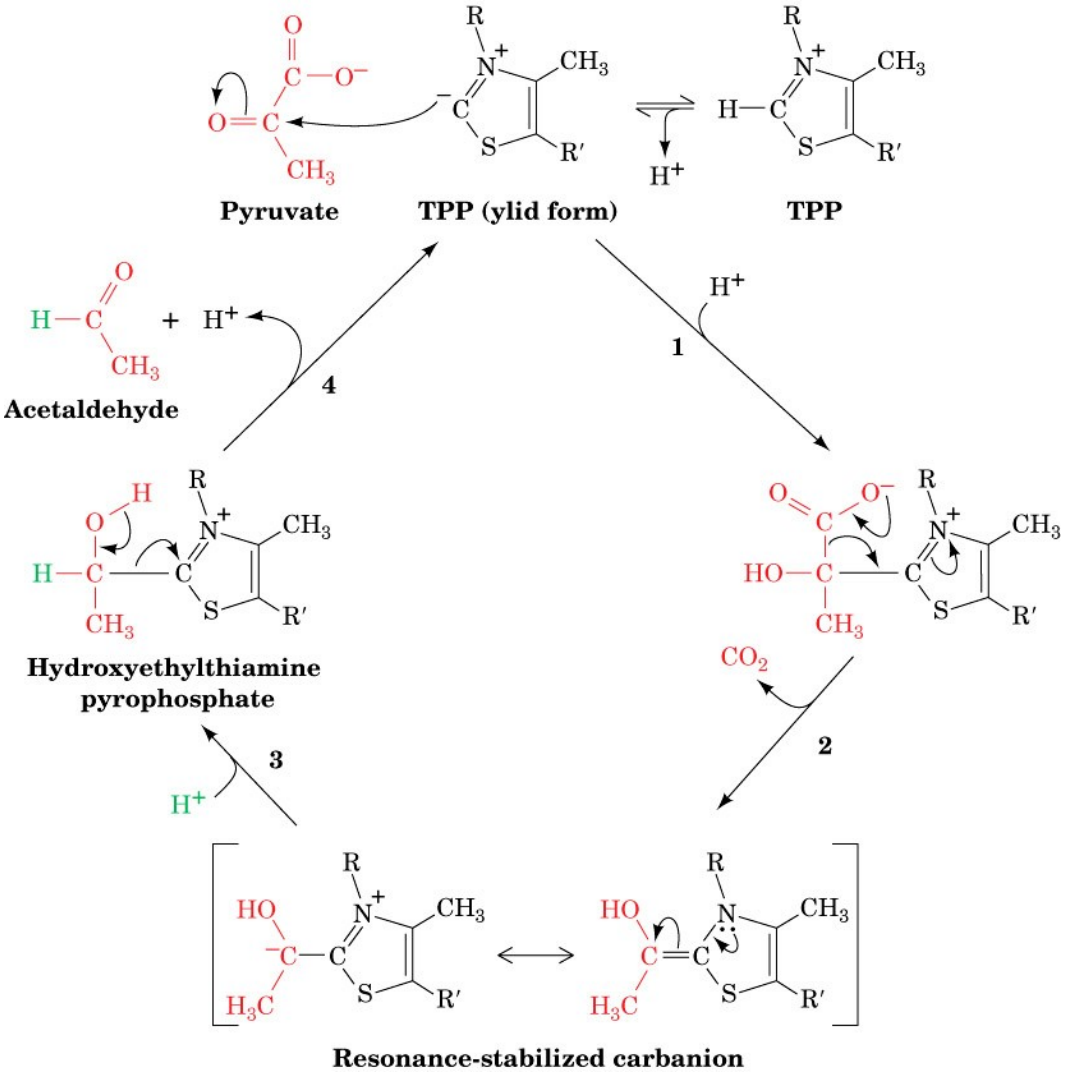
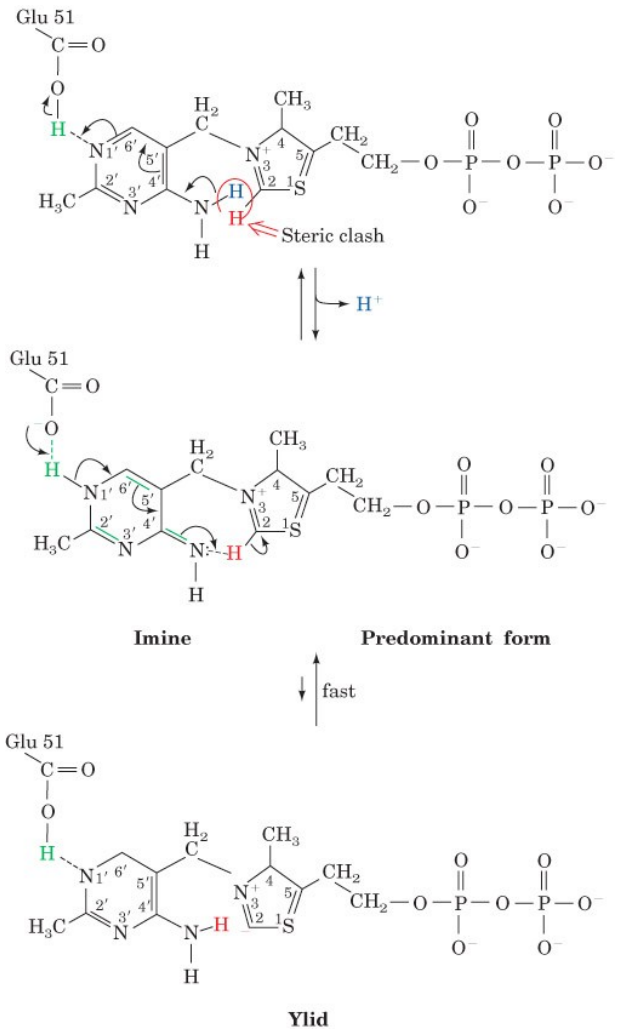


Fig. 3-12. Thiamine pyrophosphate (TPP) catalysis of the decarboxylation of pyruvate. Only the chemically essential thiazolium ring of TPP is explicitly shown in this mechanism, which is intended to focus on the chemical steps.

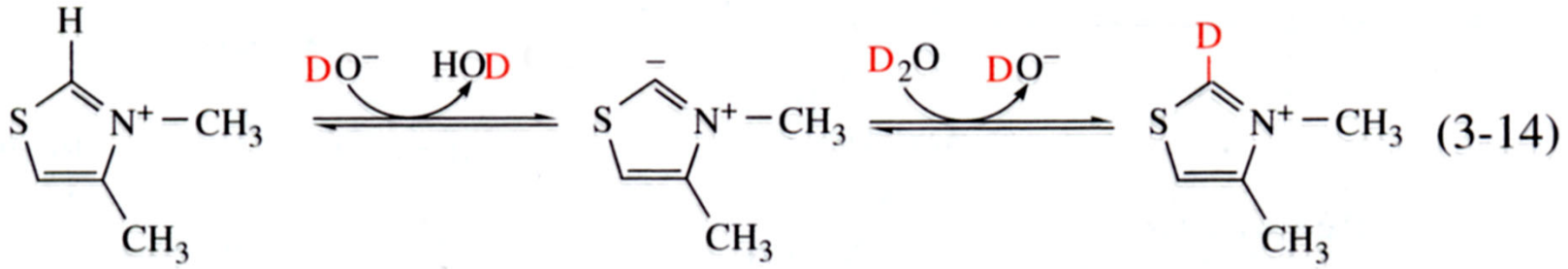
Reaction mechanism of pyruvate decarboxylase



Formation of the active ylid form of TPP in the pyruvate decarboxylase reaction

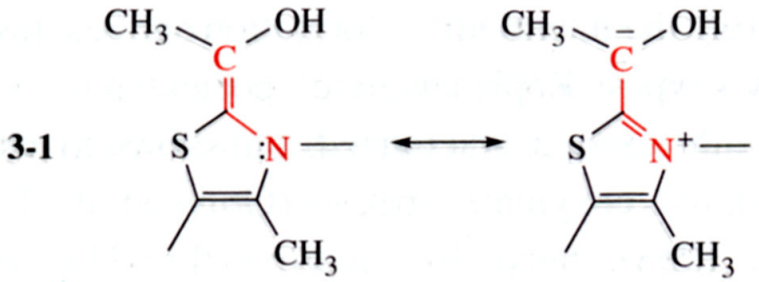


A model system: The C2-H of 1,5-dimethylthiazolium undergoes fast exchange with ^2H_2O in neutral solution



(3-14)

The pK_a for C2-H ionization of TPP in aqueous solution is ~19.
In an enzyme active site, the same ionization is orders of magnitude lower than in water.



Hydroxyethylidene-TPP is carbanionic in nature, with stabilization provided by its important resonance forms. The enamine form is likely to be more important than the charge-separated carbanion, although the polarity of the microenvironment probably influences the relative importance of the two forms.

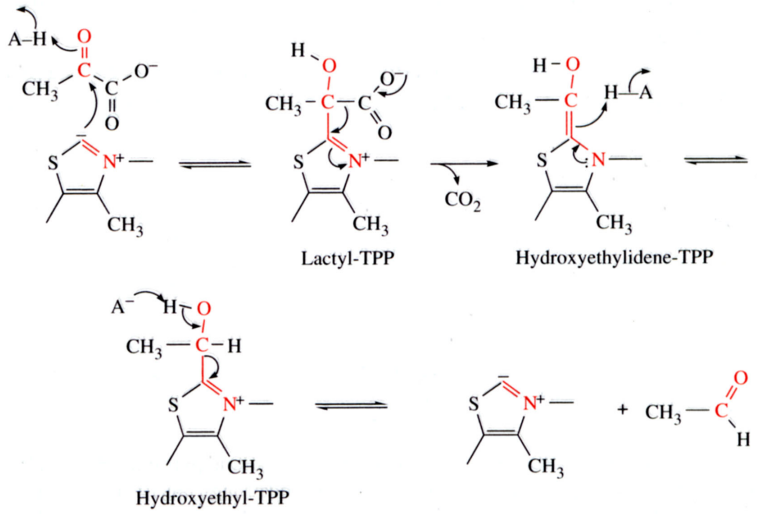
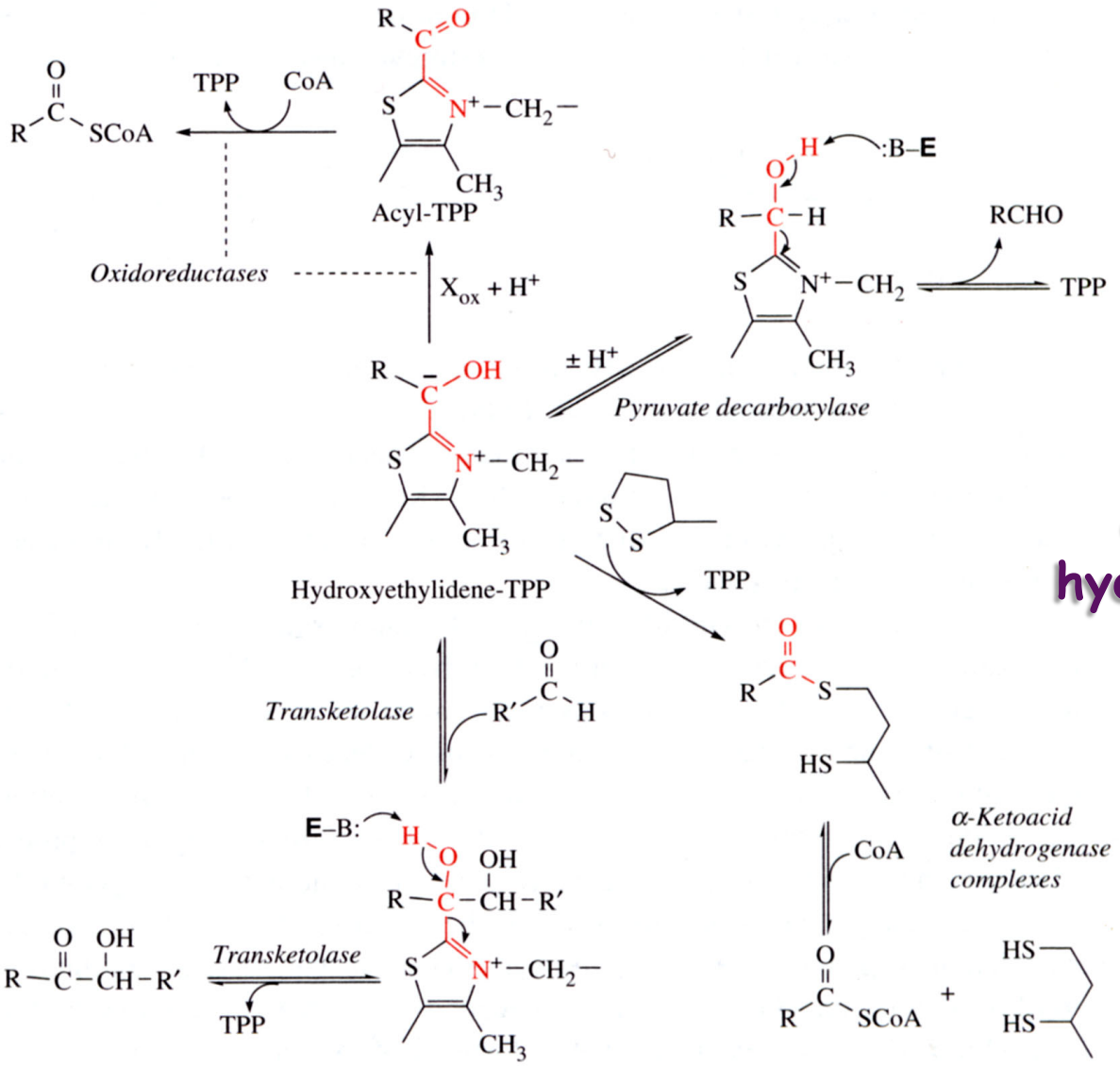


Fig. 3-12. Thiamine pyrophosphate (TPP) catalysis of the decarboxylation of pyruvate. Only the chemically essential thiazolium ring of TPP is explicitly shown in this mechanism, which is intended to focus on the chemical steps.



Different chemical fates of hydroxyethylidene-TPP

Fig. 3-13. Enzymatic reactions of hydroxyethylidene–thiamine pyrophosphate (TPP). The major enzymatic fates of hydroxyethylidene-TPP are depicted in its charge-separated carbanionic resonance form.

Proposed mechanism of phosphoketolase

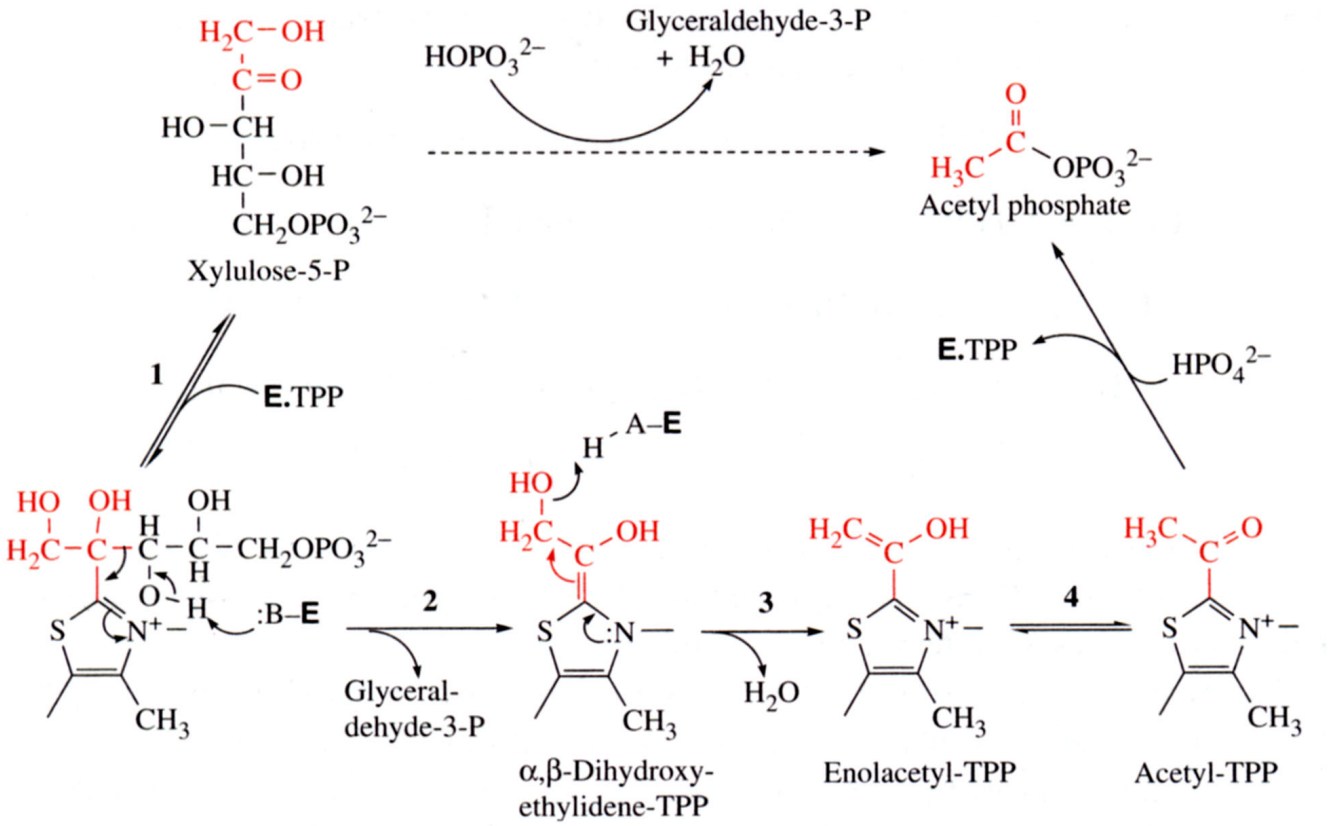
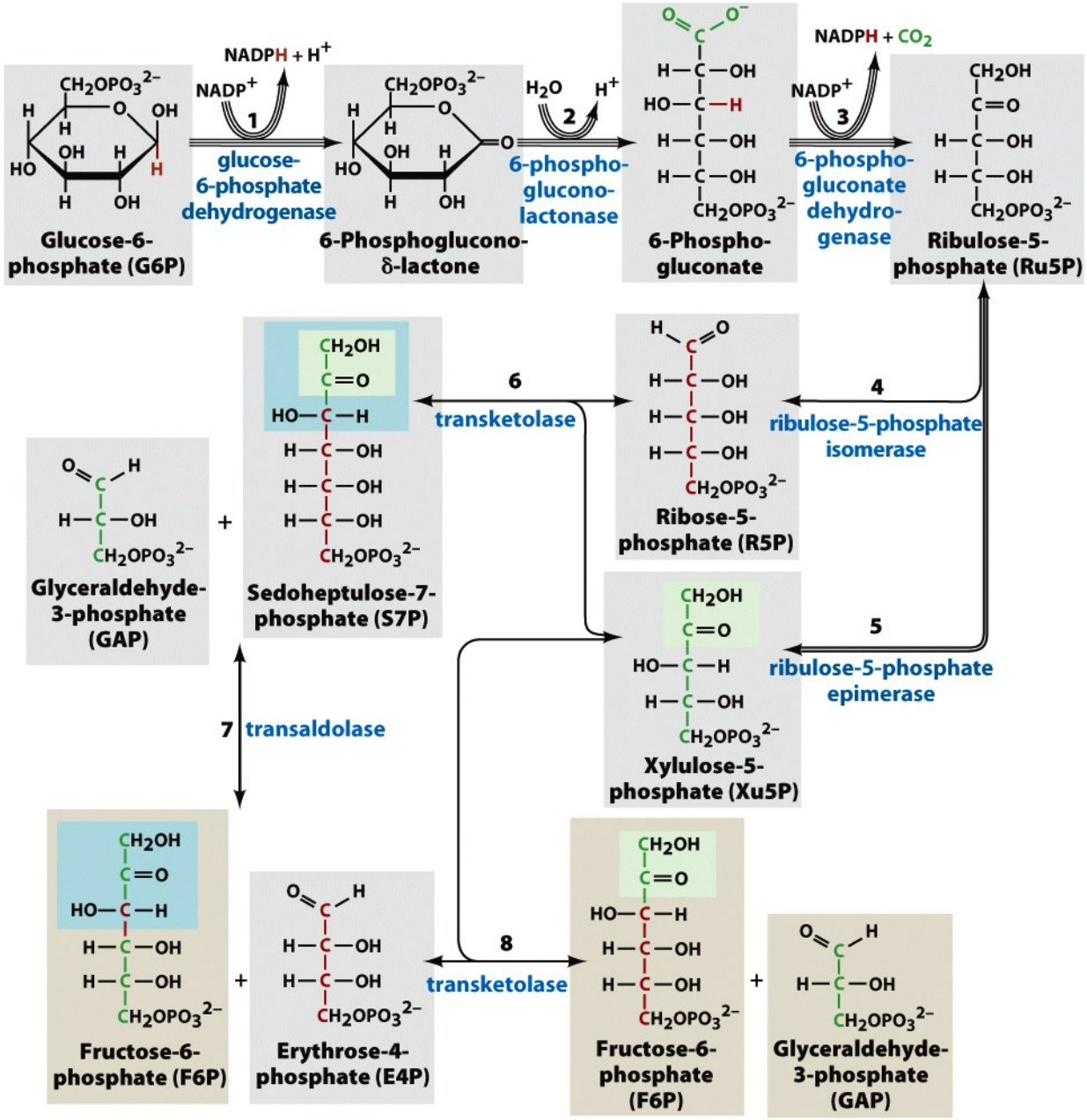


Fig. 3-14. A hypothetical mechanism for the role of TPP in the reaction of phosphoketolase. The overall transformation of xylulose-5-P and phosphate into acetyl phosphate, glyceraldehyde-3-P, and a mole of water is postulated to proceed by the mechanism shown in the lower portion of this figure. The enzyme and reaction mechanism have not been characterized.



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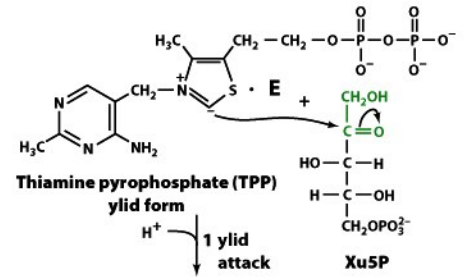
The pentose phosphate pathway



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The transketolase reaction: A TPP-requiring enzyme

Pyridoxal phosphate
(key coenzyme of amino acid metabolism)

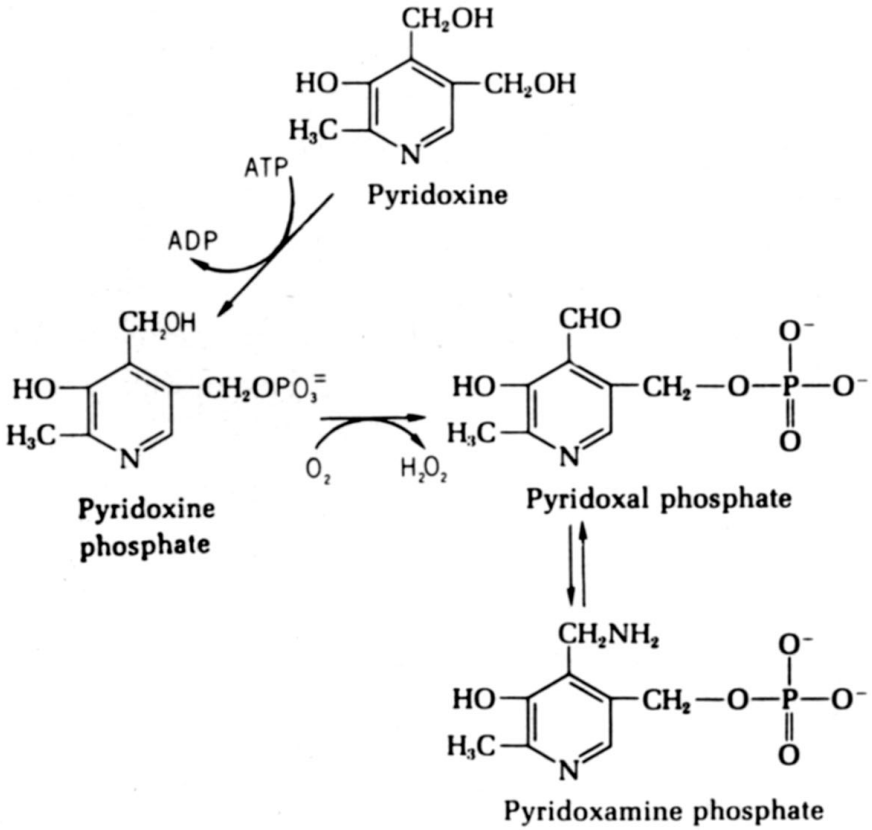


Figure 4.13 Biosynthetic pathways for the conversion of pyridoxine (top)—the most common form of vitamin B_6—first to pyridoxine phosphate (middle, left) and then to the principal coenzyme form, pyridoxal phosphate (PLP) (middle, right). PLP can secondarily be converted to the second coenzyme form of the vitamin, pyridoxamine phosphate (PMP) (bottom), which is a coenzyme of transamination (aminotransfer) reactions.

Biosynthesis of pyridoxal phosphate (PLP) from vitamin B_6

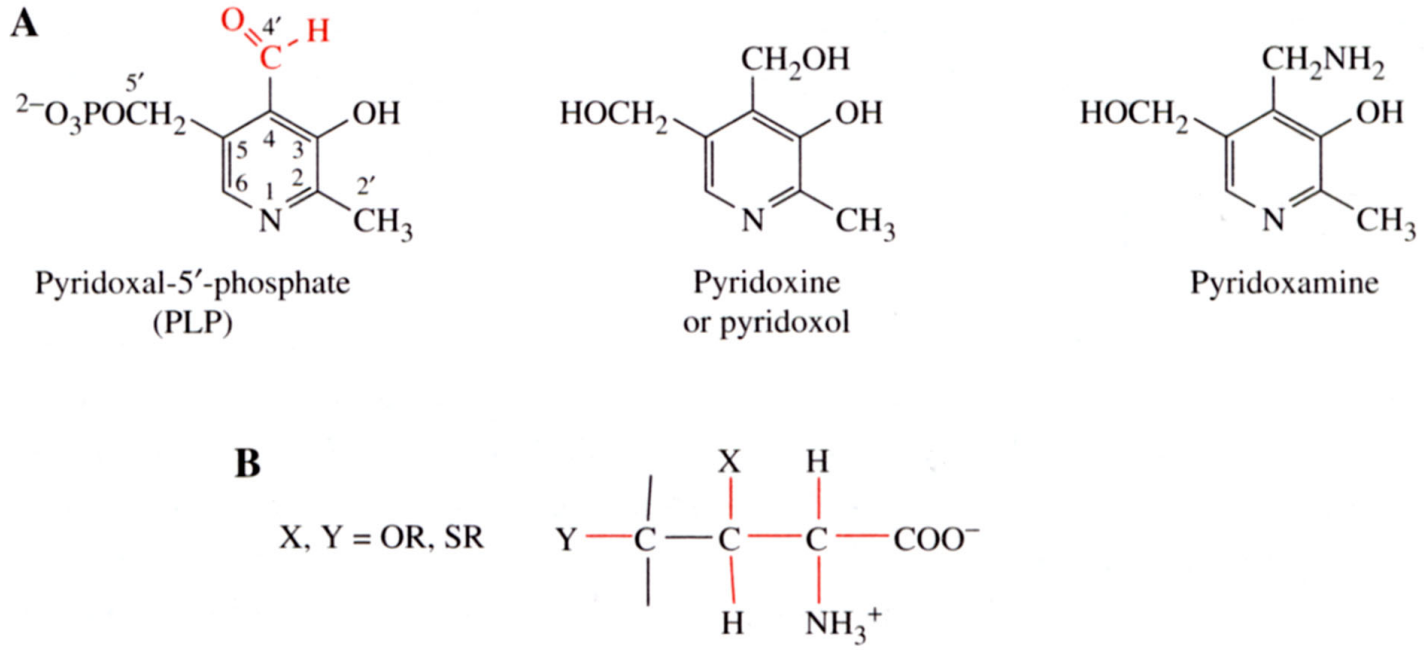
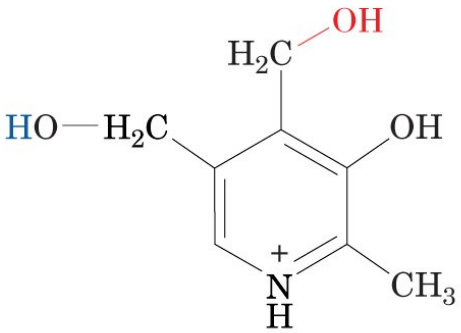
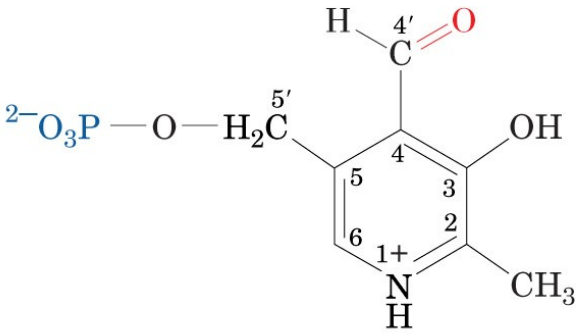


Fig. 3-16. Vitamin B_6 coenzymes and the cleavable bonds in pyridoxal-5′-phosphate (PLP) reactions. (A) Structures of vitamin B_6 and its coenzymatic forms. (B) The bonds susceptible to PLP-dependent cleavages in α-amino acids. The δ-amino group in ornithine, γ-amino group in γ-aminobutyric acid, the ε-amino group of lysine, and amino groups in substrates other than amino acids are also cleaved by the actions of PLP-dependent aminotransferases and aminomutases.

Forms of pyridoxal 5' -phosphate: (*a*) pyridoxine (vitamin B_6) and (*b*) pyridoxal 5' -phosphate (PLP)

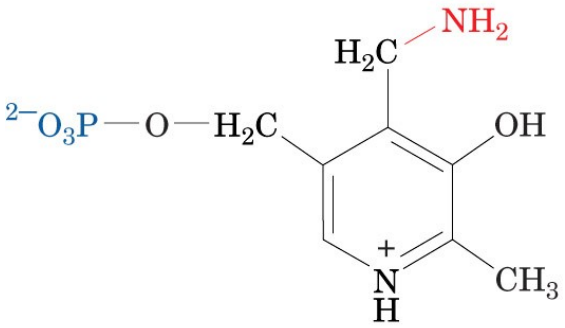


(*a*) **Pyridoxine (vitamin B_6)**

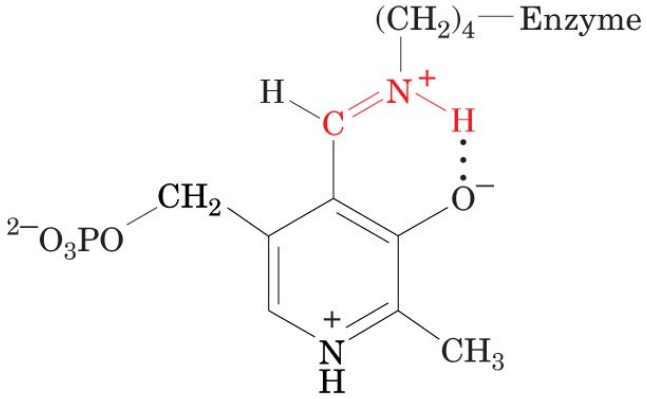


(*b*) **Pyridoxal-5′-phosphate (PLP)**

Forms of pyridoxal 5'-phosphate: (c) Pyridoxamine 5'-phosphate (PMP) and (d) the Schiff base that forms between PLP and an enzyme ε-amino group



(c) **Pyridoxamine-5′-phosphate (PMP)**



(d) **Enzyme–PLP Schiff base**

An internal aldimine

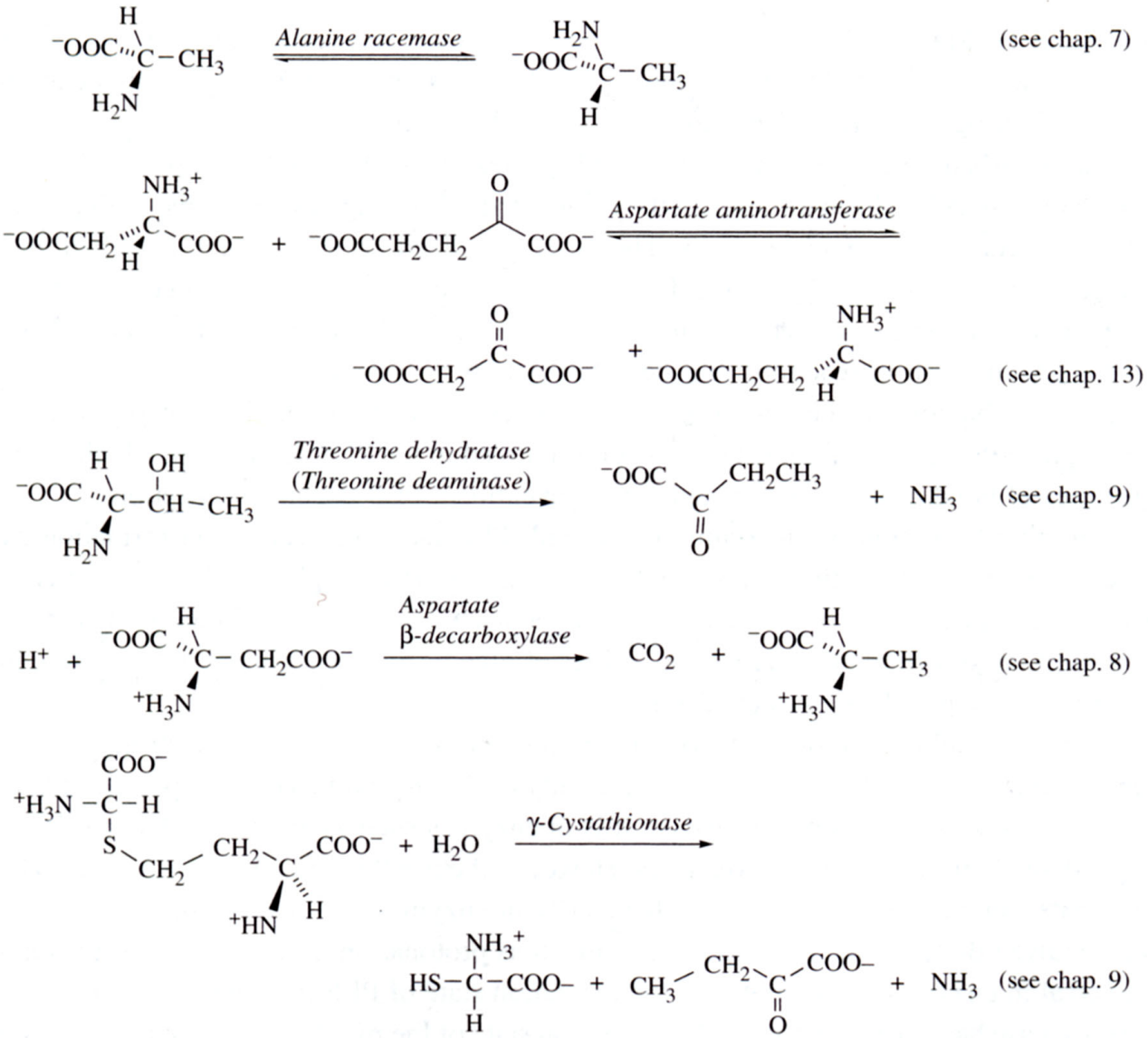
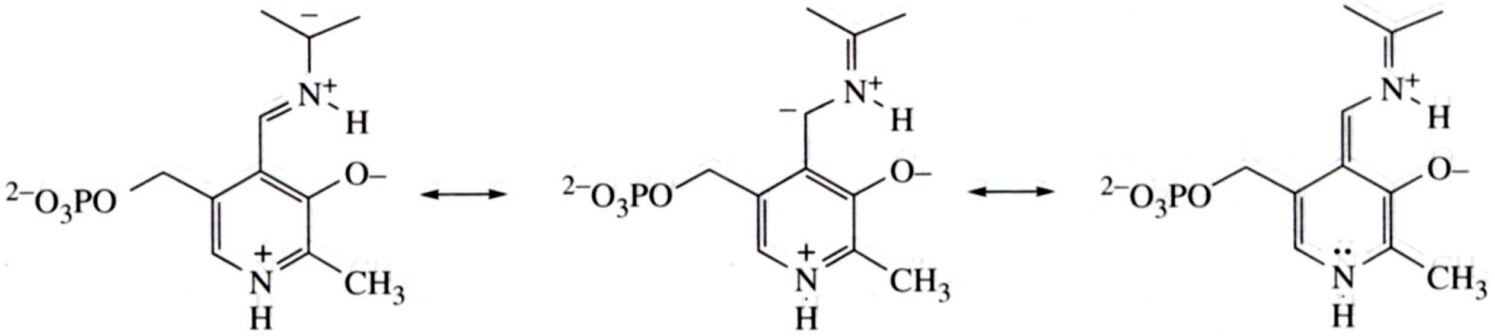


Fig. 3-17. Typical pyridoxal-5′-phosphate–dependent enzymatic reactions are shown with the chapters in which the enzymes are discussed.

α–Carbanions: aminotransferases (transamination), α-decarboxylases, racemases, aldolases, α,β-eliminations, β,γ-eliminations, aspartate-β-decarboxylase



β–Carbanions: β,γ-eliminations, aspartate-β-decarboxylase

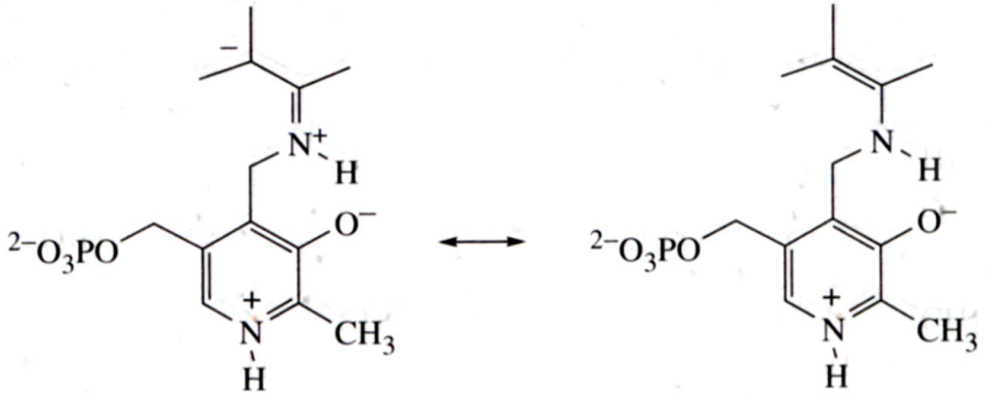


Fig. 3-18. Structures of pyridoxal-5′-phosphate–stabilized amino acid carbanionic intermediates.

Internal aldimine: maintains PLP in highly a reactive state to facilitate the formation of external aldimines (from amino groups of varied substrates); the protonated imine is considerably more electrophilic than the corresponding aldehyde or ketone.

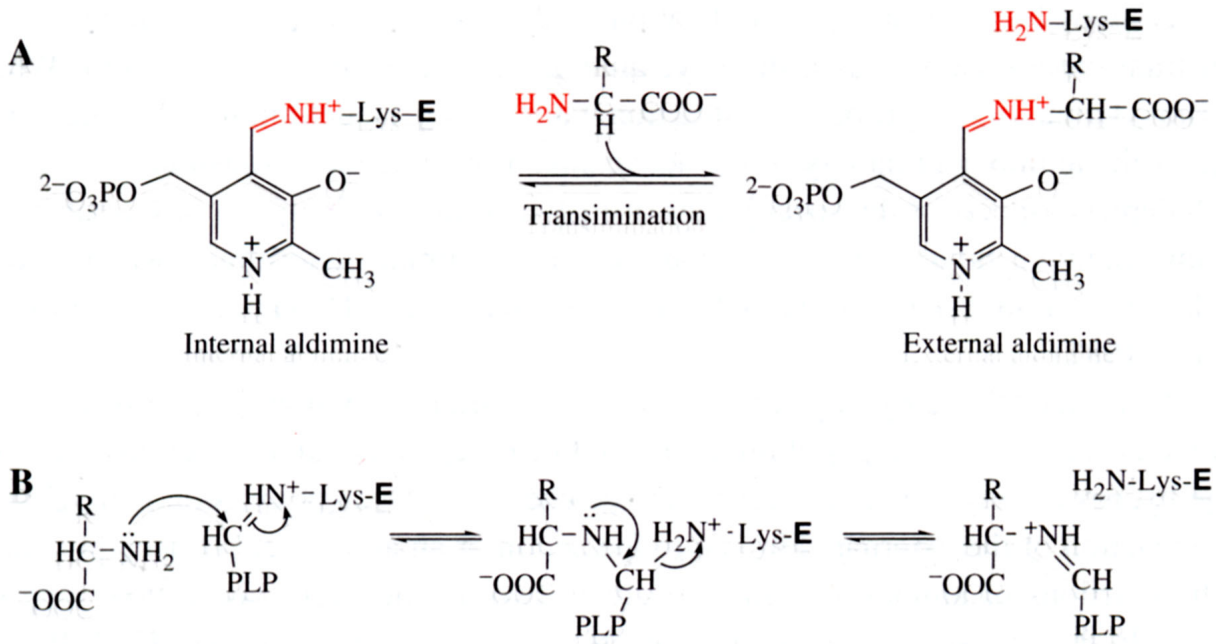


Fig. 3-19. Structure of pyridoxal-5′-phosphate (PLP) enzymes. (A) Most PLP enzymes bind PLP covalently through an imine linkage between the aldehyde group of PLP and the ε-amino group of a lysine residue at the active site. (B) The internal aldimines undergo transaldimination with amino acids to form external aldimines much faster than PLP itself would react.