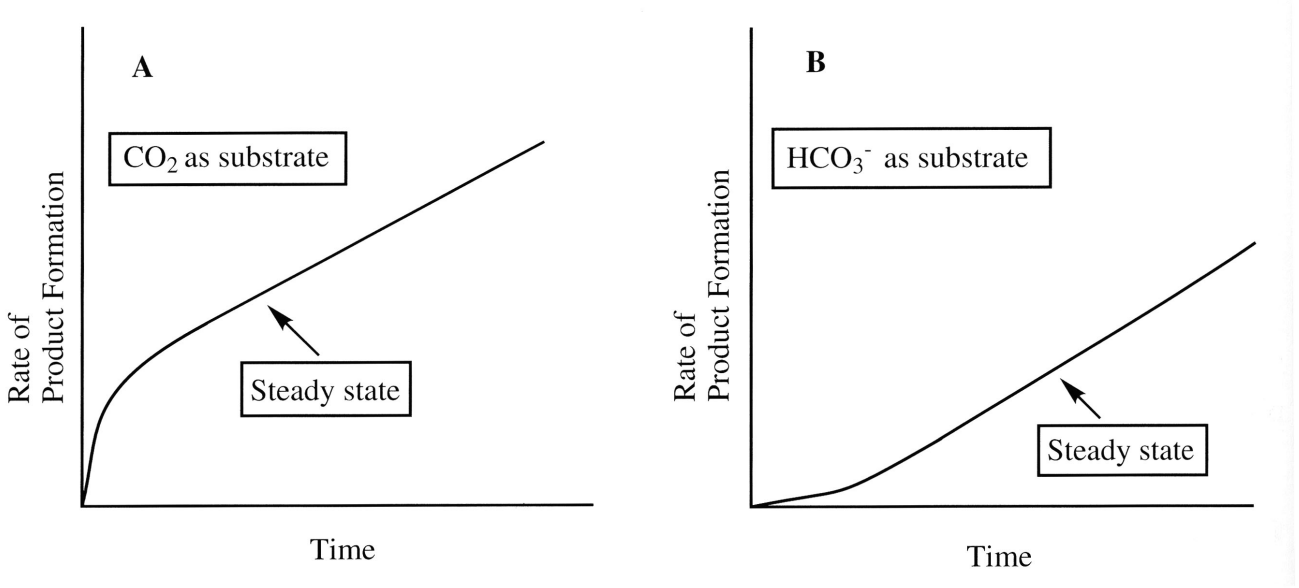
Biotin: A carboxylation coenzyme

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

Tests for whether CO_2 or HCO_3^- is the substrate for a carboxylase

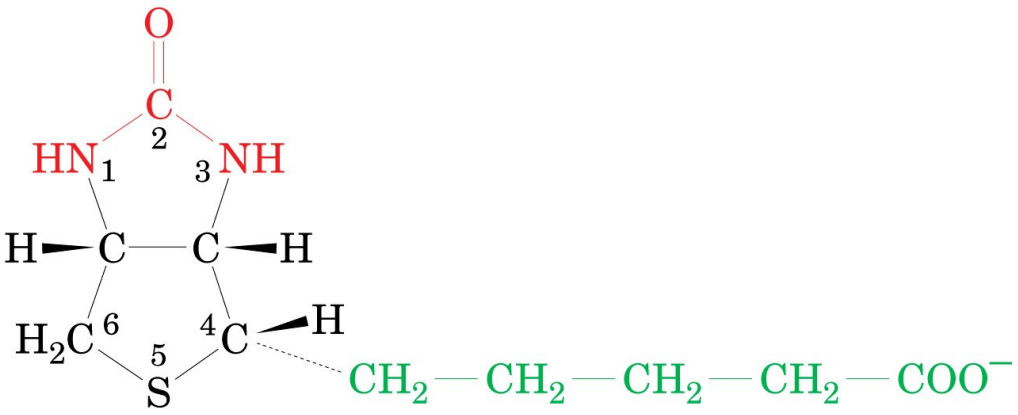


A: Rate of product formation is initially rapid, then levels to a slower steady-state rate: substrate is CO_2. The rate diminishes as HCO_3^- is converted to CO_2, then the equilibrium between HCO_3^- and CO_2 determines the steady-state rate.

B: Rate gradually increases, then levels at a faster steady-state rate: substrate is HCO_3^-. The rate rises while CO_2 is being converted into HCO_3^-, then the equilibrium between CO_2 and HCO_3^- determines the steady state rate.

Biotin and biotinyl-enzyme

Biotin consists of an imidazoline ring *cis*-fused to a tetrahydrothiophene ring bearing a valerate side-chain.

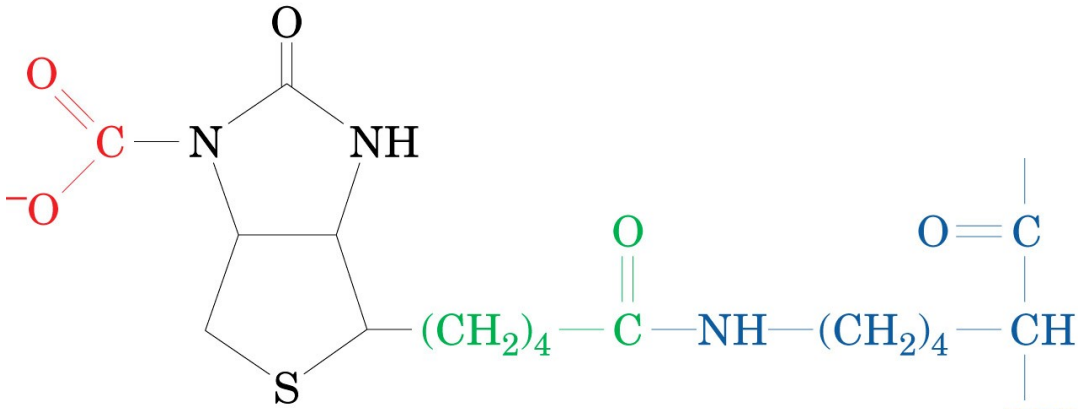


Biotin Valerate side chain

covalently bound to the ε-amino group of a lysine residue

Biotin and carboxybiotinyl–enzyme

In the carboxybiotinyl–enzyme, N1 of the biotinyl imidazoline ring is the site of coenzyme carboxylation.



Carboxybiotinyl–enzyme

"carboxylated" biotinyl group

Lys residue

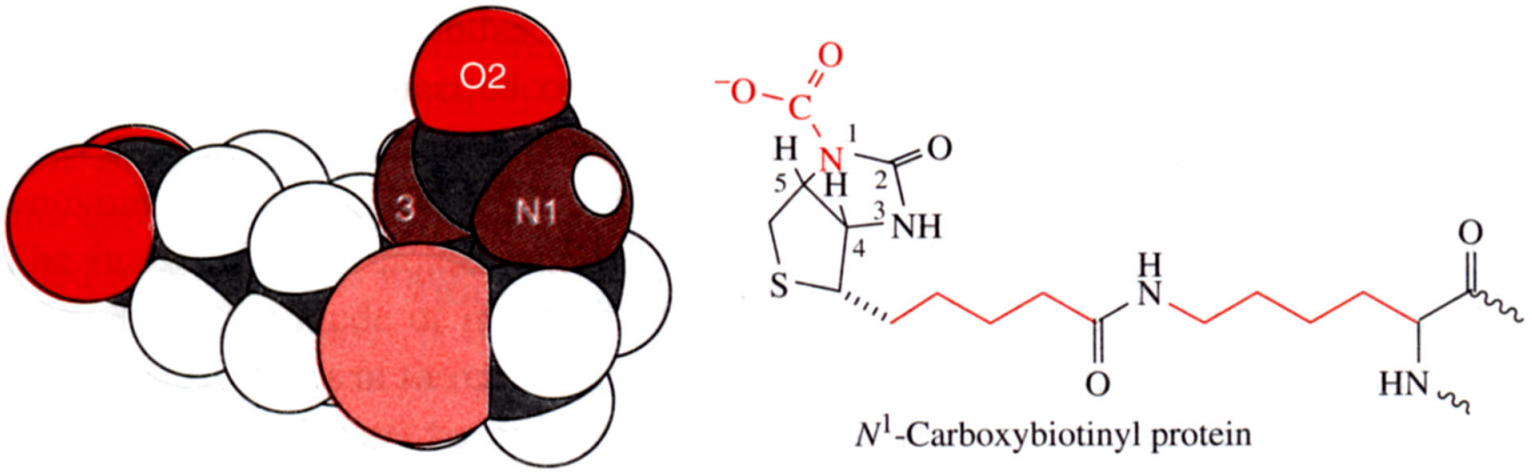
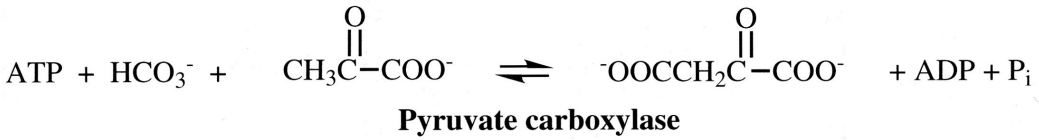
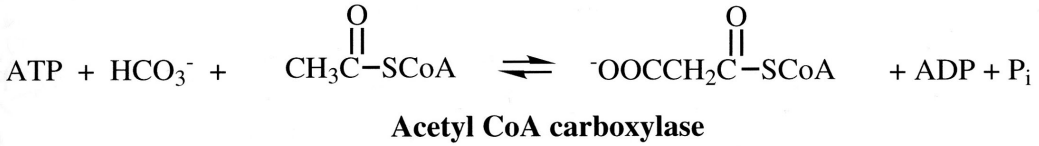


Fig. 3-31. Structures of biotin and a carboxybiotinyl protein. At the left is a space-filling model of biotin. Because of *cis*-fusion of the rings and the size of sulfur, the sulfur and carbonyl groups are sterically close. The stereochemistry of the side chain brings it within close contact to N3, so that only N1 is exposed to carboxylation. The structure of the N^1-carboxybiotinyllysyl group of a carboxylating protein is at the left. The highlighted bonds of the biotinyl and lysyl side chains are those about which rotation is allowed.

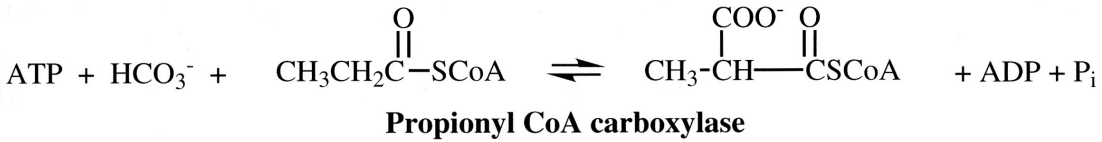
Reactions catalyzed by biotin-dependent carboxylases



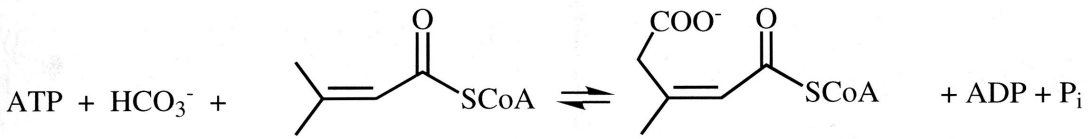
Pyruvate carboxylase



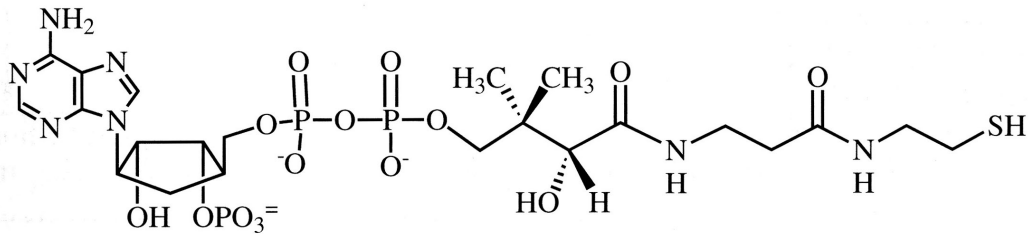
Acetyl CoA carboxylase



Propionyl CoA carboxylase

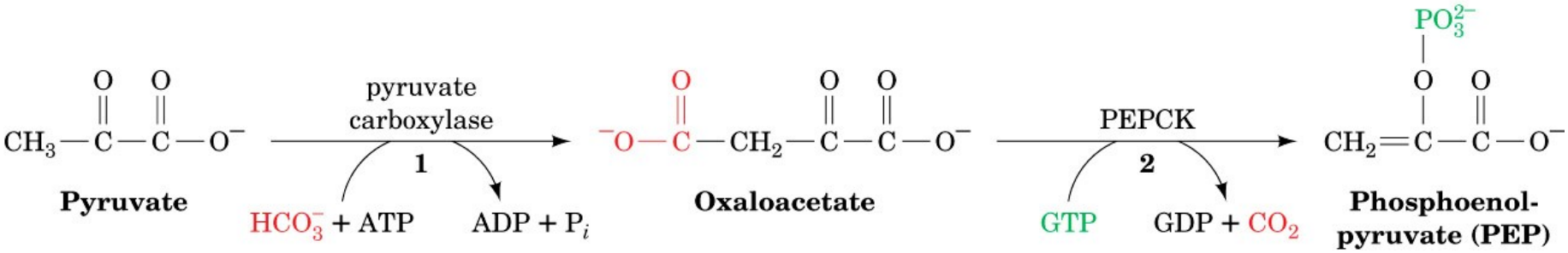


β-Methylcrotonyl CoA carboxylase



CoASH

The conversions of pyruvate to oxaloacetate (OAA) and of OAA to phosphoenolpyruvate (PEP): Anaplerotic reactions

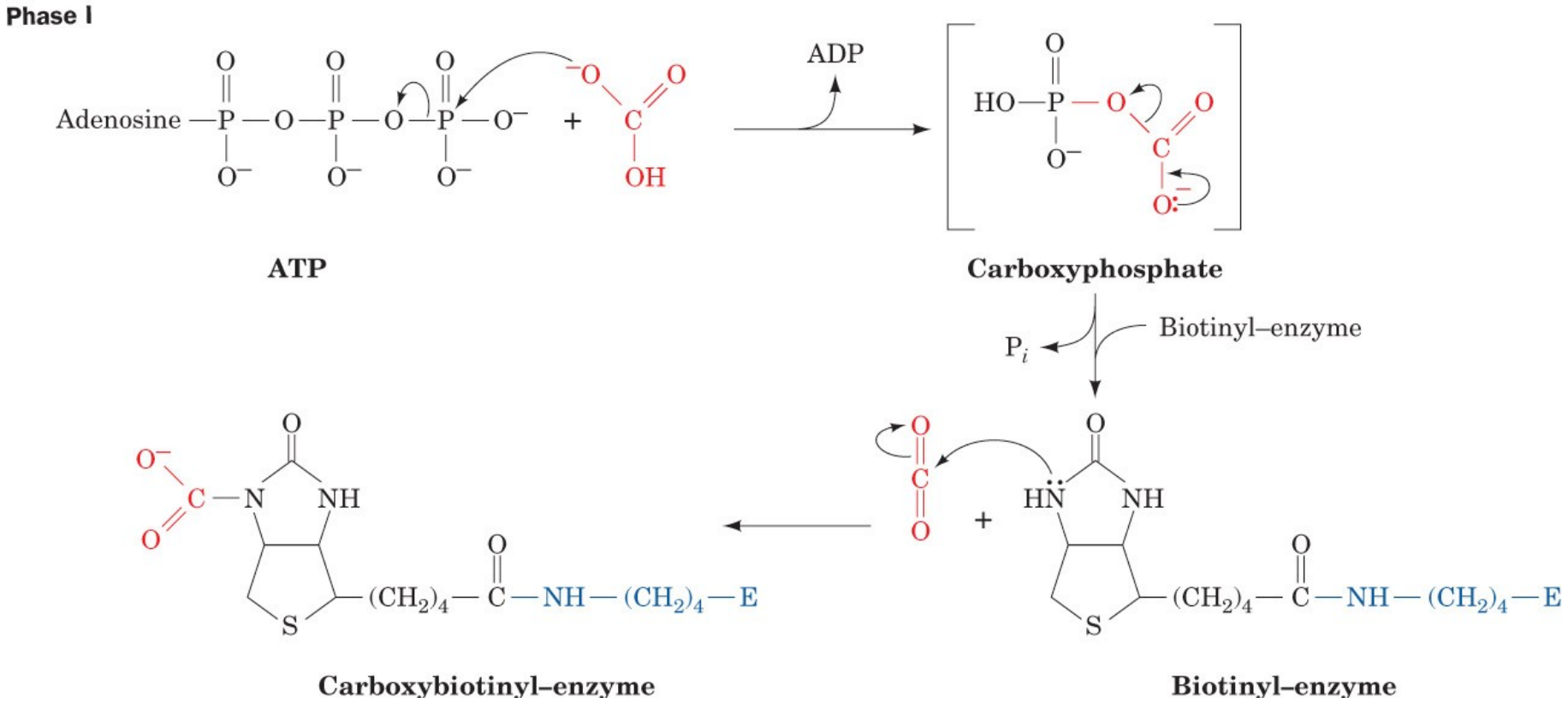


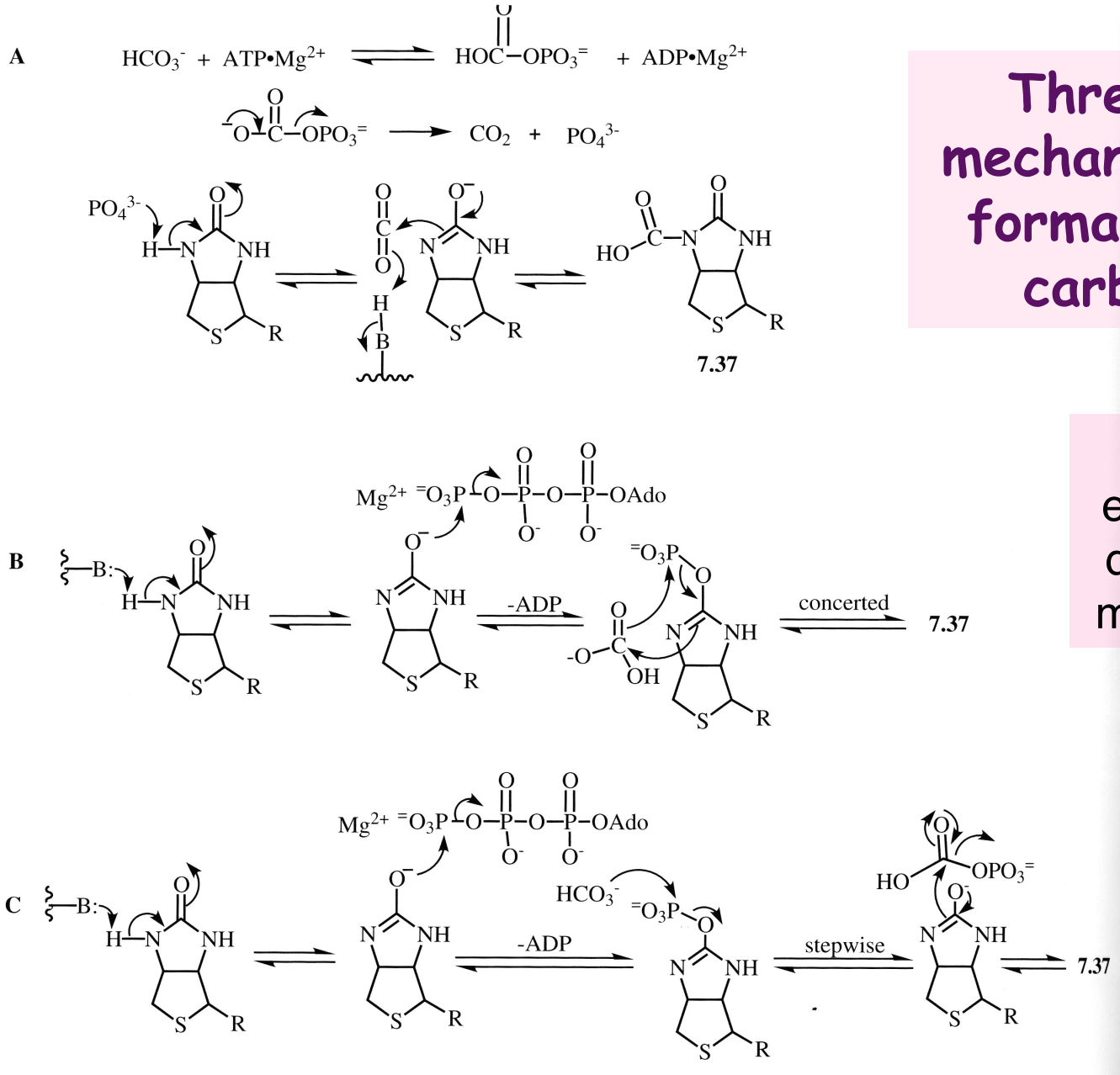
Enzyme 1: pyruvate carboxylase (PC) (requires biotin)

Enzyme 2: PEP carboxykinase (PEPCK)

What is the role of ATP in the PC reaction?

The two-phase reaction mechanism of pyruvate carboxylase: Phase I (CO_2 activation)

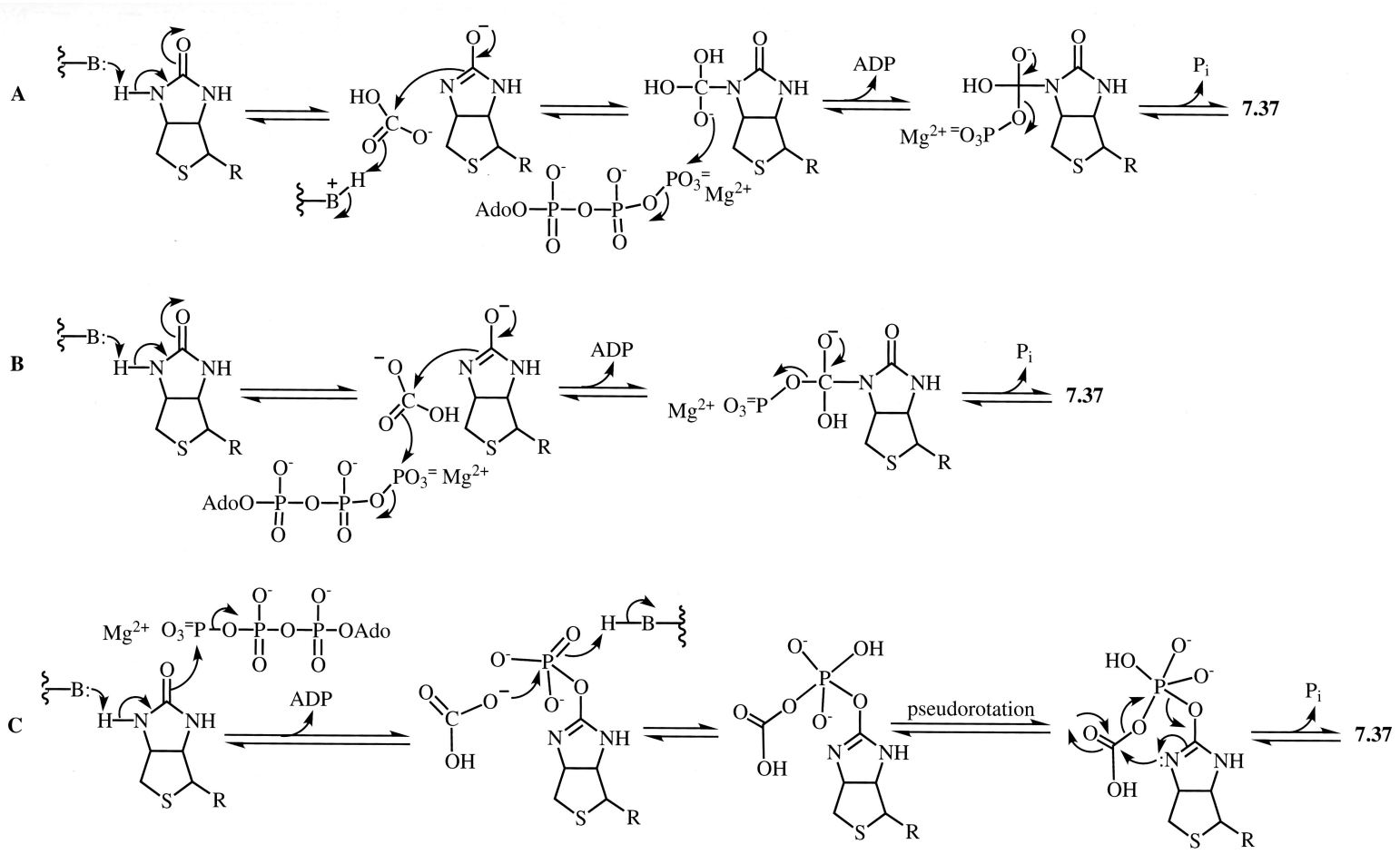




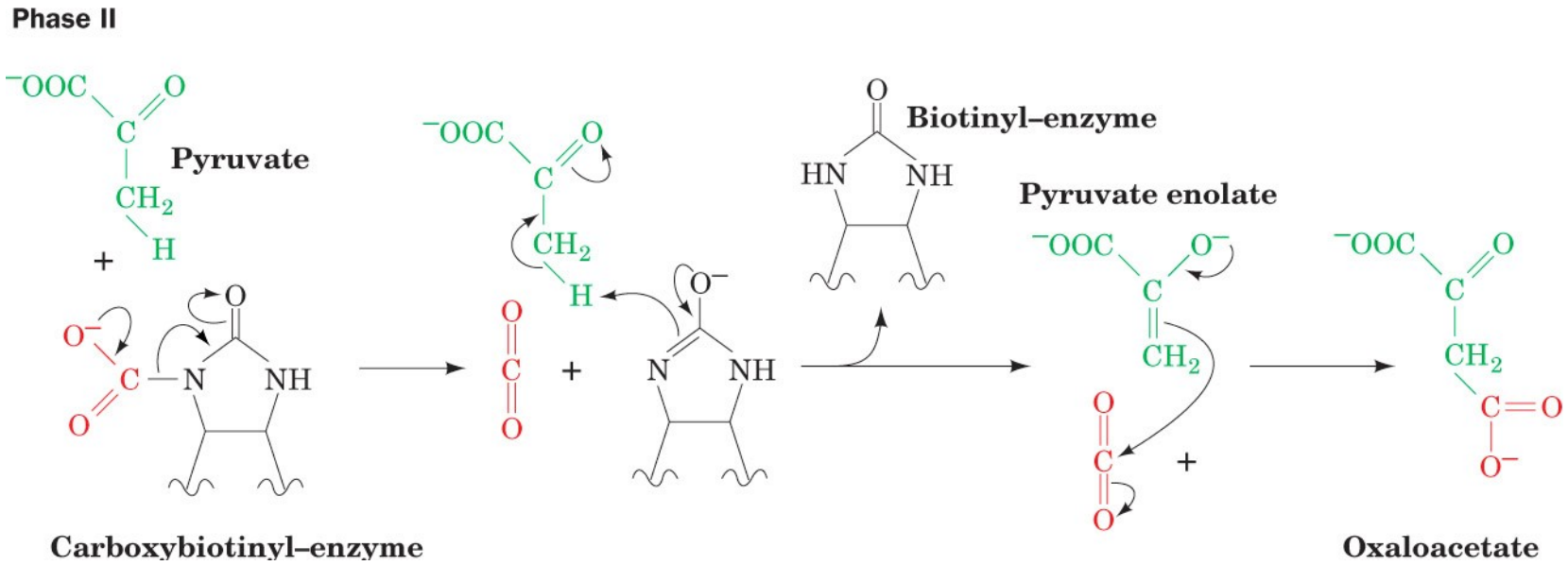
Three possible mechanisms for the formation of N1-carboxybiotin

Current experimental data support mechanism A.

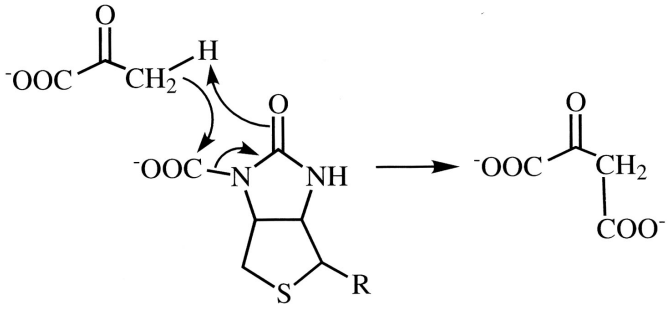
Three additional potential mechanisms for the formation of N1-carboxybiotin



The two-phase reaction mechanism of pyruvate carboxylase: Phase II (substrate carboxylation)

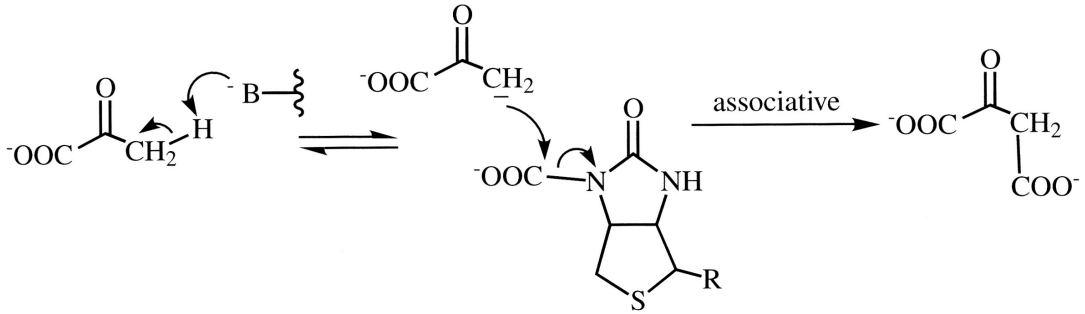


A Concerted



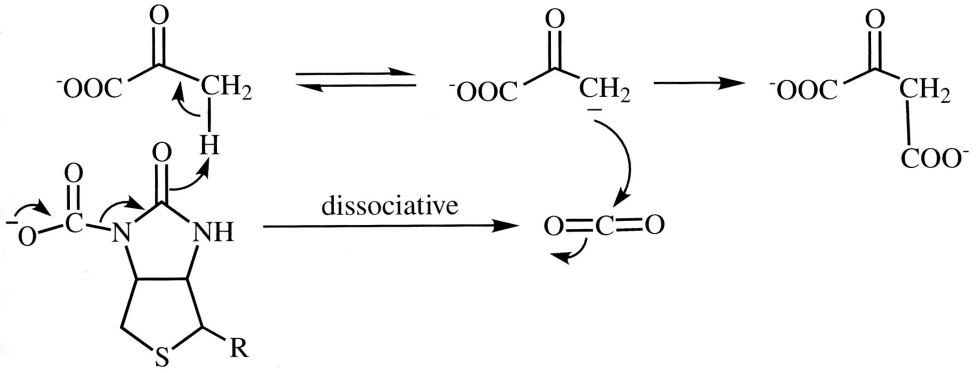
Possible mechanisms for the transfer of CO_2 from N1-carboxybiotin to substrates

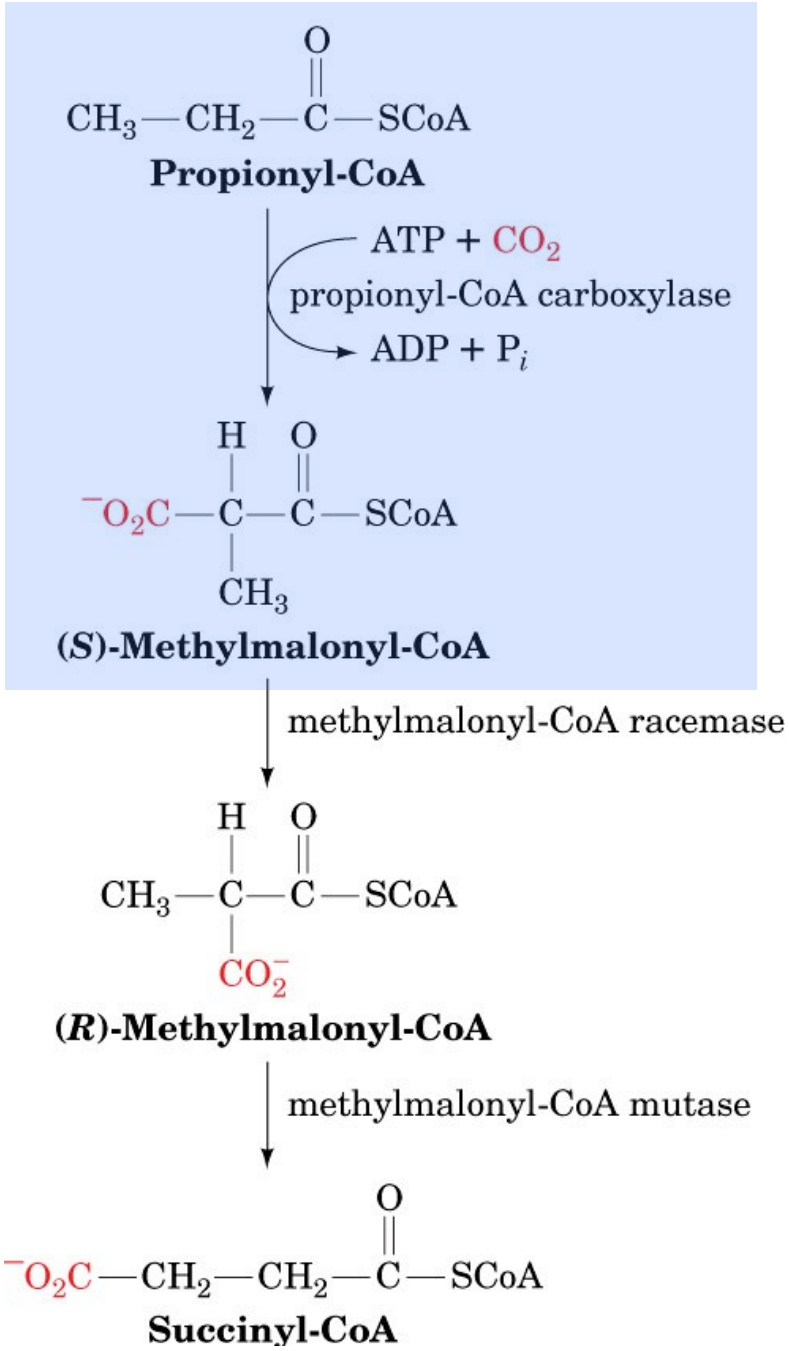
B Stepwise-associative



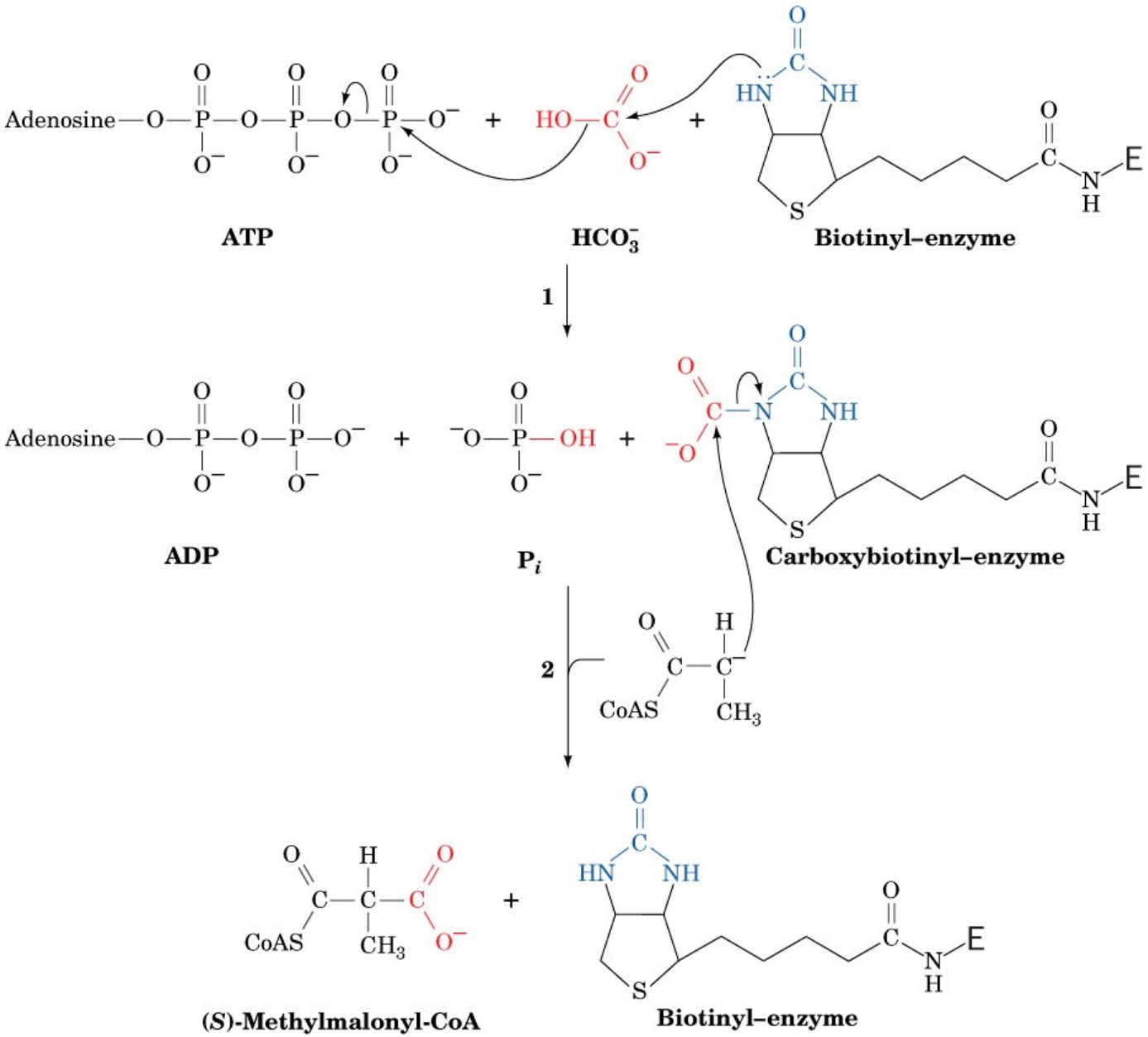
Current data support either (B) or (C).

C Stepwise-dissociative





Propionyl-CoA carboxylase: Conversion of propionyl-CoA to succinyl-CoA (degradation of odd-carbon fatty acids)



Mechanism of the propionyl-CoA carboxylase reaction; similar to that of PC

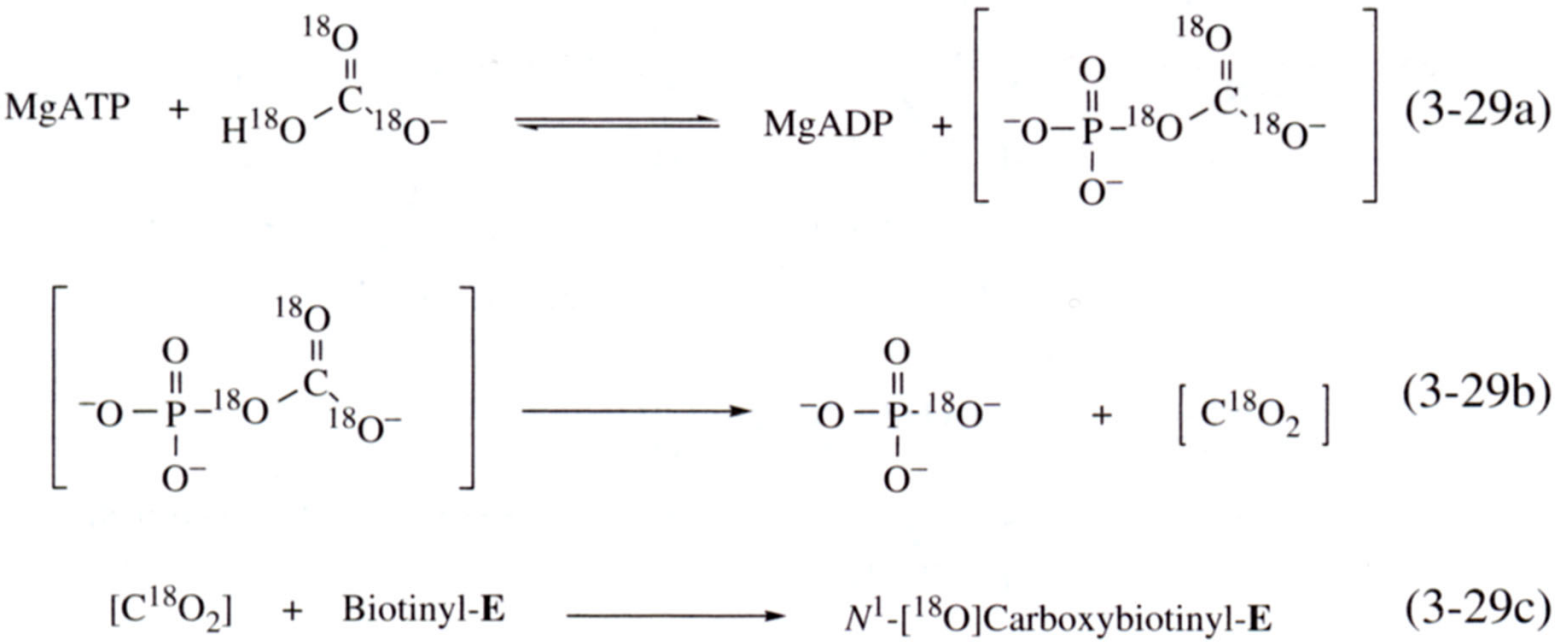
How do we know there is a carboxyphosphate intermediate?

Use of ^{18}O to monitor the fate of the bicarbonate oxygens in the propionyl-CoA carboxylase reaction

$$\text{Biotinyl-protein} + \text{ATP} + \text{HC}^{18}\text{O}_3^- \rightarrow {}^-{}^{18}\text{O}_2\text{C}\text{–Biotinyl-}\mathbf{E} + \text{ADP} + \text{H}^{18}\text{OPO}_3^{2-} \quad \text{(3-28a)}$$

$$^-{}^{18}\text{O}_2\text{C}\text{–Biotinyl-protein} + \text{CH}_3\text{–CO–SCoA} \rightarrow \text{Biotinyl-}\mathbf{E} + {}^-{}^{18}\text{O}_2\text{C}\text{–CH}_2\text{–CO–SCoA} \quad \text{(3-28b)}$$

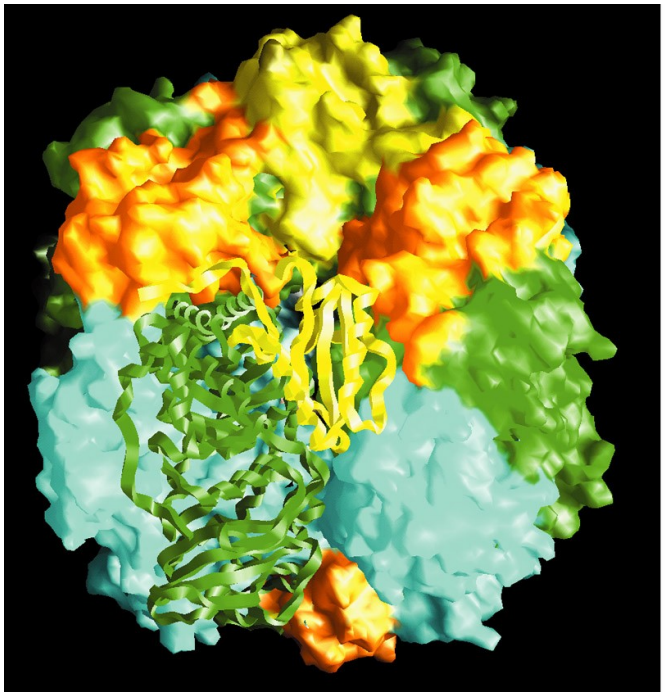
Experimental evidence of a carboxyphosphate intermediate

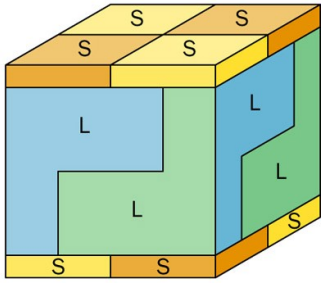


Do all enzyme-catalyzed carboxylation reactions require biotin as a coenzyme? No!

X-ray structure of tobacco ribulose 1,5-bisphosphate (RuBP) carboxylase

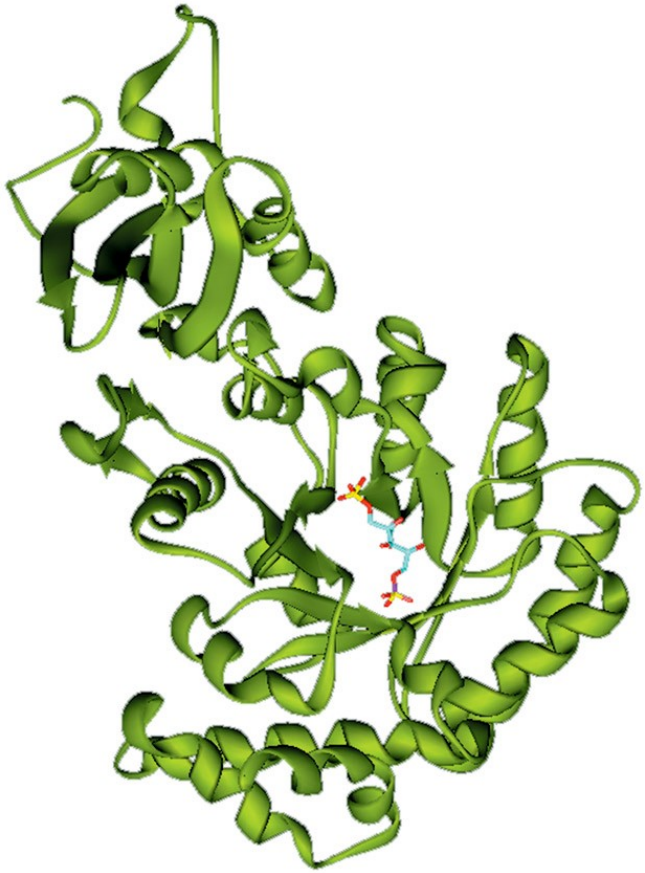
The quaternary structure of the L_8S_8 protein



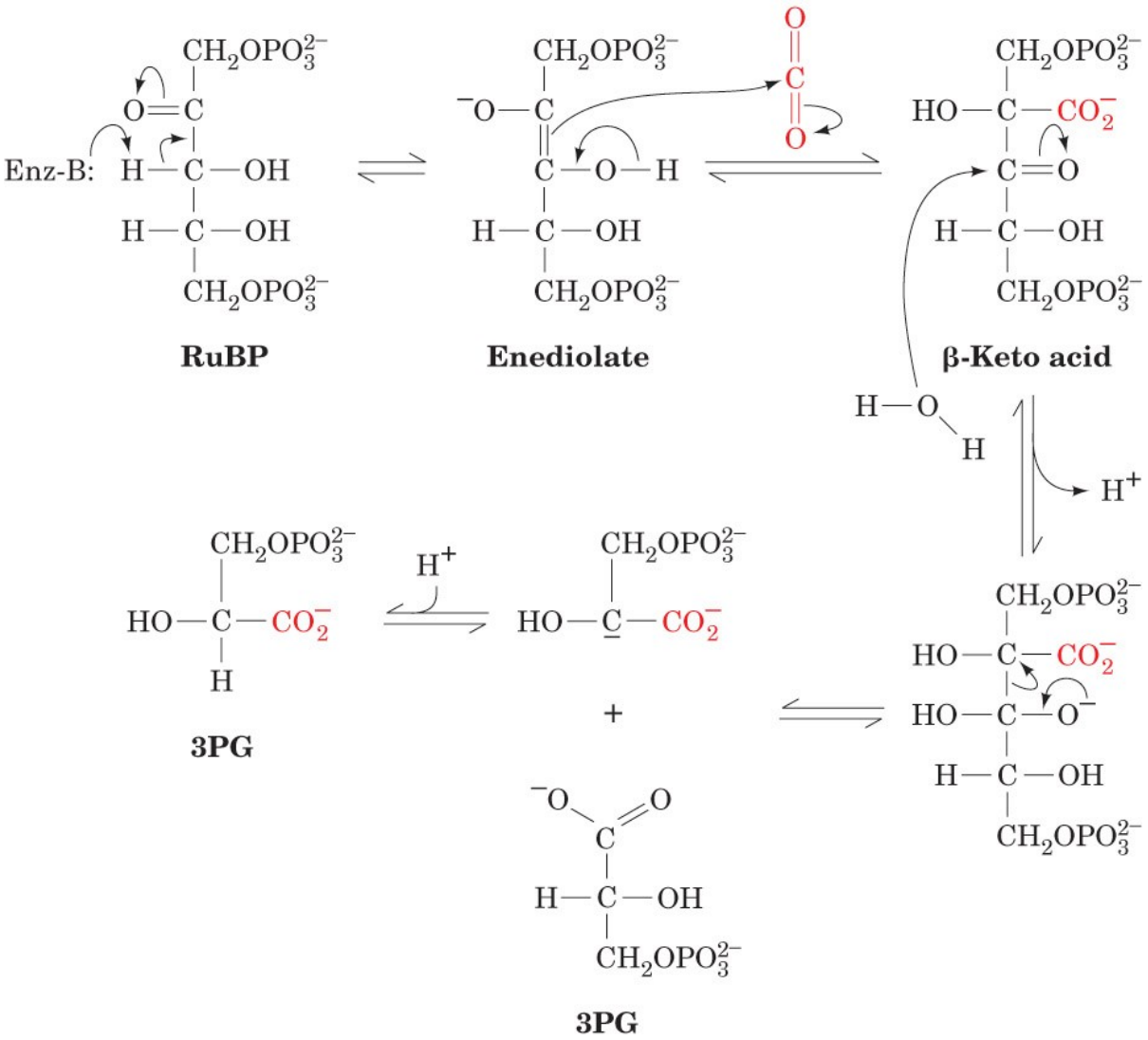


X-Ray structure of tobacco RuBP carboxylase

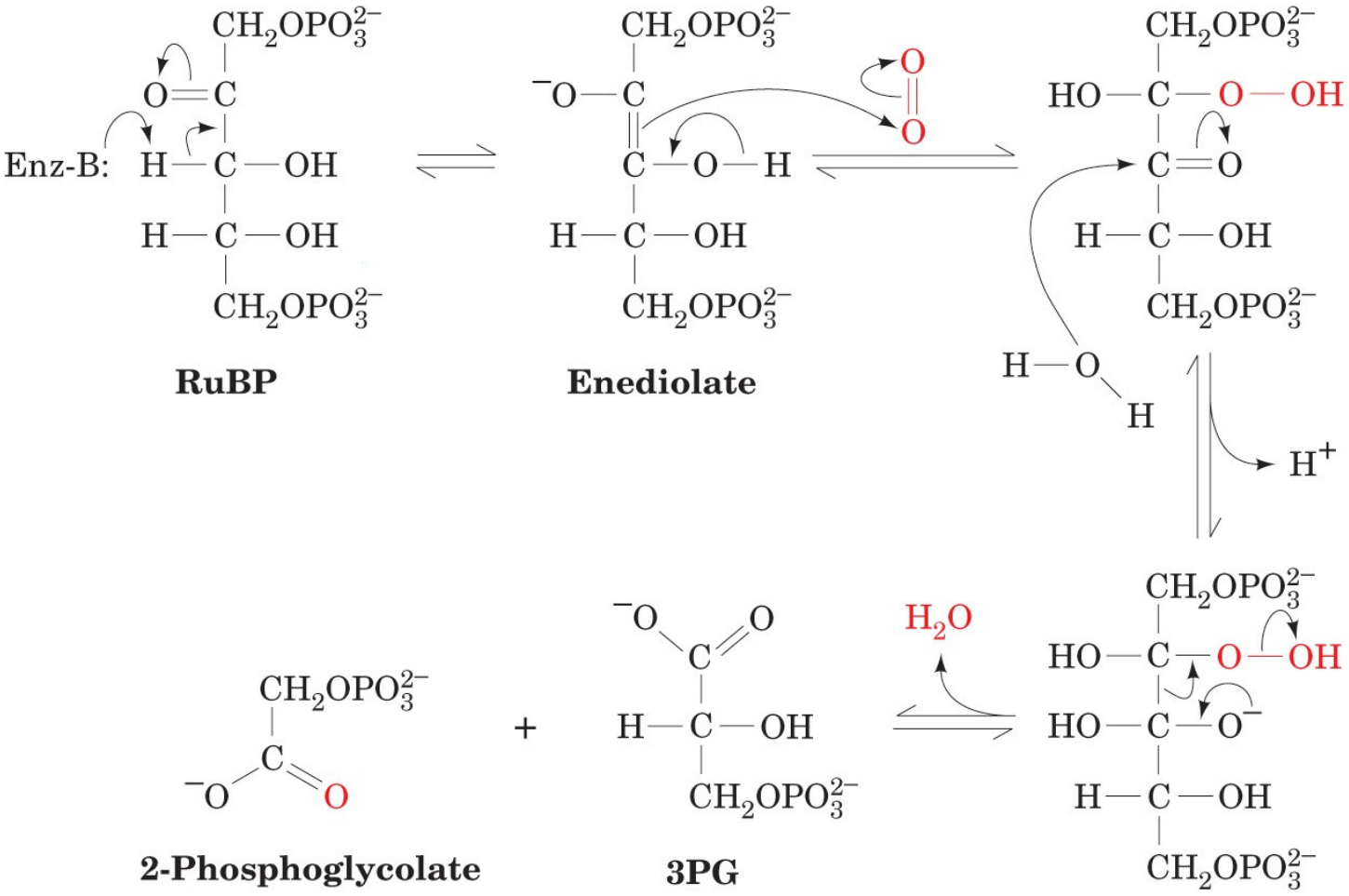
An L subunit complexed with the transition state inhibitor, 2-carboxyarabinitol-1,5-bisphosphate (CABP)



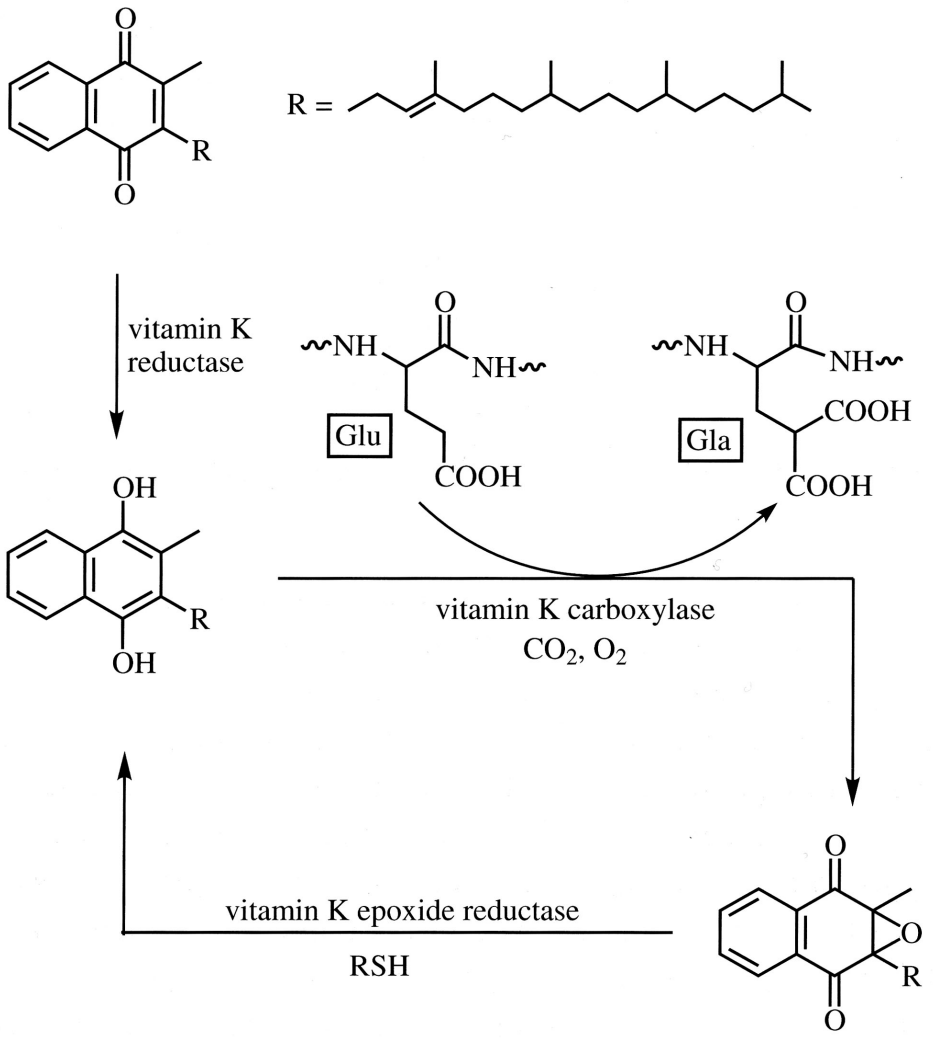
Probable reaction mechanism of the carboxylation reaction catalyzed by RuBP carboxylase



Probable mechanism of the oxygenase reaction catalyzed by RuBP carboxylase-oxygenase



CO_2 as the carboxylating agent: Vitamin K-dependent carboxylation of proteins (post-translational modification)



γ-Carboxyglutamylation of proteins: reduced vitamin K is an obligatory substrate for a carboxylase that activates proteins in the blood-clotting cascade

Example of a blood-clotting cascade

Carboxylation of prothrombin: vitamin K-dependent

↓

Ca^{2+}-prothrombin + factors Va and Xa:
bind to membrane surface

↓

Cleavage of prothrombin by factor Xa gives thrombin

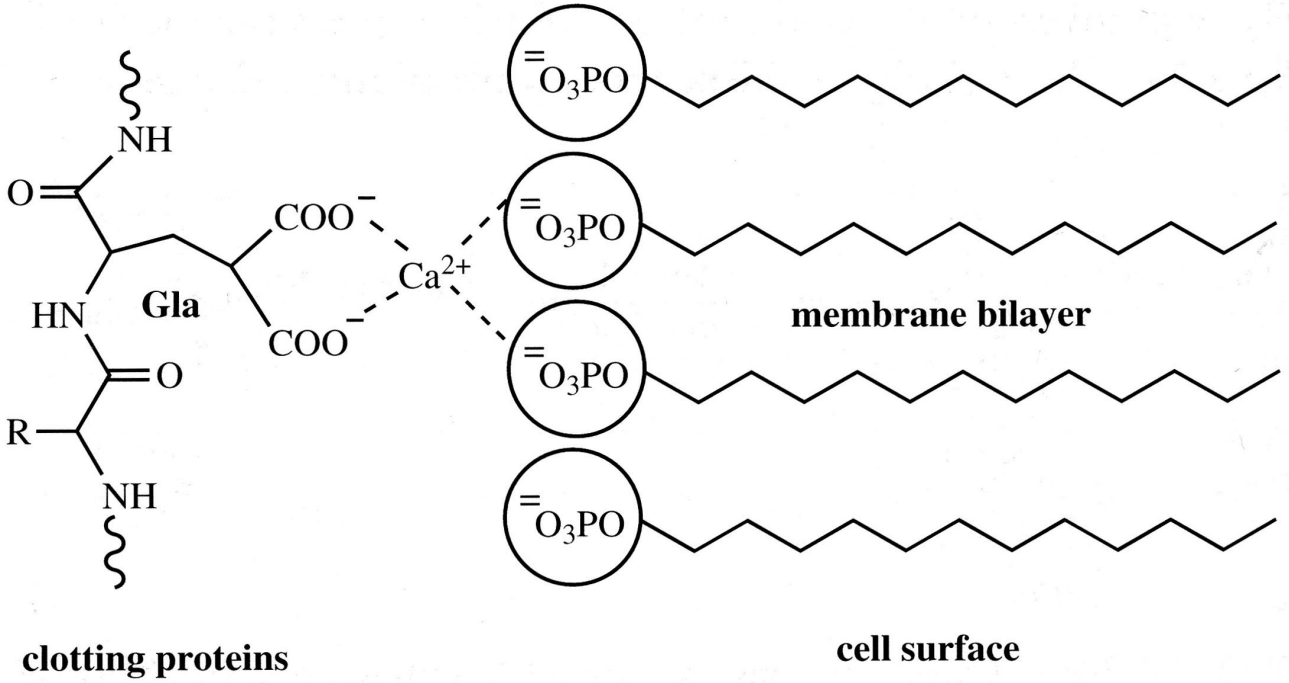
↓

Thrombin cleaves fibrinogen to fibrin

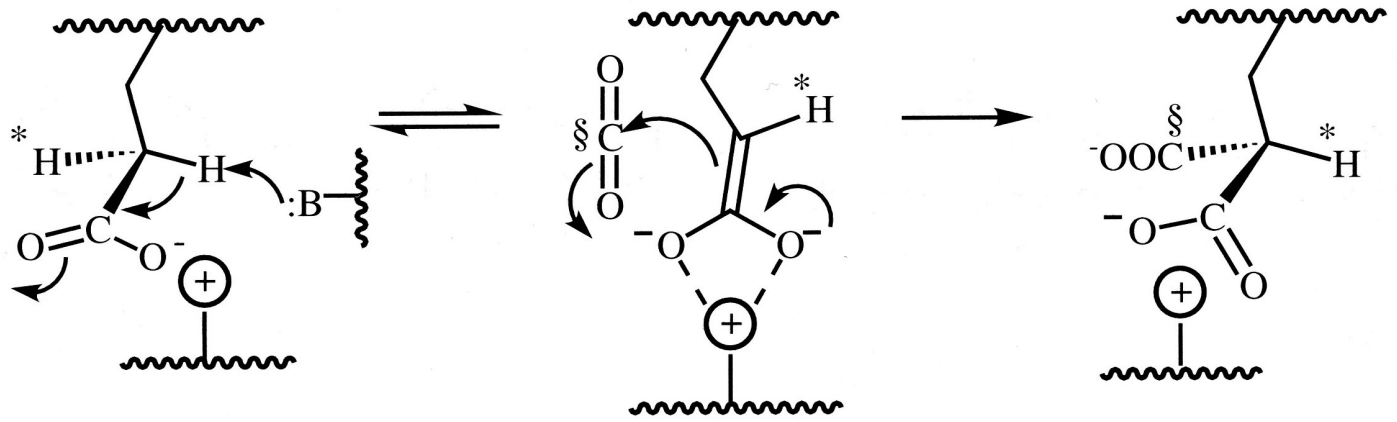
↓

Fibrin induces blood-clotting

Calcium-dependent binding of clotting proteins to cell surfaces

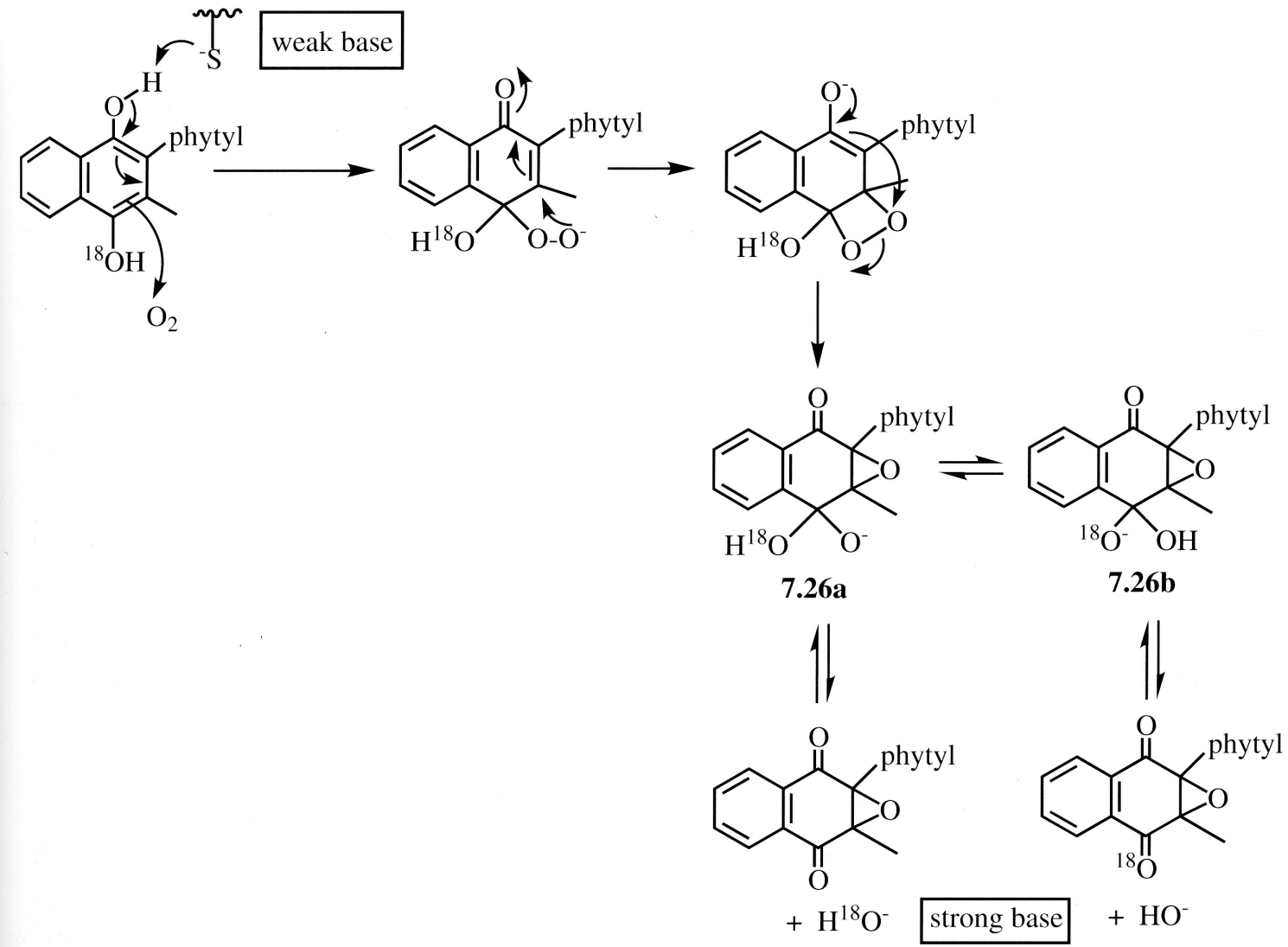


Proposed vitamin K carboxylase-catalyzed carboxylation of glutamate residues of proteins via a carbanionic intermediate



What is the role of reduced vitamin K?

The *base strength amplification* (BSA) mechanism

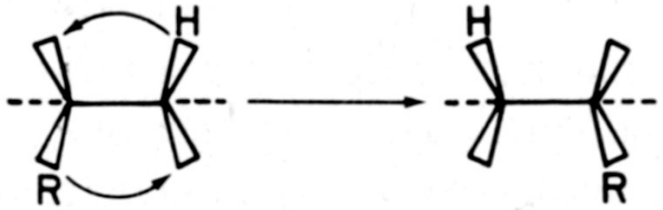


Explanation of the BSA mechanism

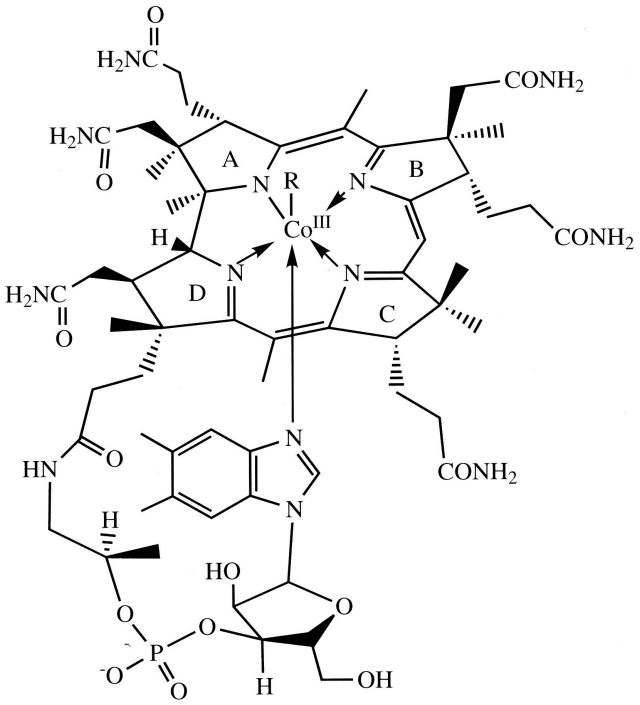
A weak base (active site cysteine) removes the hydroquinone proton from reduced vitamin K, which then reacts with O_2, leading to the strong base, the ketal anion. This anion does not remove the glutamate proton directly, but rather, the elimination product, *hydroxide ion*, is proposed to be the strong base involved in this abstraction.

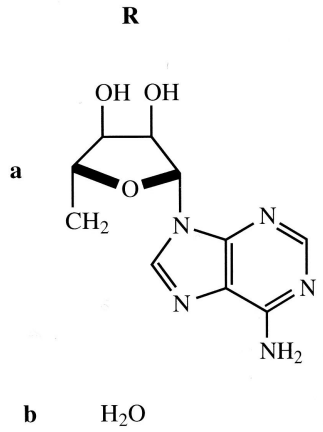
The function of vitamin K appears to be to convert O_2 into hydroxide anion in a hydrophobic environment where it can deprotonate Glu residues. This is more effective than aqueous hydroxide because bases are known to be stronger in hydrophobic solvents than in aqueous media.

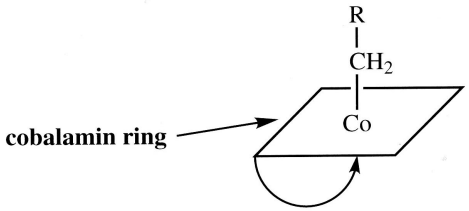
Coenzyme B_{12} Mediator of 1,2-shift rearrangements



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



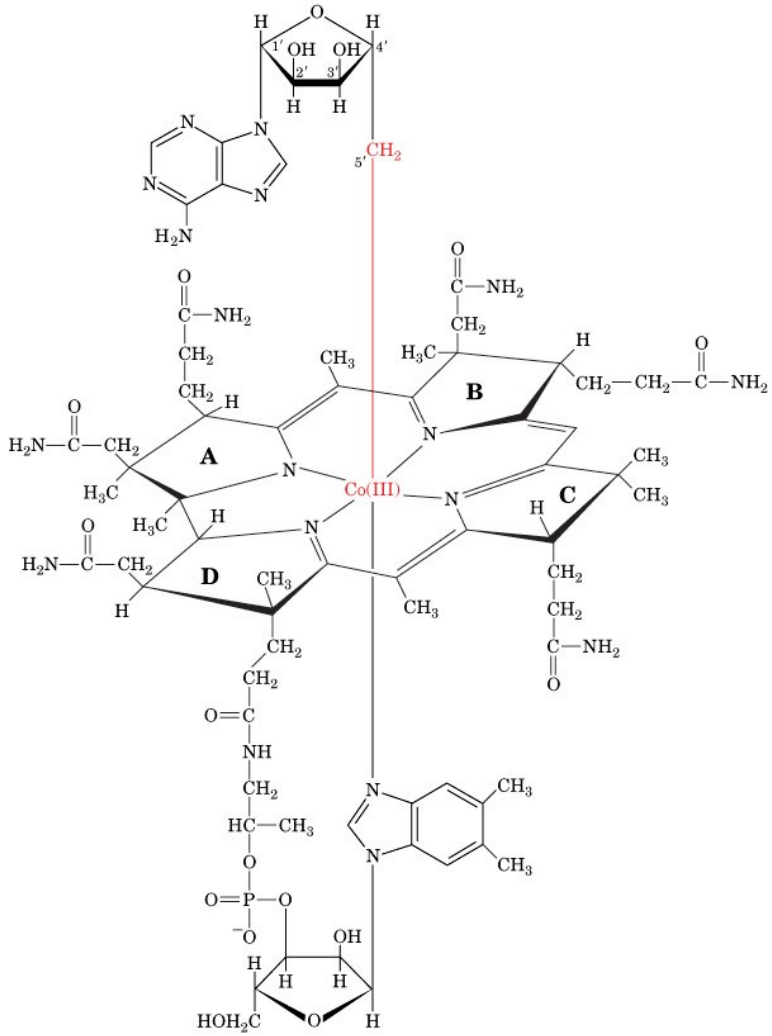




Vitamin B_{12}: R = b

Coenzyme B_{12}: R = a
(adenosylcobalamin
or AdoCbl)

Structure of 5' -deoxyadenosylcobalamin (coenzyme B_{12})



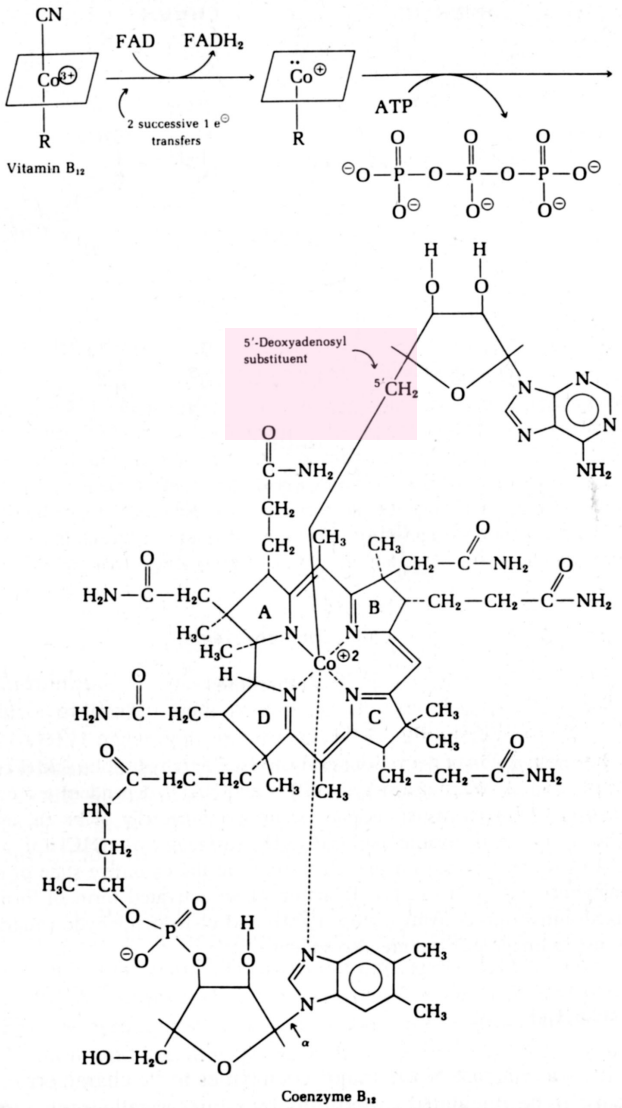
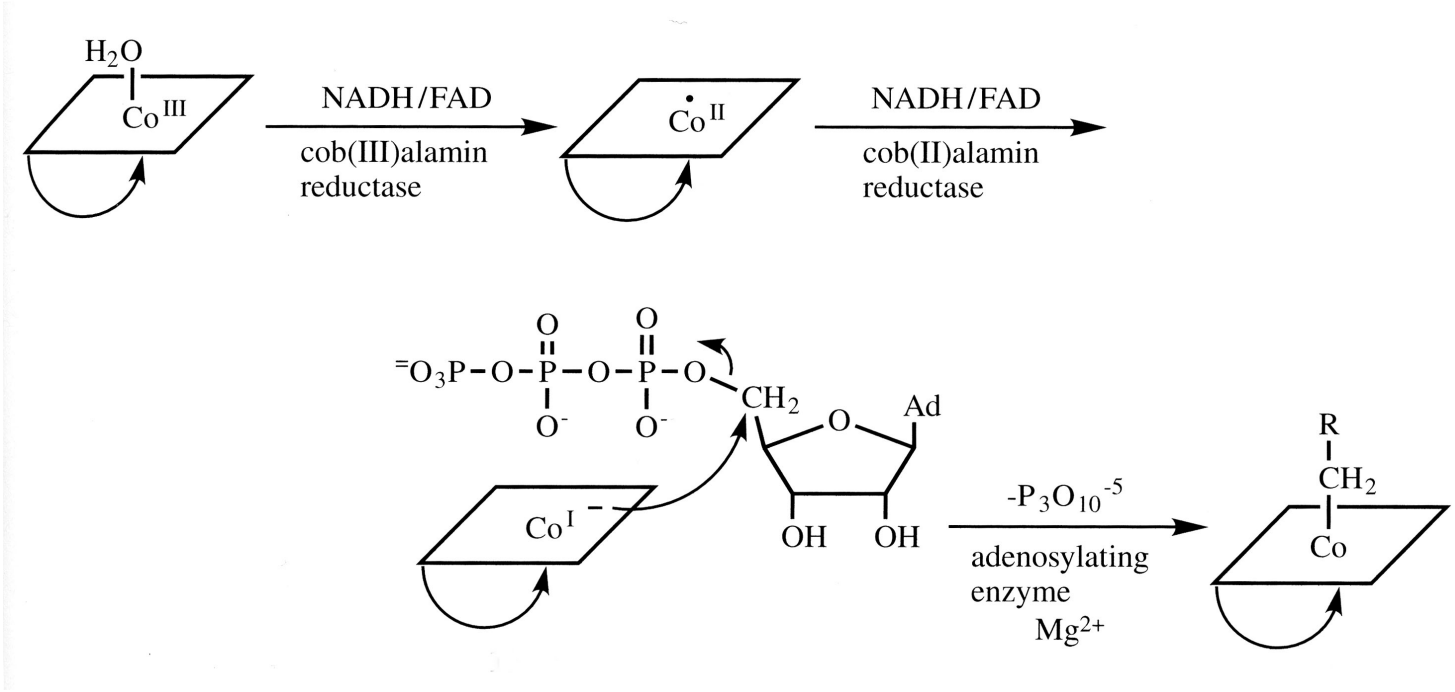


Figure 4.18 Conversion of vitamin B_{12} to coenzyme B_{12}, 5′-deoxyadenosyl cobalamine. The conversion process involves reduction of the cobalt atom in the vitamin from Co^{+3} to Co^{+}. Co^{+} is a good nucleophile which displaces the triphosphate moiety of ATP yielding 5′-deoxyadenosyl cobalamine (coenzyme B_{12}). The 5′-methylene group of the deoxyadenosyl substituent, which is covalently bound to the cobalt atom in the corrin ring, is the reactive moiety in most coenzyme B_{12}-dependent enzyme-catalyzed reactions.

Biosynthetic conversion of vitamin B_{12} to coenzyme B_{12} (5' -deoxyadenosyl cobalamin)

Another scheme on the biosynthesis of coenzyme B_{12}



Cob(I)alamin is one of the most powerful nucleophiles known; the absolute reactivities of Co(I) nucleophiles are up to 10^7 times greater than those of iodide ion.

Coenzyme B_{12}-dependent enzyme-catalyzed reactions

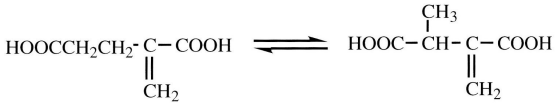
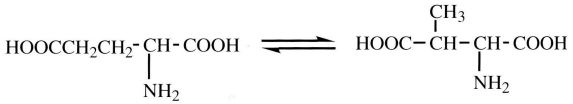
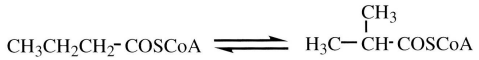
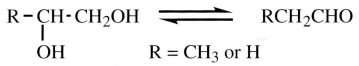
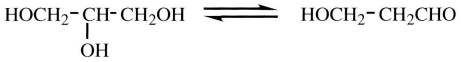
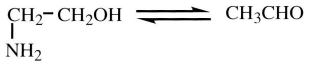
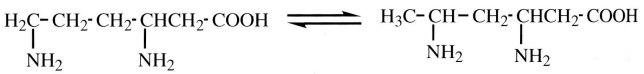
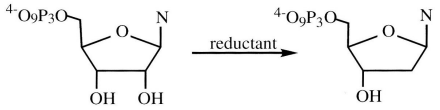
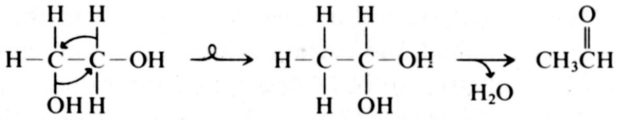
Enzyme	Reaction catalyzed
CARBON SKELETAL REARRANGEMENTS	
Methylmalonyl-CoA mutase	
2-Methyleneglutarate mutase	
Glutamate mutase	
Isobutyryl-CoA mutase	
ELIMINATIONS	
Diol dehydratase	
Glycerol dehydratase	
Ethanolamine ammonia lyase	
ISOMERIZATIONS	
L-β-Lysine-5,6-aminomutase	
D-Ornithine-4,5-aminomutase	
REDUCTION	
Ribonucleotide reductase	

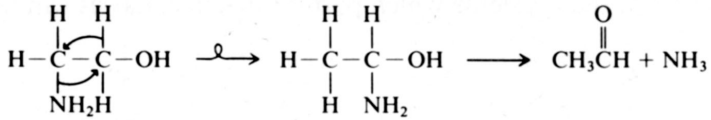
TABLE 4.3 REPRESENTATIVE REACTIONS CATALYZED BY COENZYME B_{12}-DEPENDENT ENZYMES

I. 1,2-shift reactions (internal oxido-reduction)

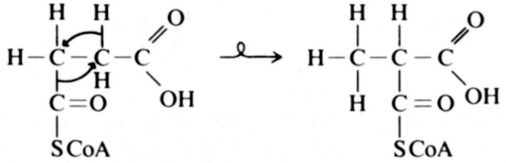
A. C—O bond broken: (diol dehydrase)



B. C—N bond broken: (ethanolamine ammonia lyase)

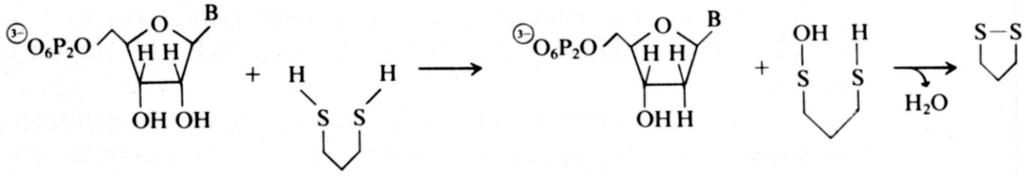


C. C—C bond broken: (methyl malonyl-CoA mutase)



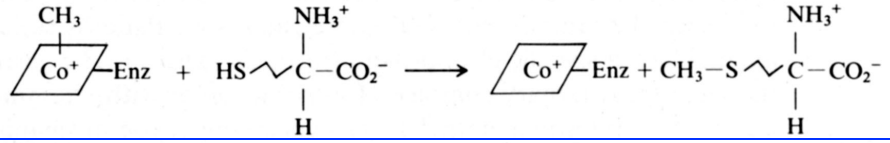
II. Shift reaction between two molecules (external oxido-reduction)

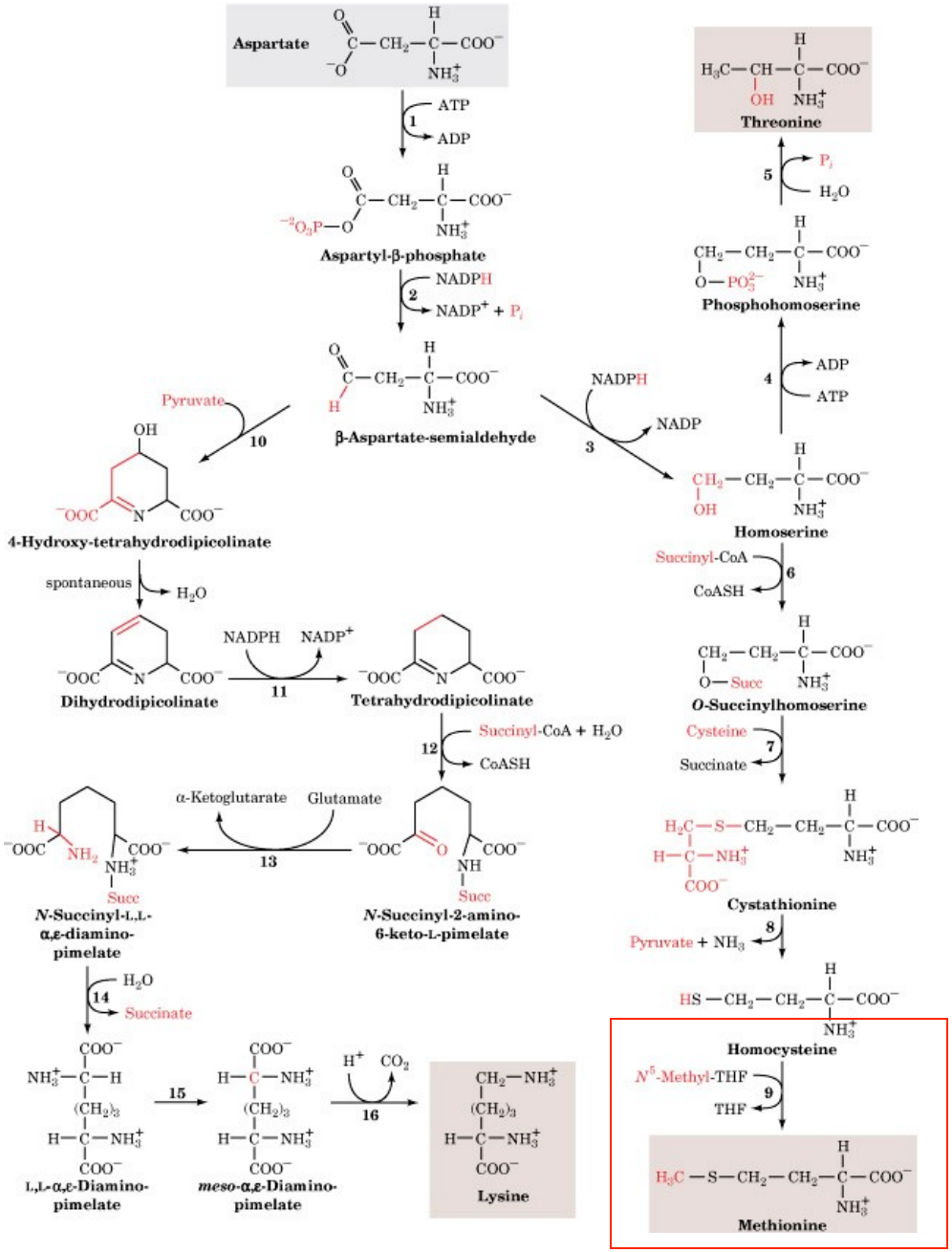
A. C—O bond broken: (ribonucleotide reductase)



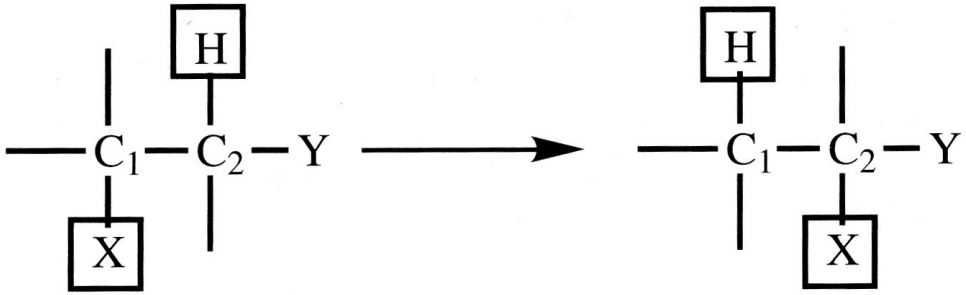
III. Methyl transfer reaction (methyl transferase)

A. C—S bond formed: (methionine synthase)

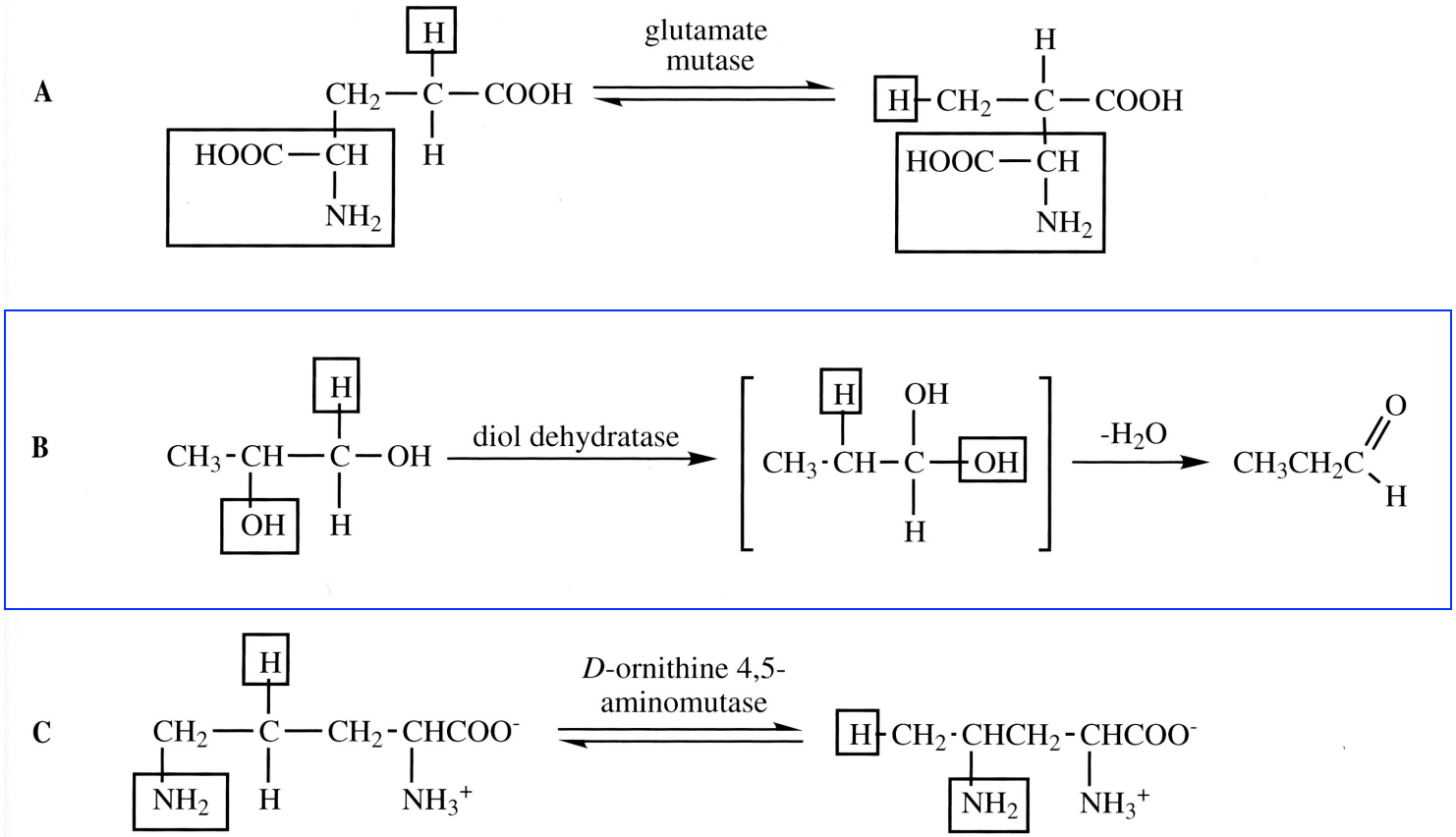



Aspartate
ATP
1
ADP
Aspartyl-β-phosphate
NADPH
2
NADP+ + Pi
β-Aspartate-semialdehyde
Pyruvate
10
4-Hydroxy-tetrahydrodipicolinate
spontaneous
H2O
Dihydrodipicolinate
NADPH
NADP+
11
Tetrahydrodipicolinate
12
Succinyl-CoA + H2O
CoASH
α-Ketoglutarate
Glutamate
13
N-Succinyl-L,L-α,ε-diamino-pimelate
N-Succinyl-2-amino-6-keto-L-pimelate
14
H2O
Succinate
L,L-α,ε-Diamino-pimelate
15
meso-α,ε-Diamino-pimelate
H+
CO2
16
Lysine
NADPH
3
NADP
Threonine
5
Pi
H2O
Phosphohomoserine
4
ADP
ATP
Homoserine
Succinyl-CoA
6
CoASH
O-Succinylhomoserine
Cysteine
7
Succinate
Cystathionine
8
Pyruvate + NH3
Homocysteine
N5-Methyl-THF
9
THF
Methionine

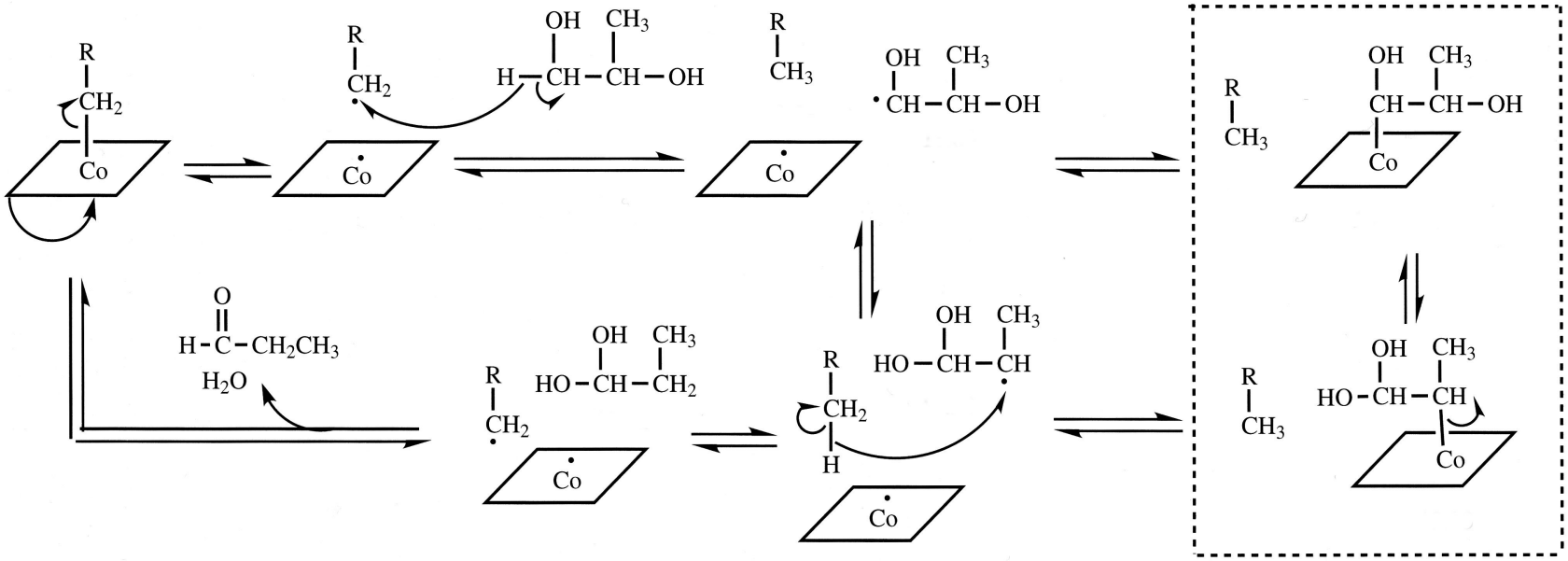
The general form of coenzyme B_{12}-dependent rearrangements



Three examples of coenzyme B_{12}-dependent rearrangements showing how the hydrogen and an adjacent group appear to exchange places



Proposed mechanism of diol dehydratase. The part shown in the dashed box is more speculative than the rest of the mechanism.



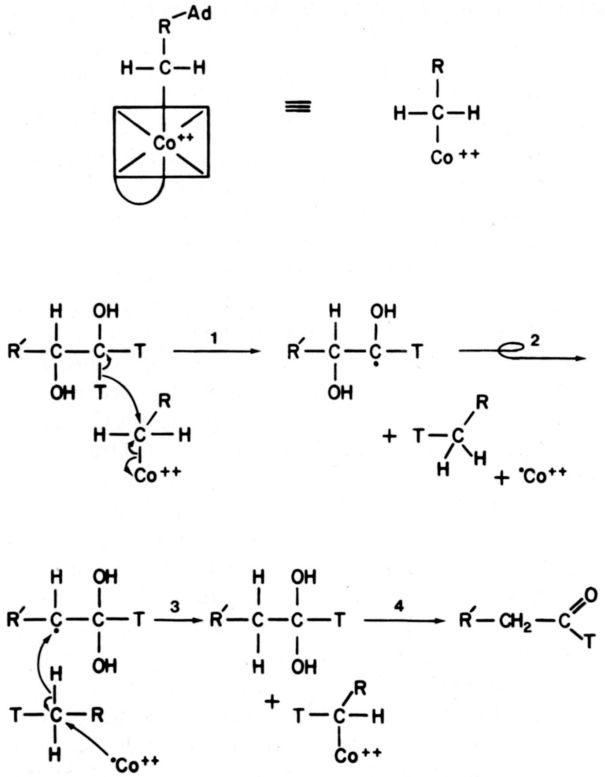


Figure 4.19 Proposed mechanism of the diol dehydrase reaction. A free radical mechanism is proposed. T in the figure represents a tritium (^3H) atom which in reaction 1 is transferred from the substrate to the 5′-methylene group in the coenzyme, generating two free radical species. The substrate then rearranges, resulting in transfer of the 2-hydroxyl group to the carbonyl carbon, C1 (reaction 2). Next, a hydrogen atom (H) is transferred to the substrate from the coenzyme while the cobalt free radical attacks the methyl group in the 5′-deoxyadenosyl moiety of the coenzyme (reaction 3). Because of the equivalence of the three hydrogen atoms in the methyl group of the 5′-deoxyadenosyl moiety, a tritium atom can be retained in the coenzyme as is shown. Finally, loss of water from the substrate (reaction 4) occurs spontaneously. Note that one third of the tritium atoms incorporated into coenzyme B_{12} from the 1-position of the substrate, propanediol, should be incorporated into the 2-position of propionaldehyde in a single stoichiometric transfer reaction involving a single substrate and a single enzyme molecule.

Proposed mechanism of diol dehydrase, a coenzyme B_{12}-requiring enzyme

Use of tritium
to determine the
fate of hydrogen
during enzyme
catalysis involving B_{12}