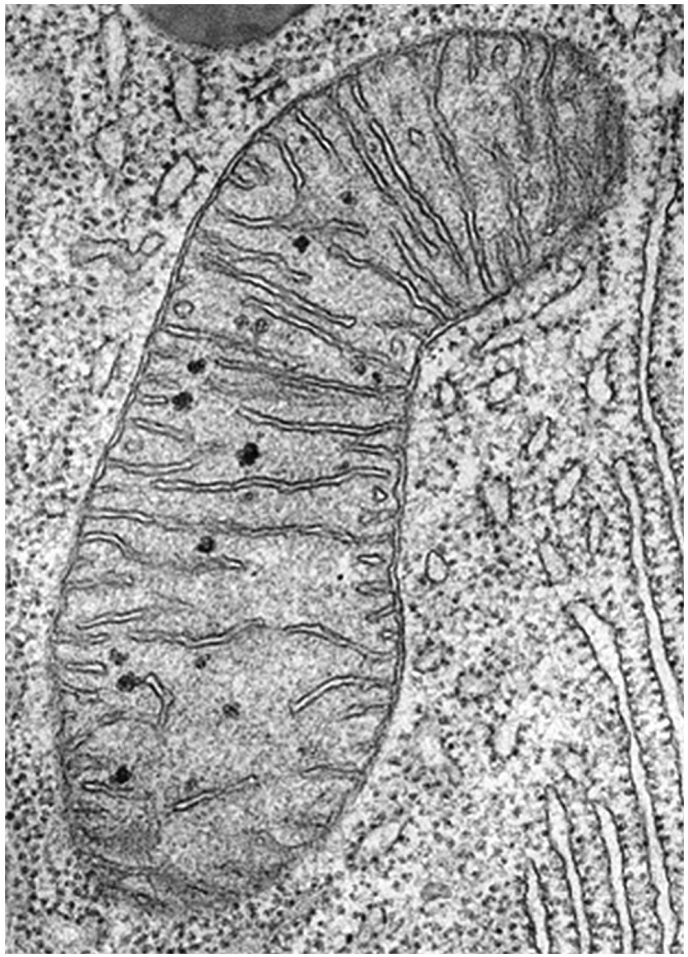
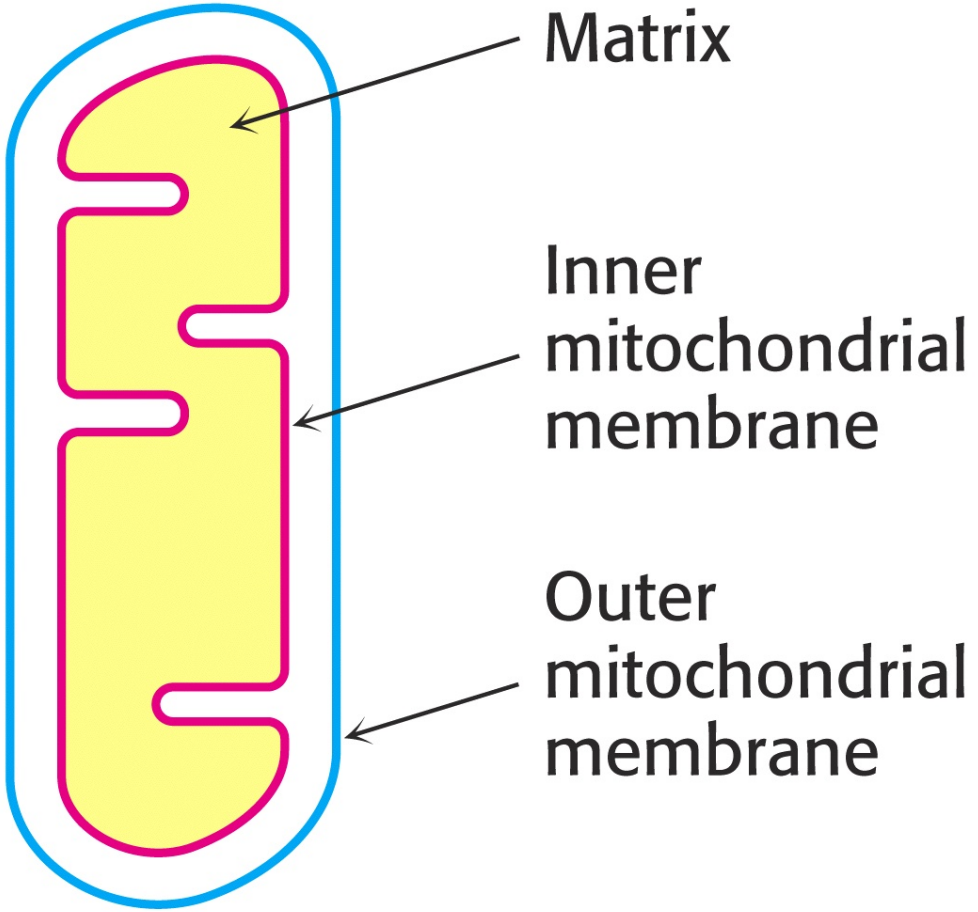
Krebs cycle

Stryer's 6th edition, chapter 17

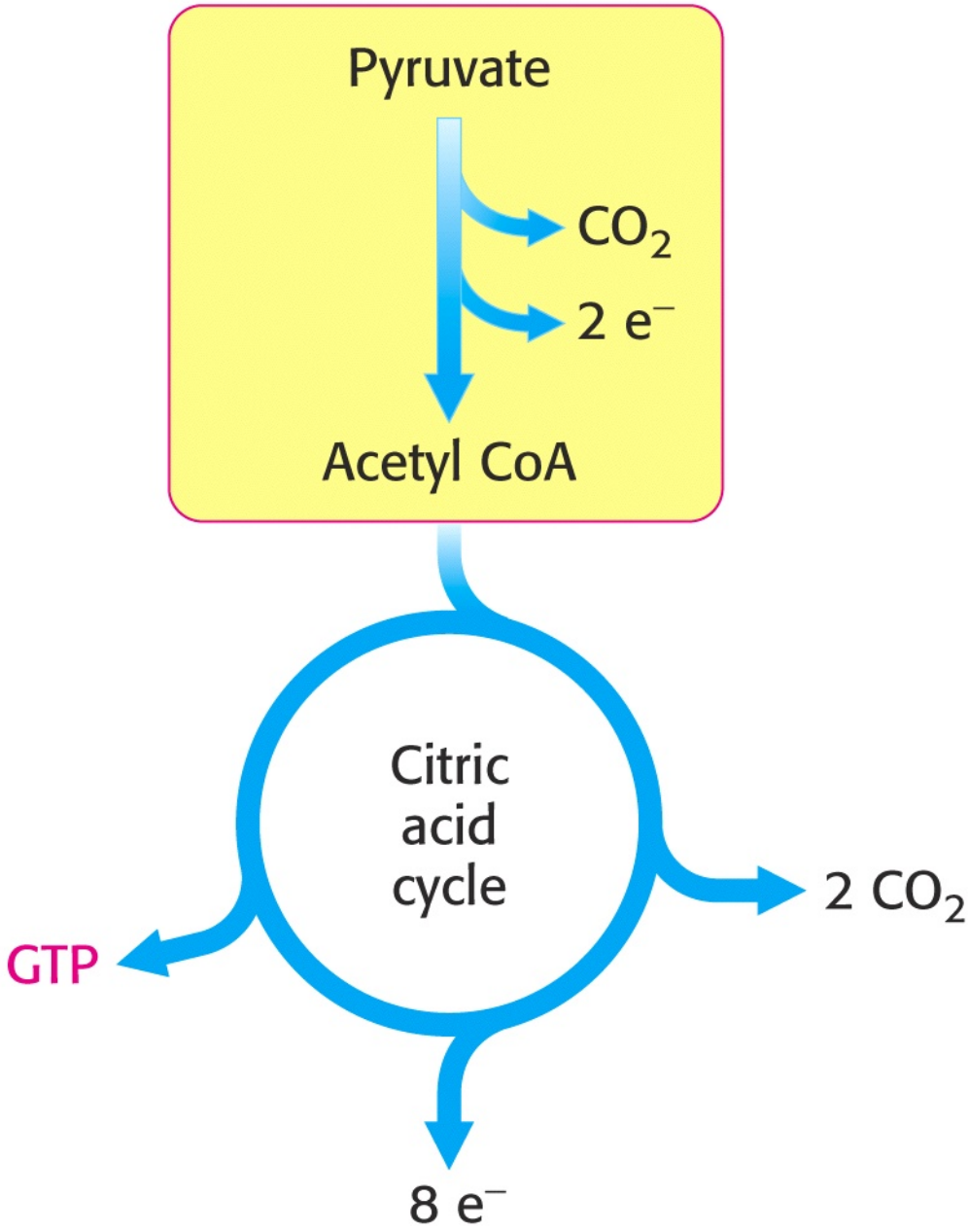
The Krebs cycle

- The final pathway for the oxidation of fuel molecules: carbohydrates, fatty acids, and amino acids.
- Hence it is an aerobic process.
- More than 90% of our energy.
- It also supplies building blocks
- Also known as the:
  - Citric acid cycle
  - Tricarboxylic acid cycle

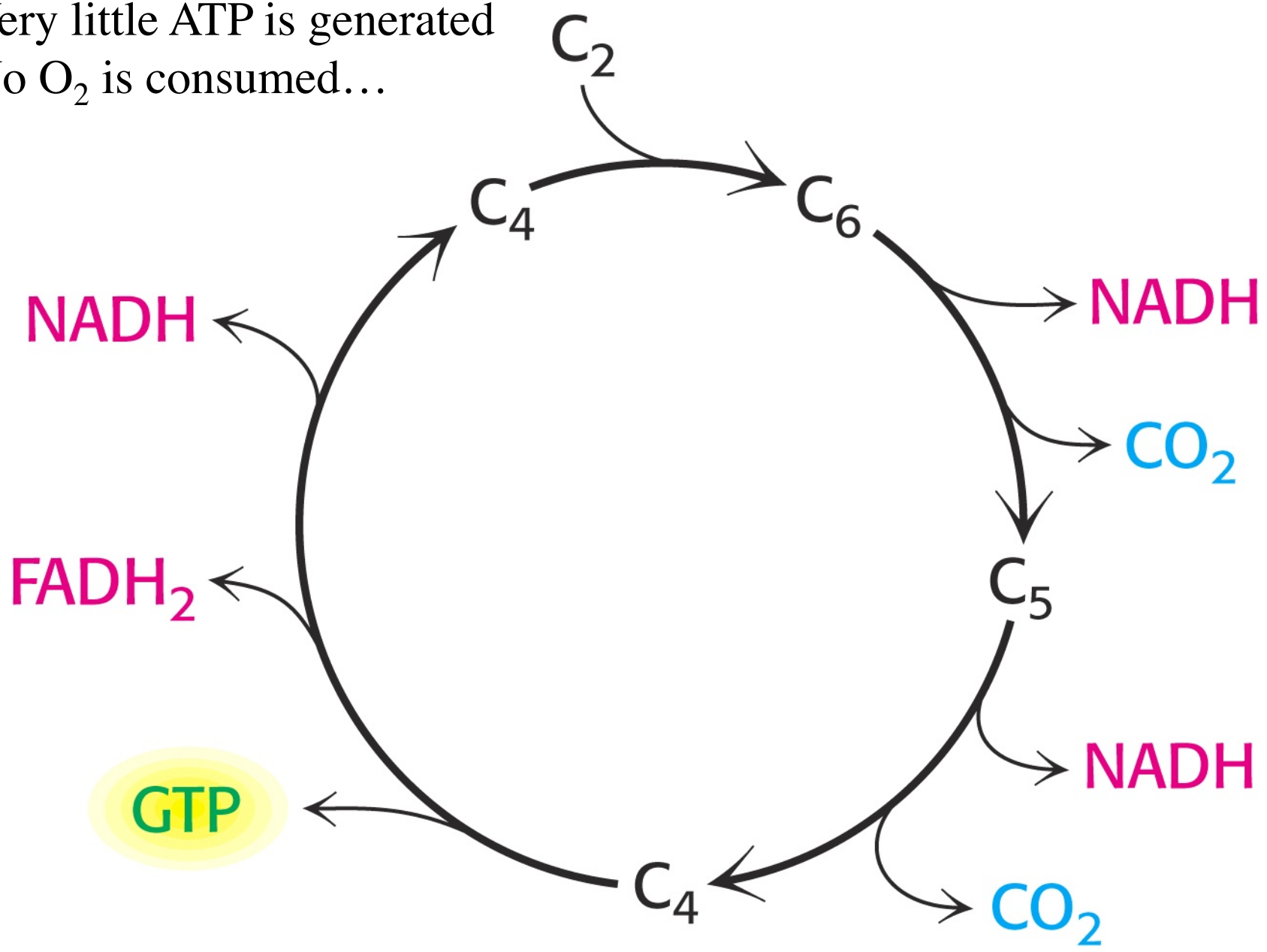



Matrix
Inner mitochondrial membrane
Outer mitochondrial membrane

The input from glycolysis



Very little ATP is generated
No O_2 is consumed…



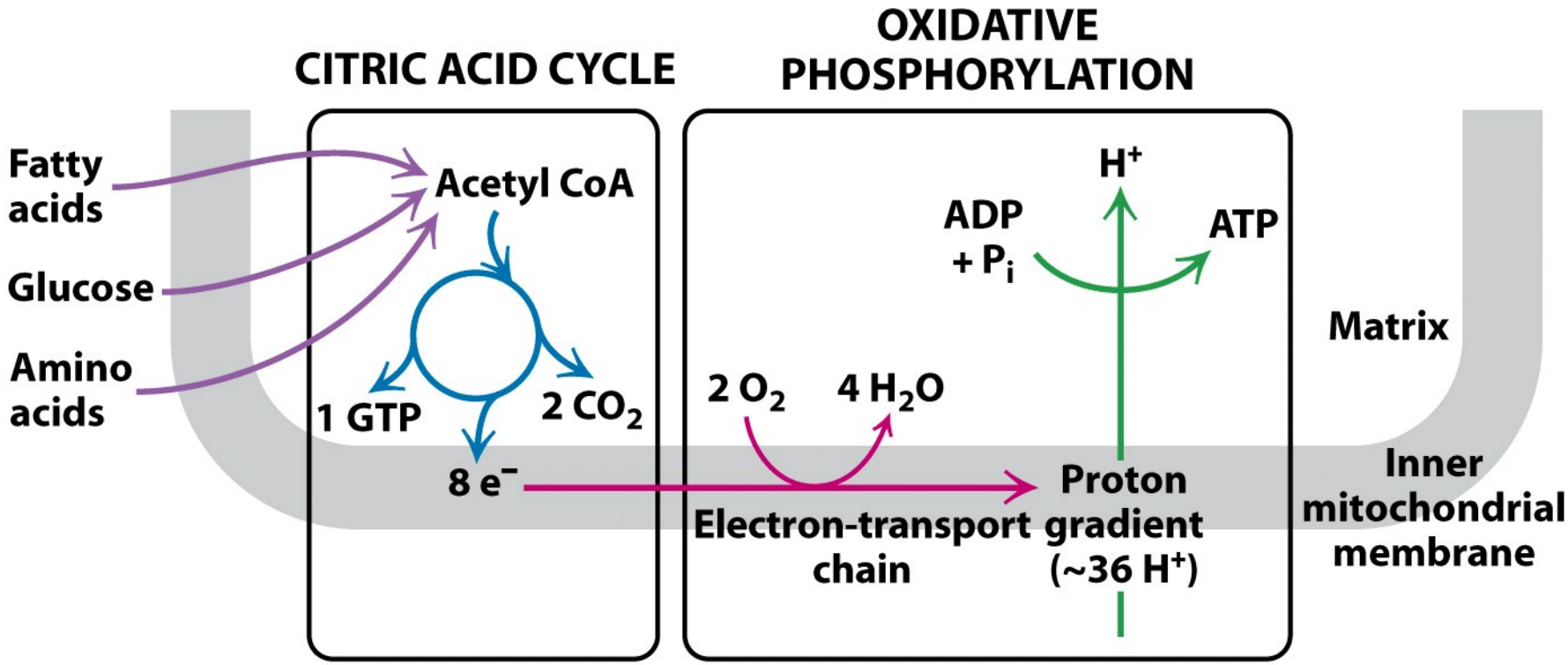
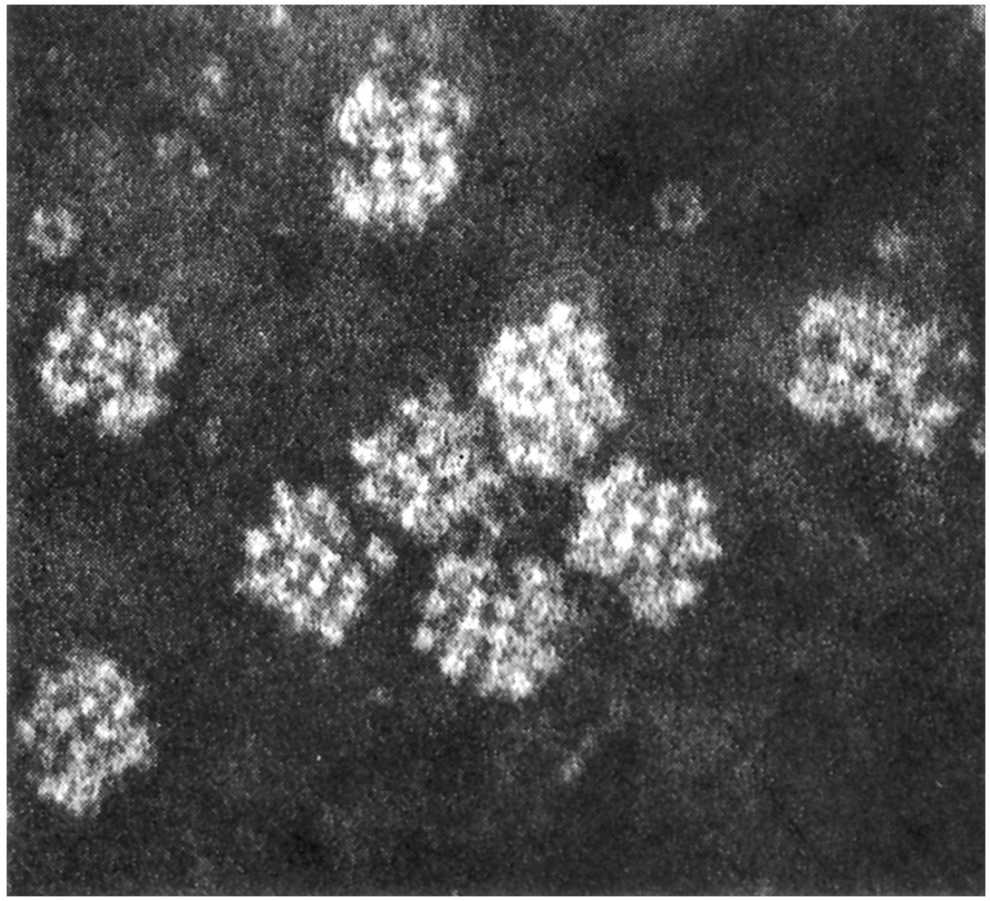


Figure 17-3
Biochemistry, Sixth Edition

Linking glycolysis and Krebs

- Under aerobic conditions glycolysis terminates at pyruvate.
- Pyruvate dehydrogenase (4-10 MD) links the two pathways/cycles.
- It oxidatively decarboxylates pyruvate into acetyl CoA in several steps…



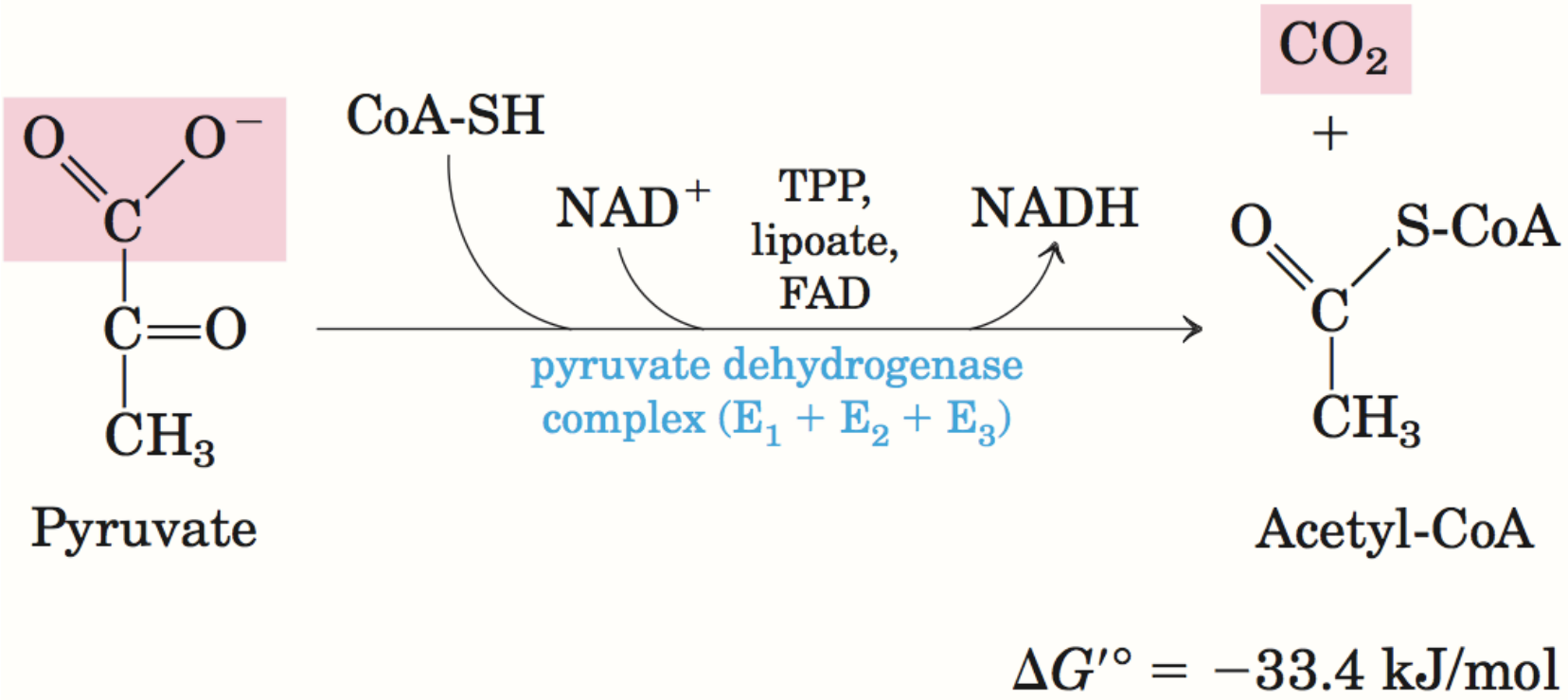
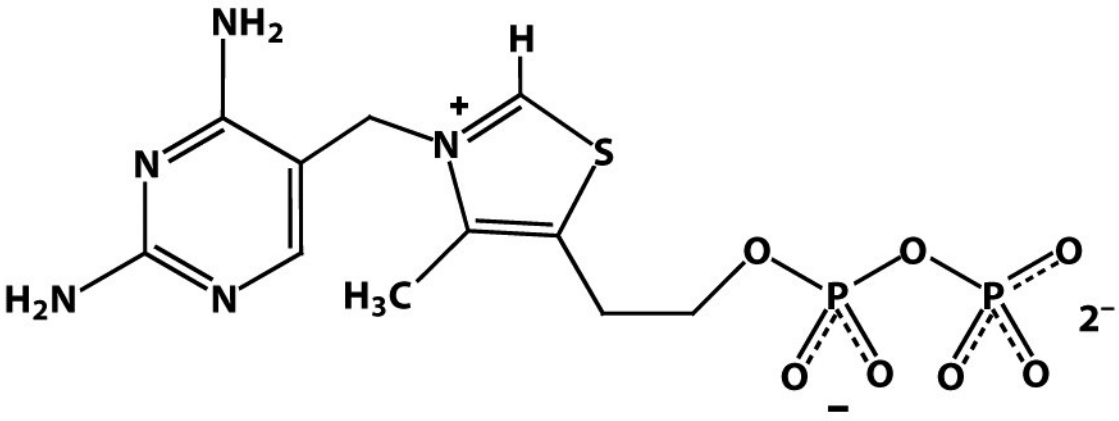


TABLE 17.1 **Pyruvate dehydrogenase complex of *E. coli***

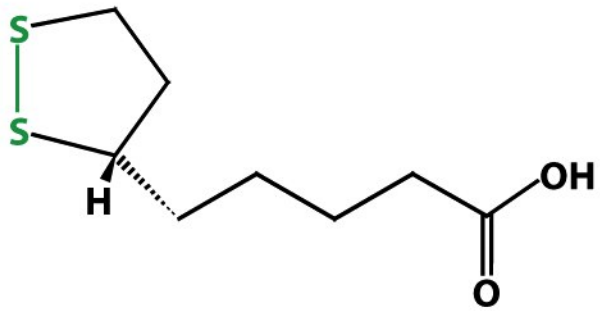
Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of the acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide

Pyruvate dehydrogenase

- Three enzymes: E1, E2 & E3.
- Three catalytic cofactors:
  - Thiamine pyrophosphate (TPP, vitamin B1)
  - Lipoic acid
  - FAD
- Two stoichiometric cofactors
  - CoA
  - NAD^+.



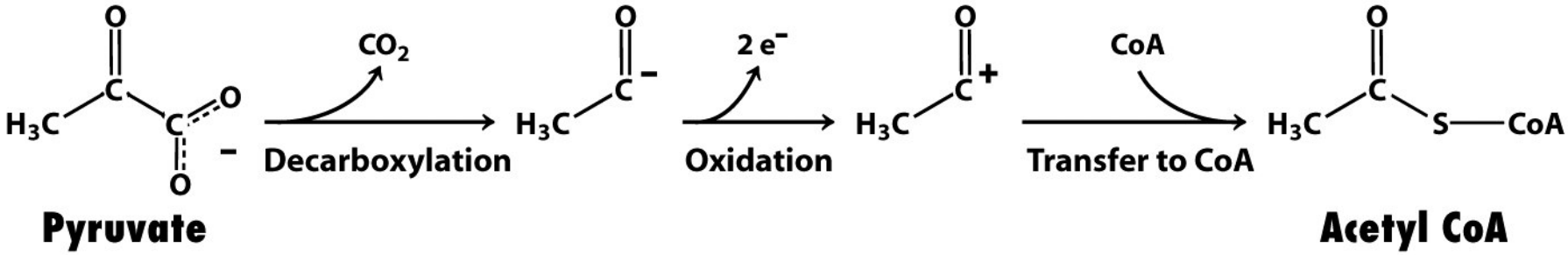
Thiamine pyrophosphate (TPP)



Lipoic acid

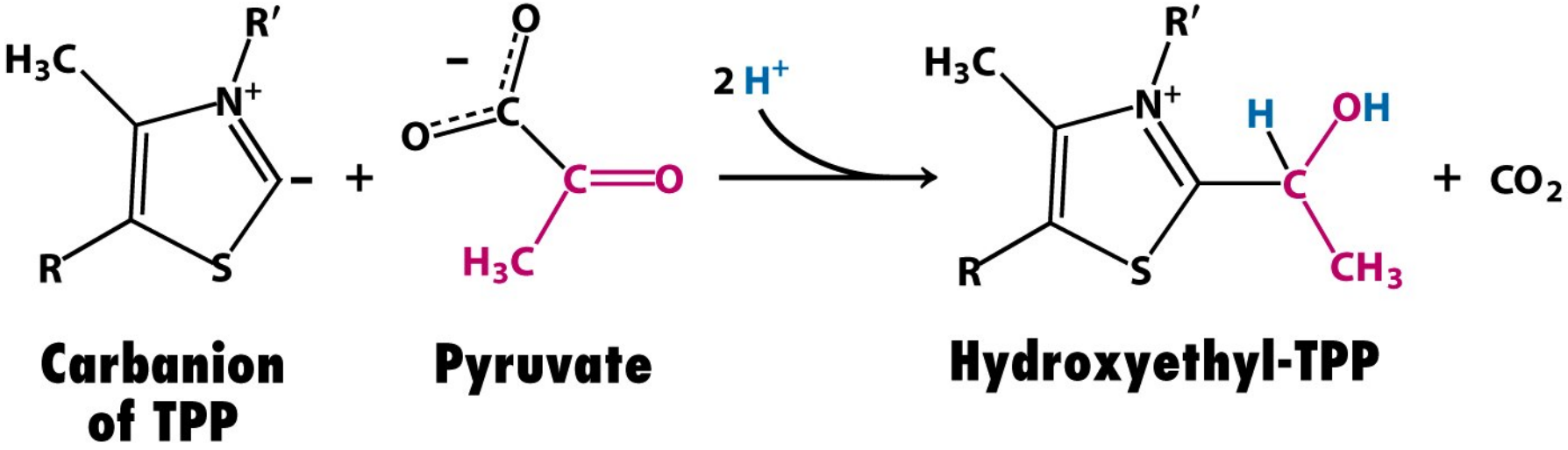
Unnumbered figure pg 478a
Biochemistry, Sixth Edition

A three step reaction



Unnumbered figure pg 478b
Biochemistry, Sixth Edition

1. Decarboxylation (E1)



Unnumbered figure pg 478c
Biochemistry, Sixth Edition

Thiazole ring

Electron sink

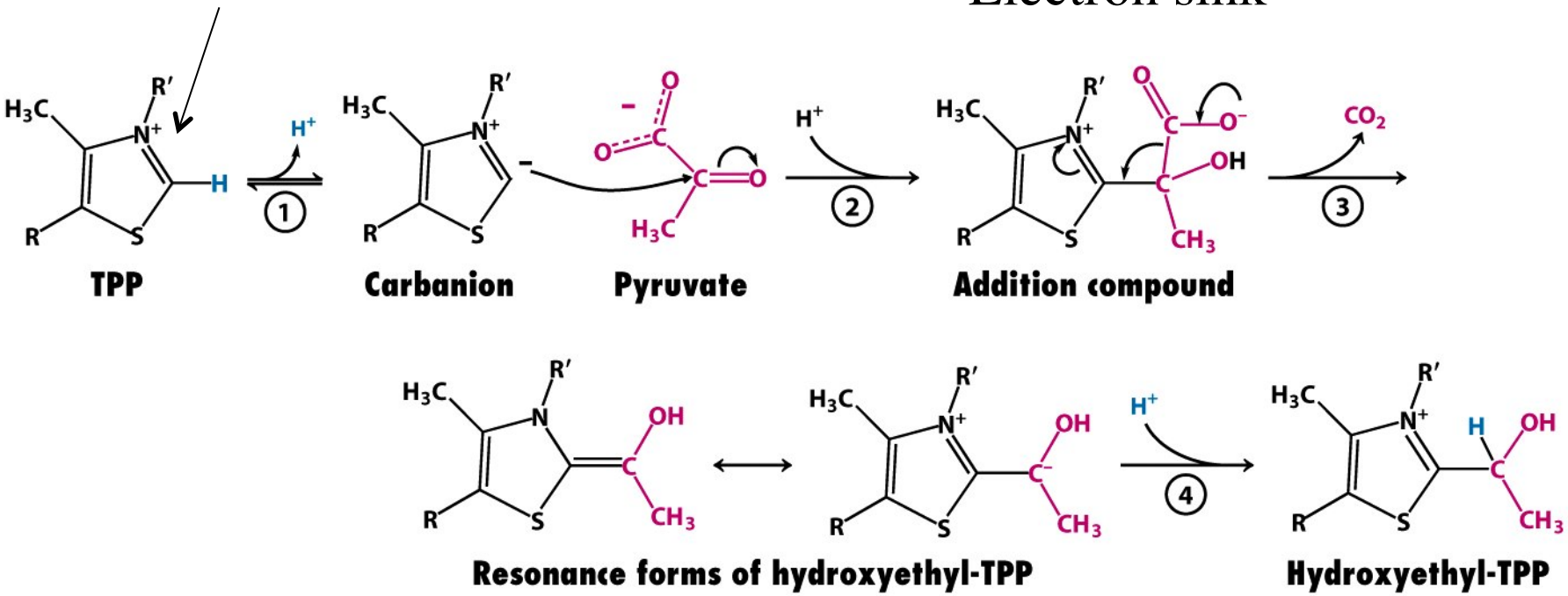
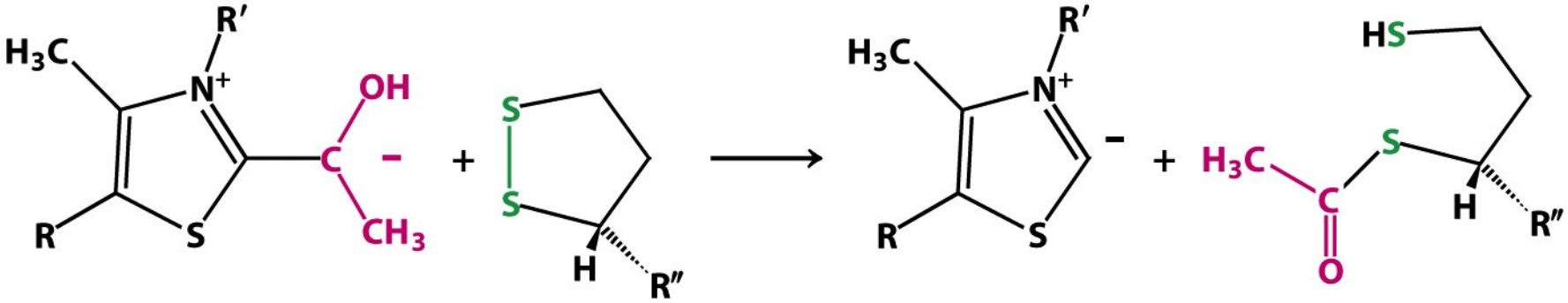


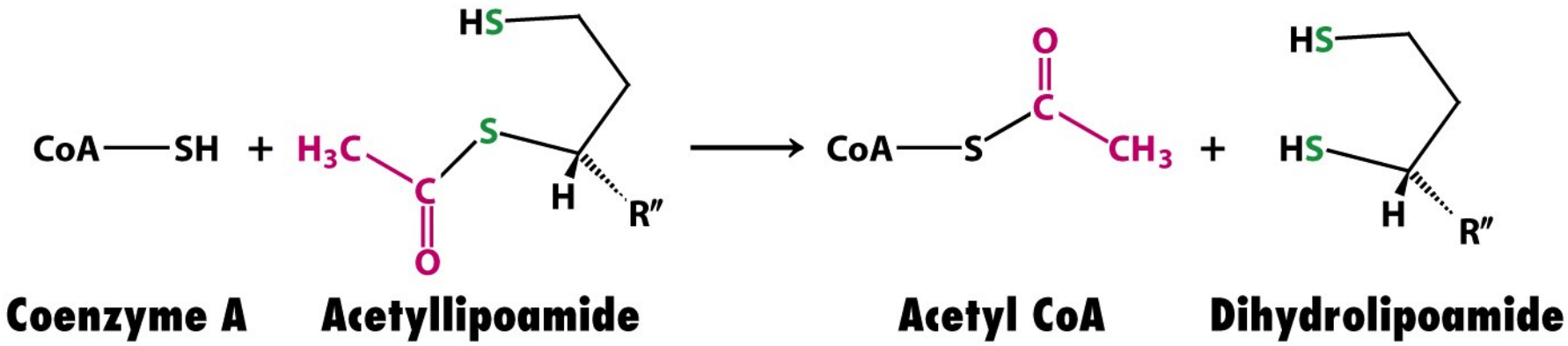
Figure 17-6
Biochemistry, Sixth Edition

2. Oxidation (E1)



Unnumbered figure pg 478d
Biochemistry, Sixth Edition

3. Formation of Acetyl CoA (E2)



Unnumbered figure pg 479a
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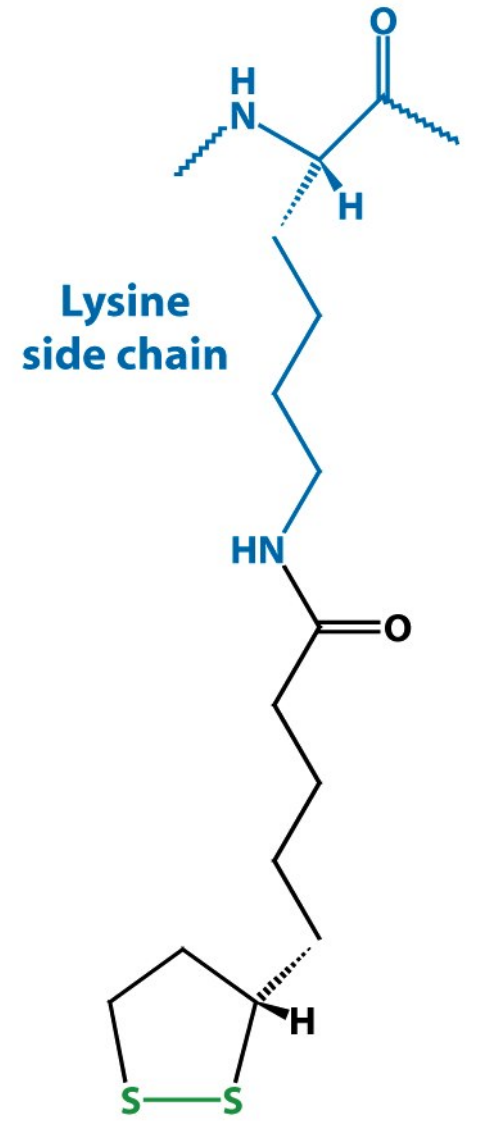
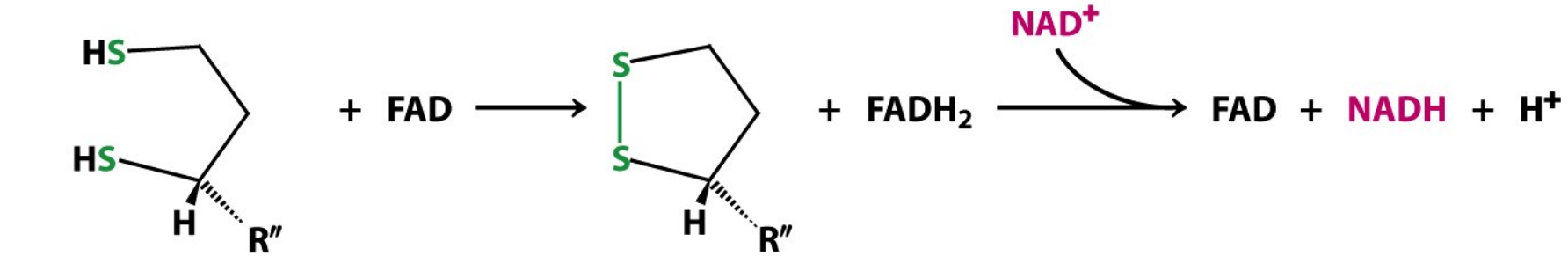
Energy rich thioester is preserved

Flexibility



Unnumbered figure pg 479b
Biochemistry, Sixth Edition

4. Regeneration



Unnumbered figure pg 479c
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The protein changes the redox potential of FAD

A multi enzyme complex

- Substrate channeling makes it very effective and reduces side reactions.
- 24 copies of E1
- 24 copies of E2
- 12 copies of E3

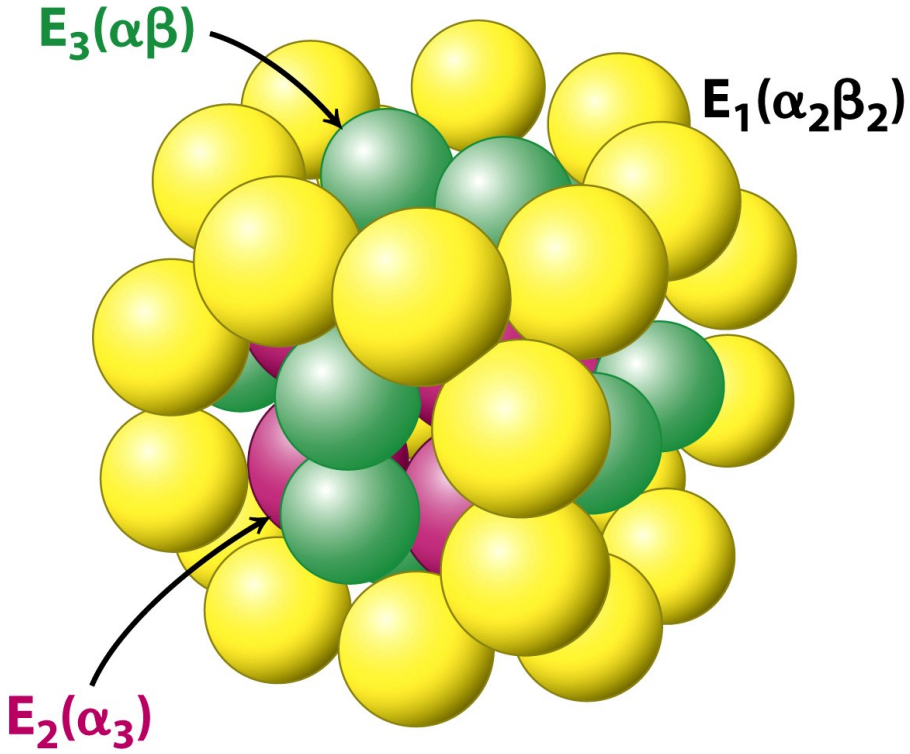
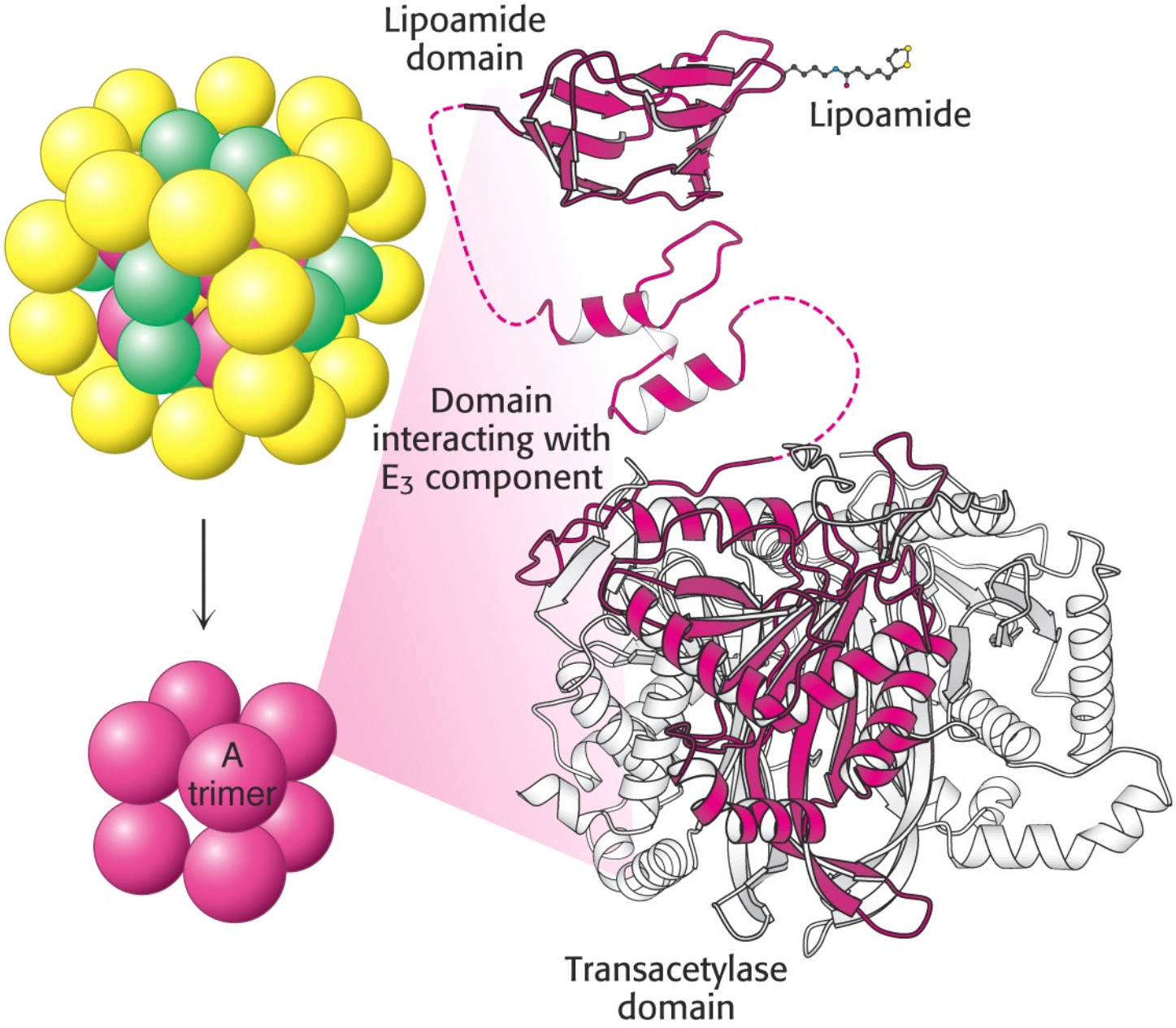
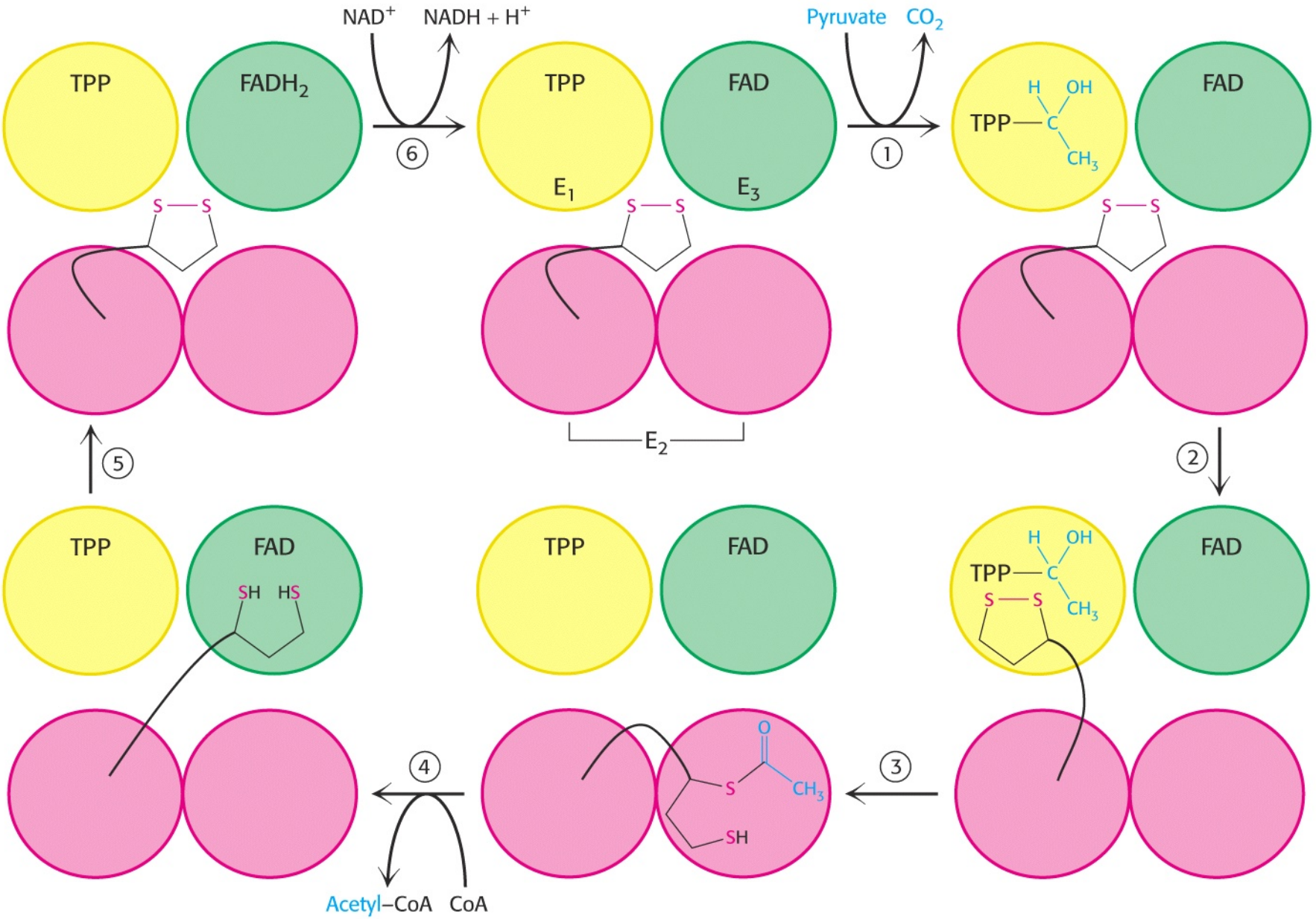
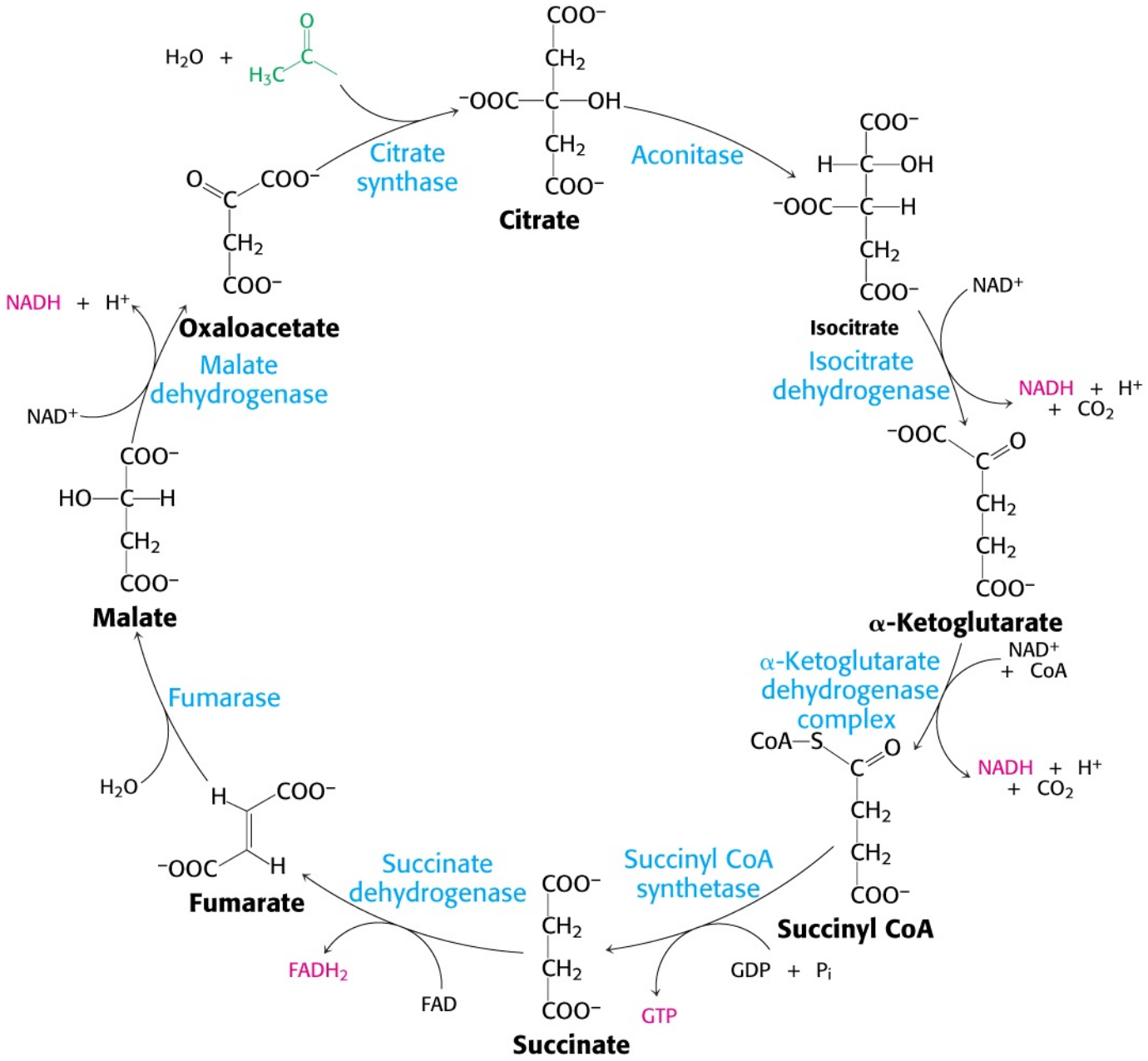


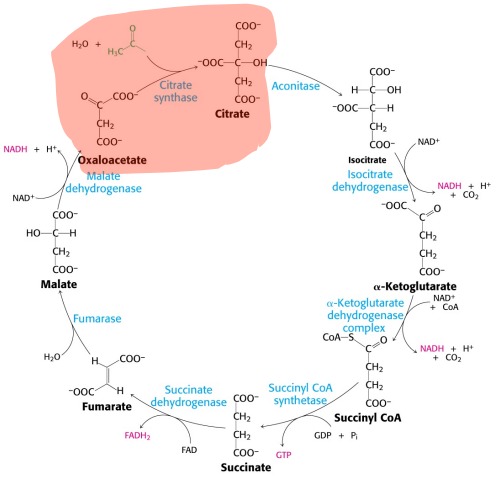
Figure 17-7
Biochemistry, Sixth Edition


Lipoamide domain
Lipoamide
Domain interacting with E_3 component
A trimer
Transacetylase domain


NAD+
NADH + H+
Pyruvate
CO2
TPP
FADH2
TPP
FAD
E1
E3
TPP
FAD
H
OH
CH3
S—S
E2
1
2
3
4
5
6
TPP
FAD
SH
HS
TPP
FAD
TPP
FAD
S—S
O
CH3
SH
Acetyl–CoA
CoA

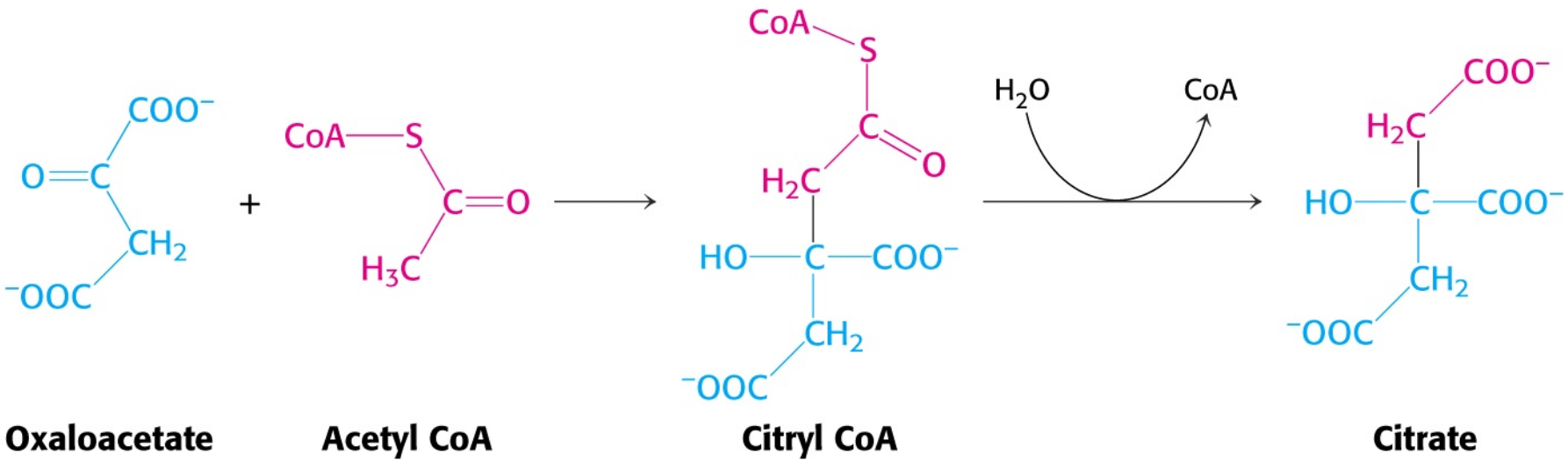
The Krebs Cycle



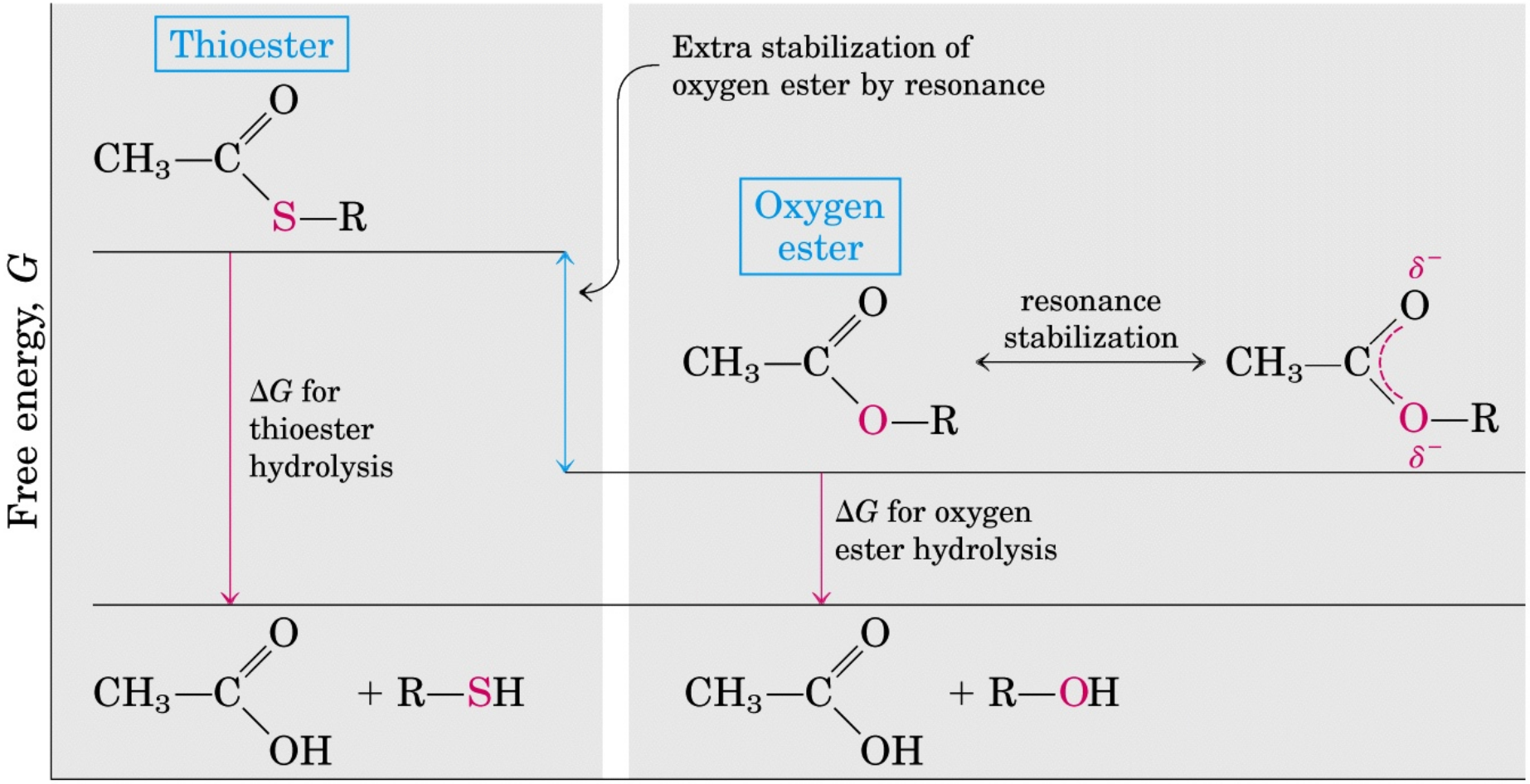


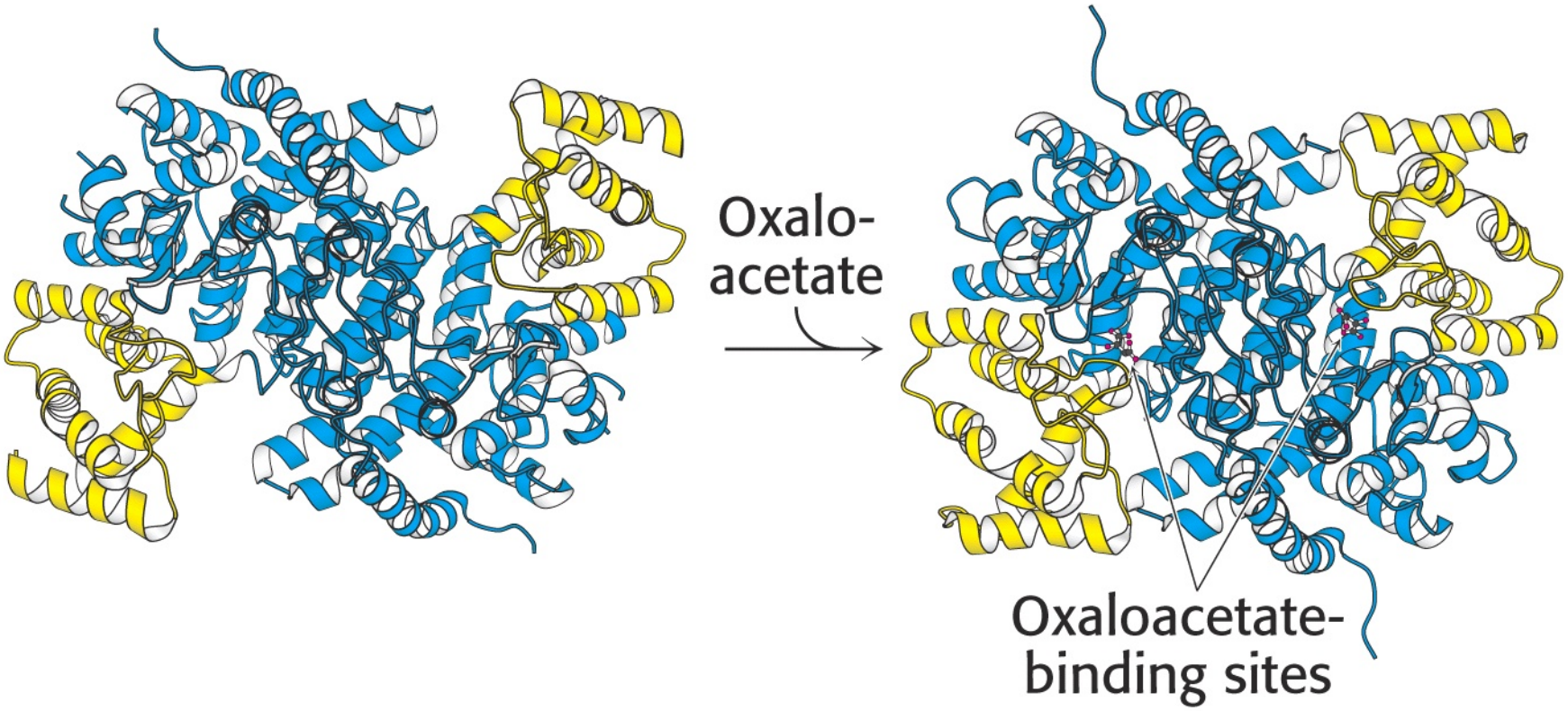
1. Condensation of acetyl-CoA with oxaloacetate to form citric acid

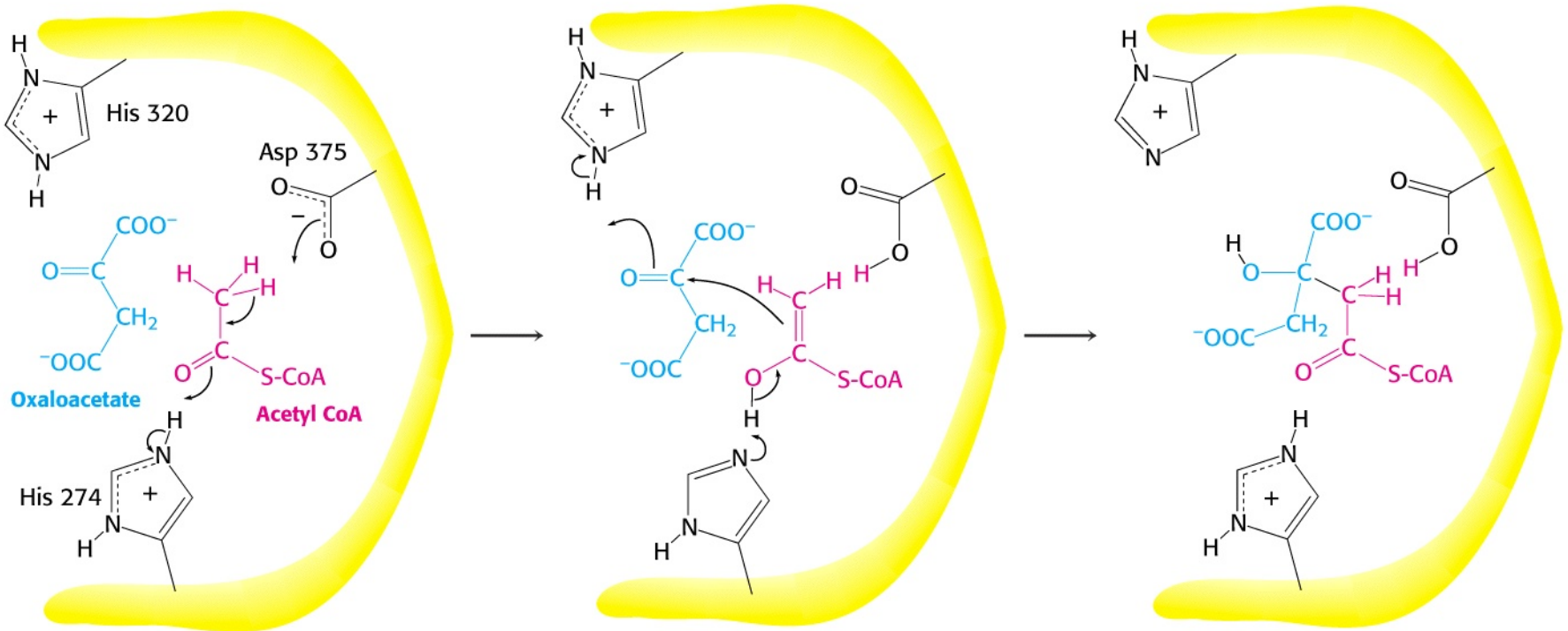
$\Delta G = -7.5$ kcal/mol

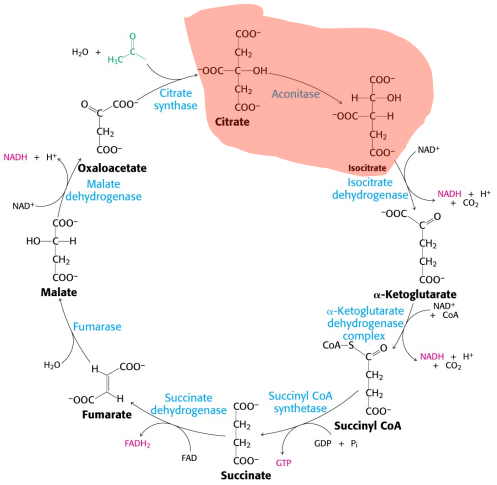


- Hydrolysis of the thioester drives this reaction.
- The concentration of oxaloacetate is normally very low in the cell.
- You will see why this is so in the last step in the cycle.


Thioester
Extra stabilization of oxygen ester by resonance
Oxygen ester
resonance stabilization
Free energy, G
ΔG for thioester hydrolysis
ΔG for oxygen ester hydrolysis
+ R—SH
+ R—OH

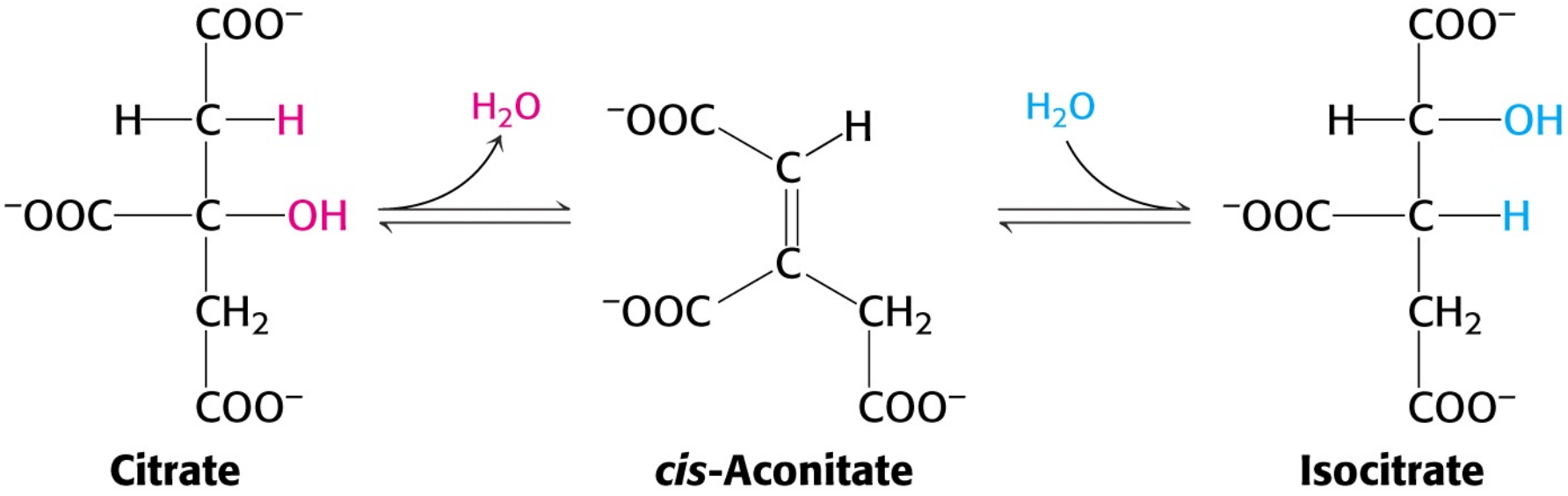

Oxalo-
acetate
Oxaloacetate-
binding sites


His 320
Asp 375
Oxaloacetate
Acetyl CoA
His 274
S-CoA
Substrate complex
Enol intermediate
Citryl CoA complex

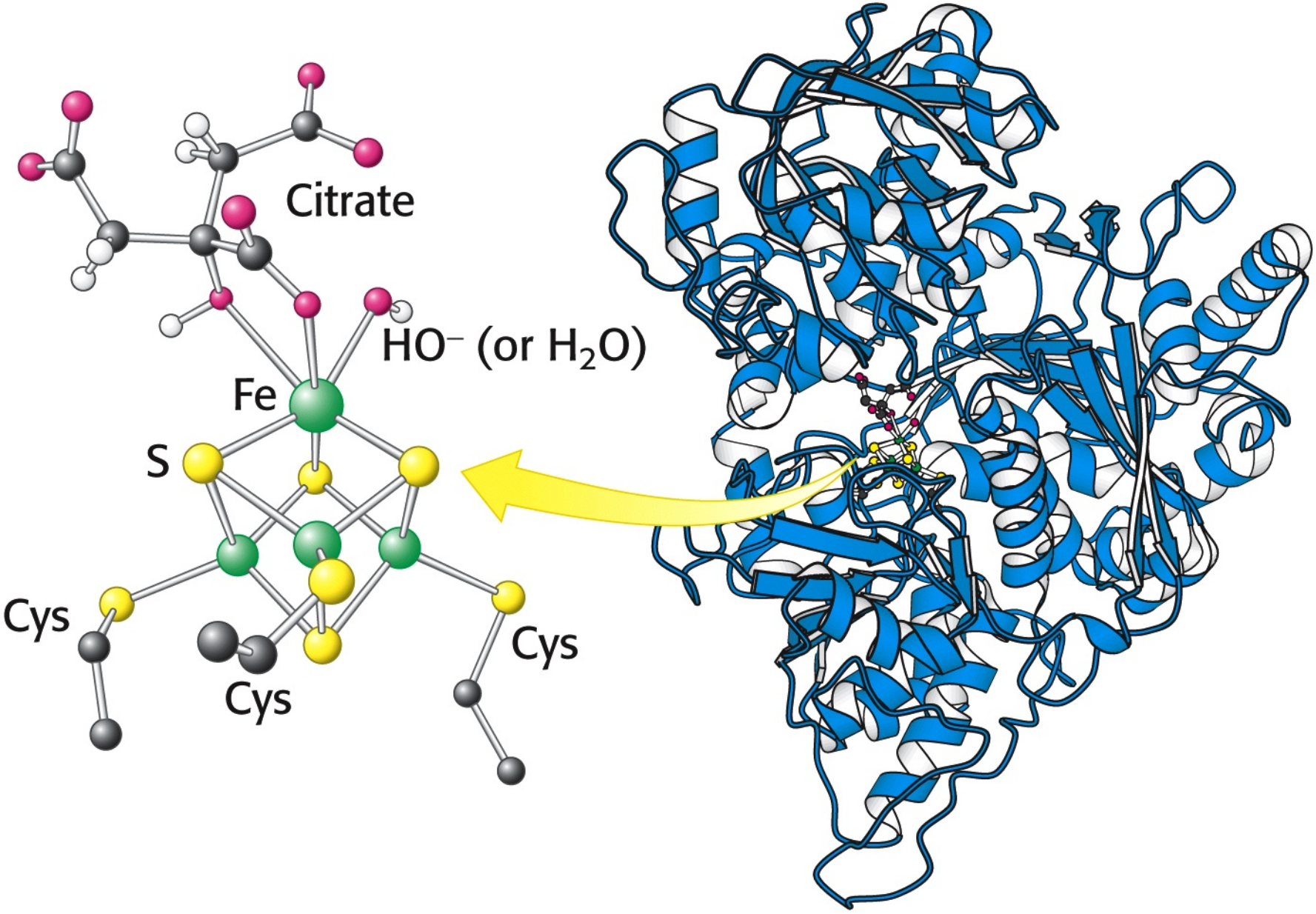


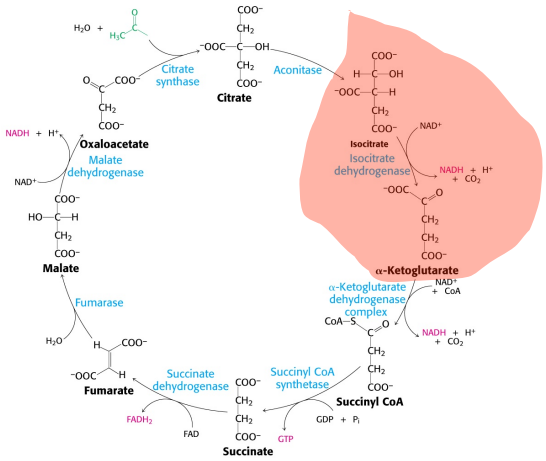
2. Cis-aconiatase can reversibly hydrate citric acid to isocitric acid

$\Delta G = 1.5$ kcal/mol



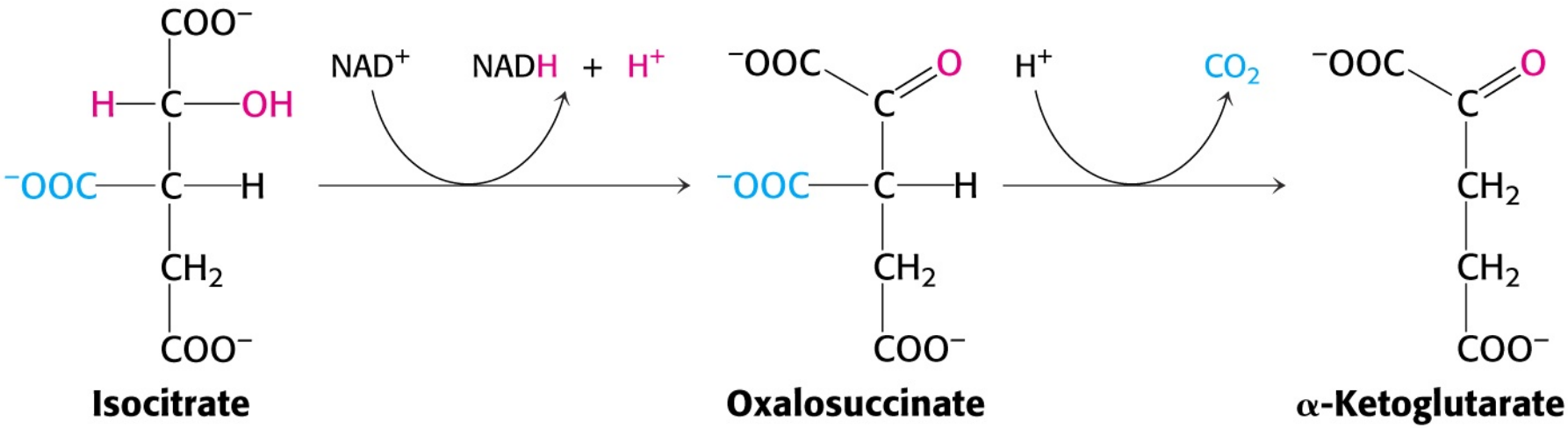
- Mass action drives this reaction.
- Note that citrate is a symmetrical, achiral molecule.
- It is however, a pro-chiral molecule.


Citrate
HO⁻ (or H2O)
Fe
S
Cys
Cys
Cys

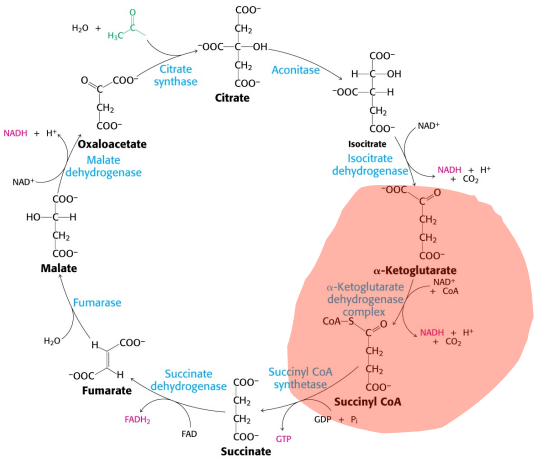


3. Oxidative Decarboxylation of isocitrate to α-ketoglutarate

$\Delta G = -2$ kcal/mol

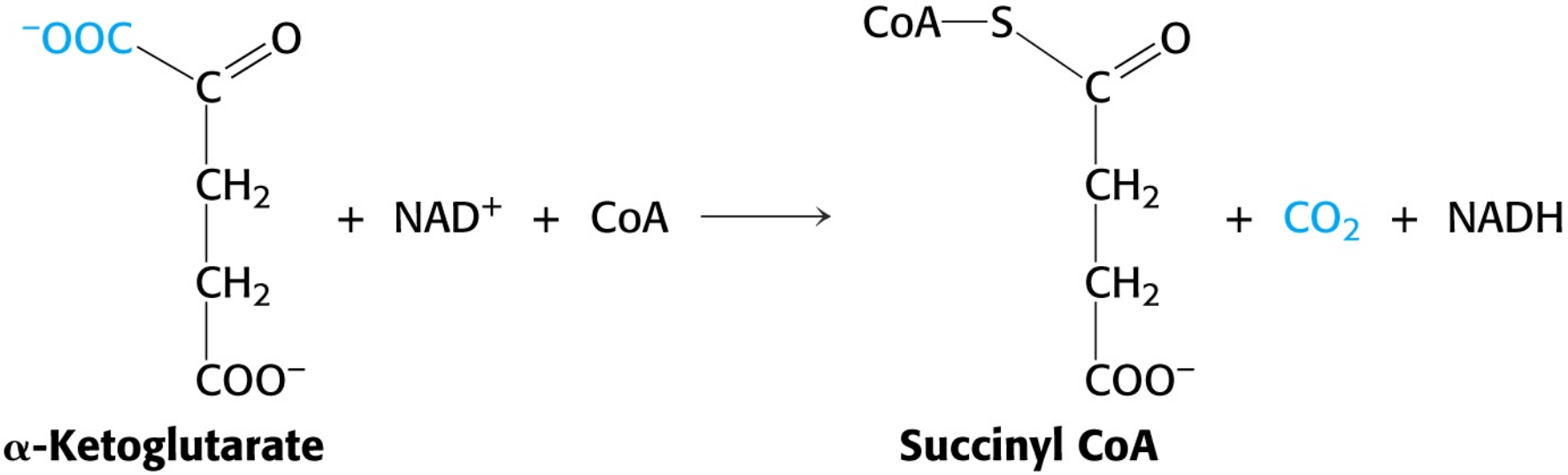


- There are two different forms of the enzyme.
- One that uses NAD^+ and the other that uses $NADP^+$.



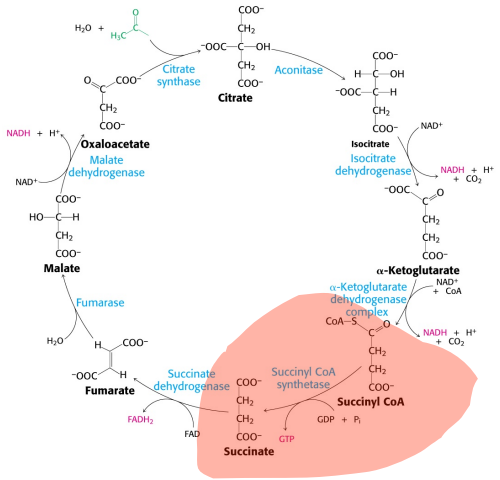
4. Oxidative Decarboxylation of α-ketoglutarate succinyl-CoA

$\Delta G = -7.2$ kcal/mol



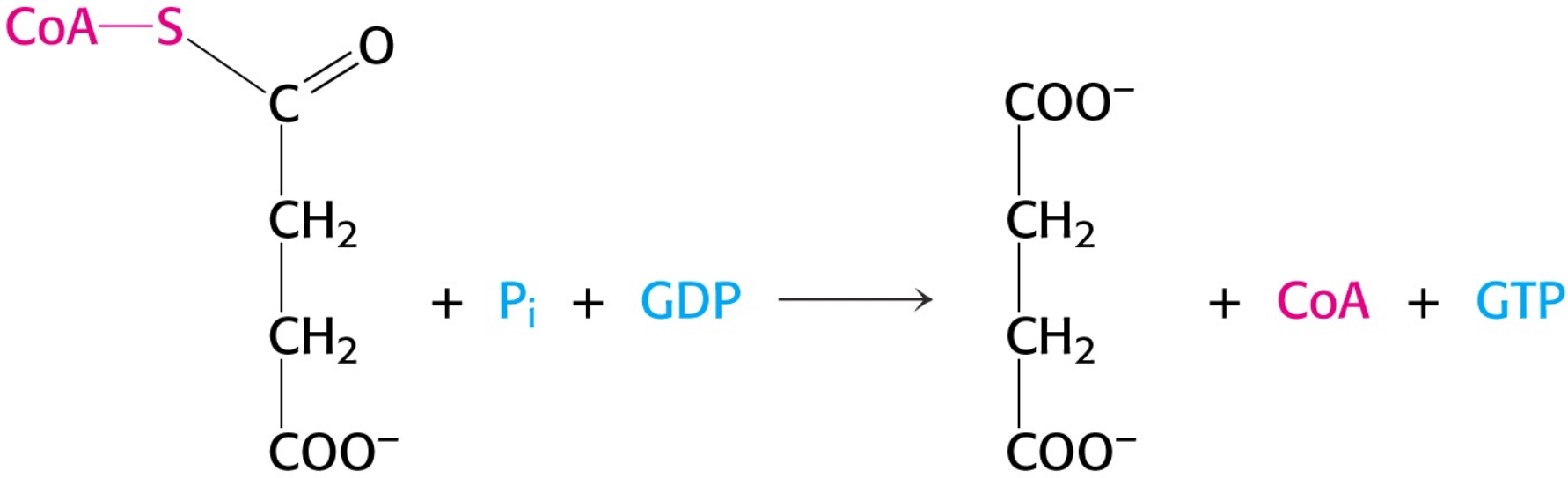
α-Ketoglutarate **Succinyl CoA**

- Another oxidative Decarboxylation.
- The energy of the oxidation in conserved in the form of a thioester.
- The enzymes and mechanism of this reaction are very similar to that of pyruvate dehydrogenase.



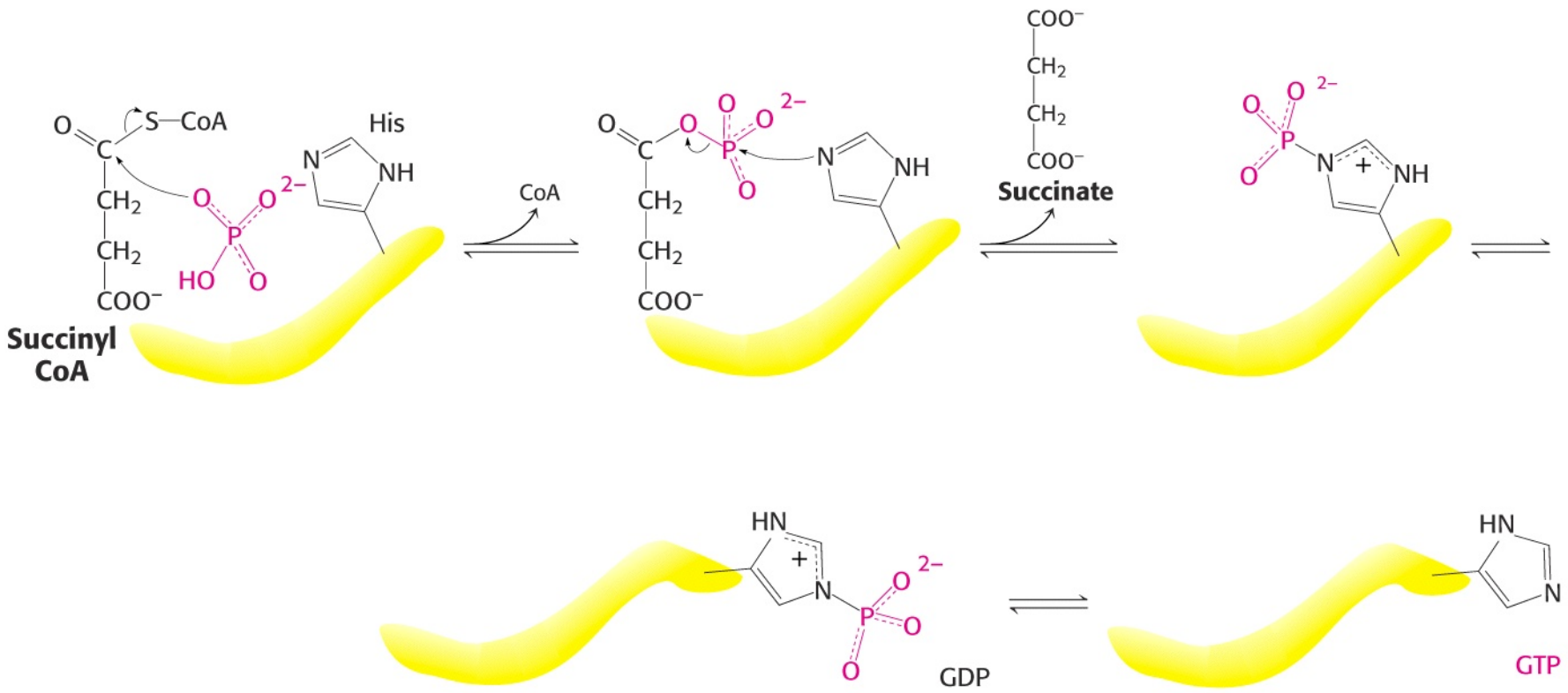
5. Conversion of succinyl-CoA to succinate

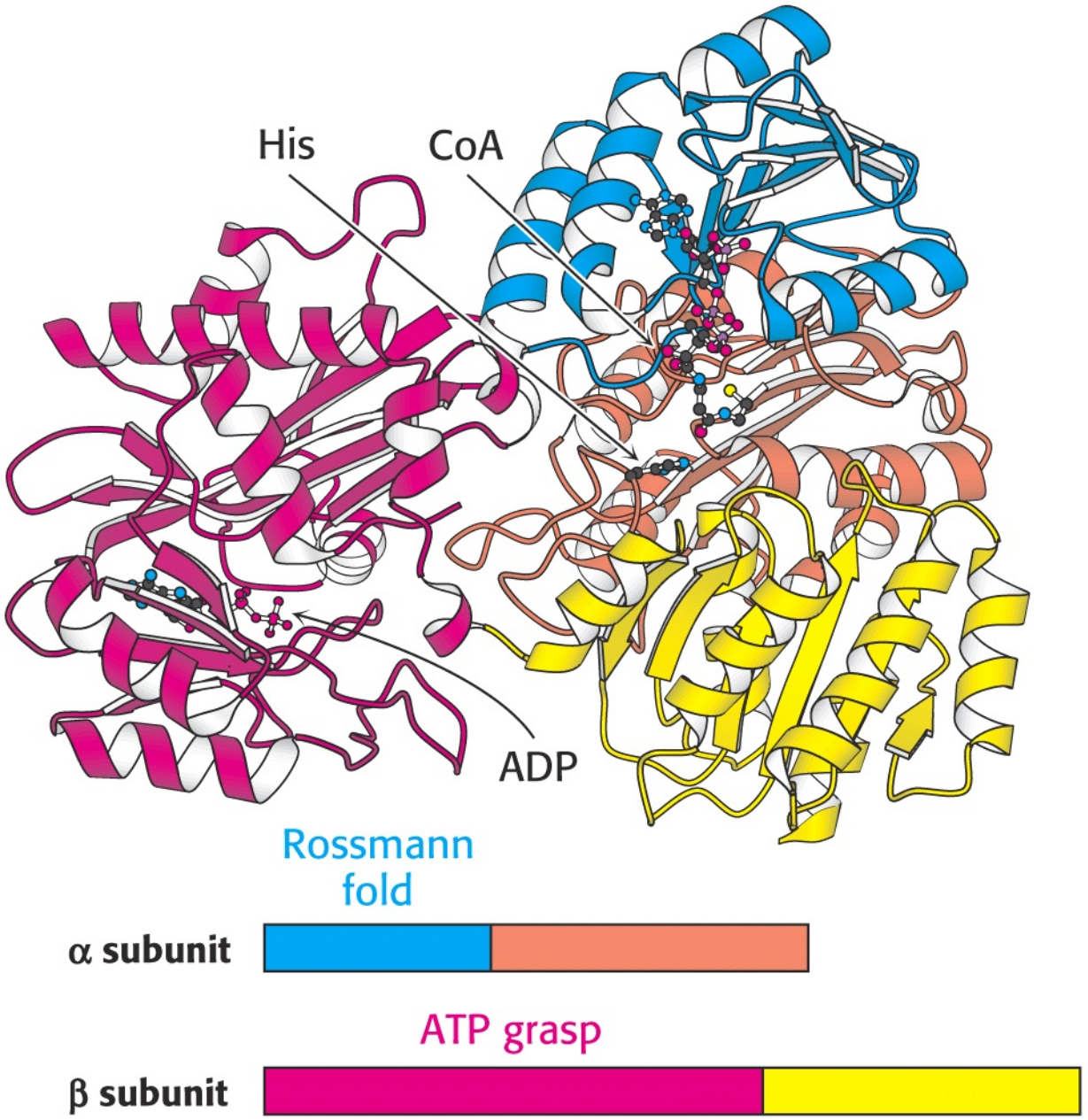
$\Delta G = -0.8$ kcal/mol

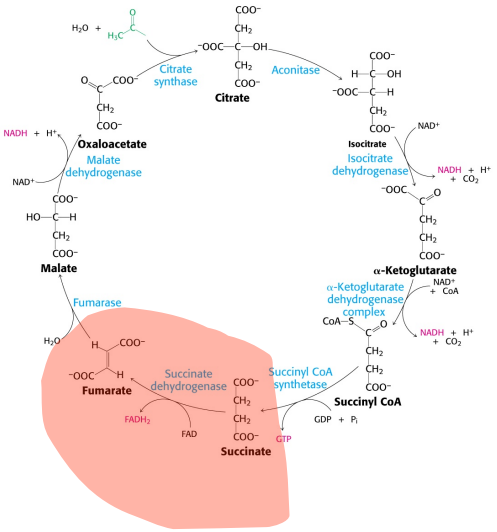


Succinyl CoA **Succinate**

- Another example of substrate level phosphorylation, similar to those found in glycolysis.
- The GTP can be converted to ATP readily.

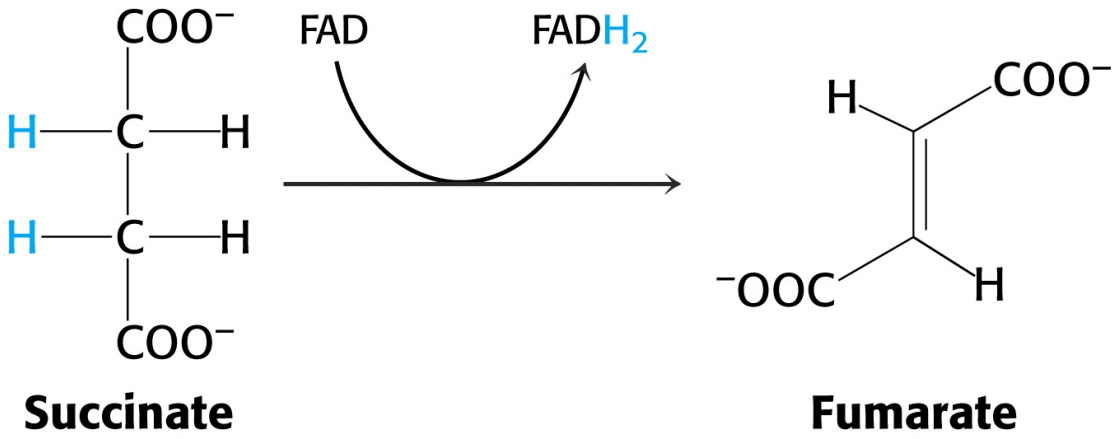

Succinyl CoA
His
CoA
Succinate
GDP
GTP


His
CoA
ADP
Rossmann fold
α subunit
ATP grasp
β subunit

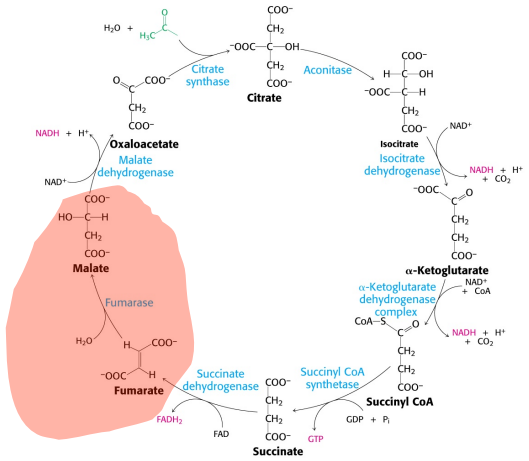


6. Oxidation of succinate to fumarate

$\Delta G = 0$ kcal/mol

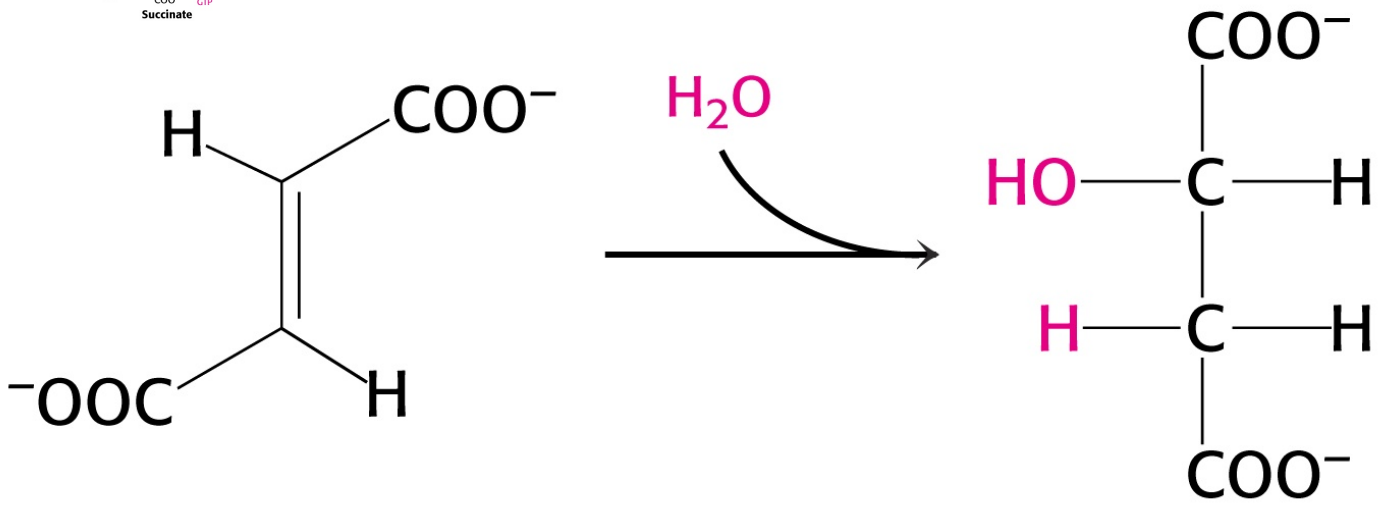


- This enzyme is the only membrane bound enzyme in the cycle. It directly connects the Krebs cycle to oxidative phosphorylation.
- The enzyme is stereo-selective in that it produces only trans fumarate.



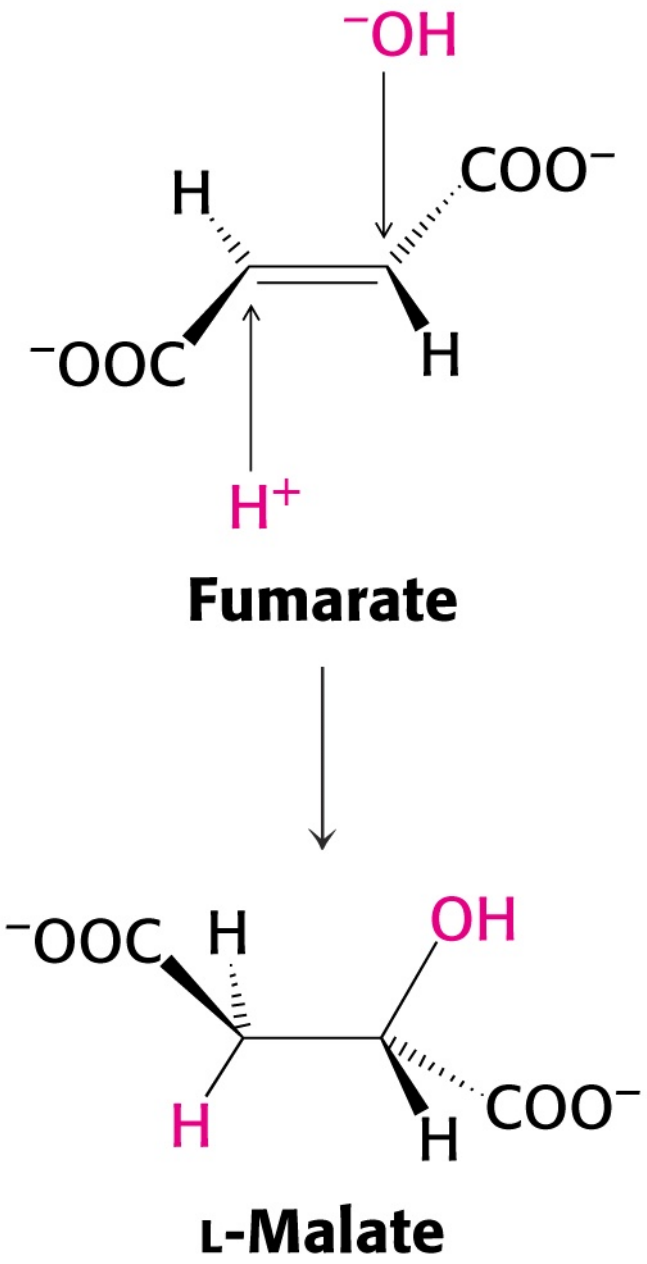
7. Hydration of fumarate to malate

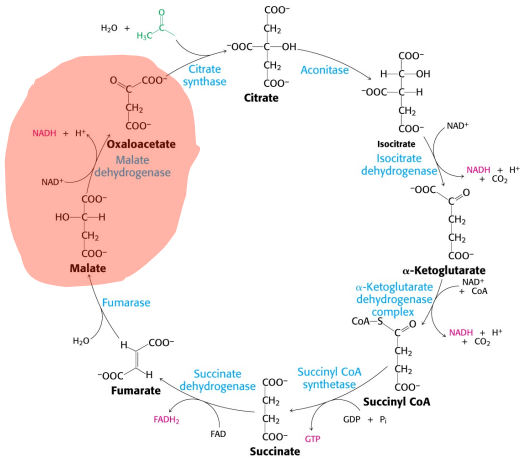
$\Delta G = -0.9$ kcal/mol



Fumarate **Malate**

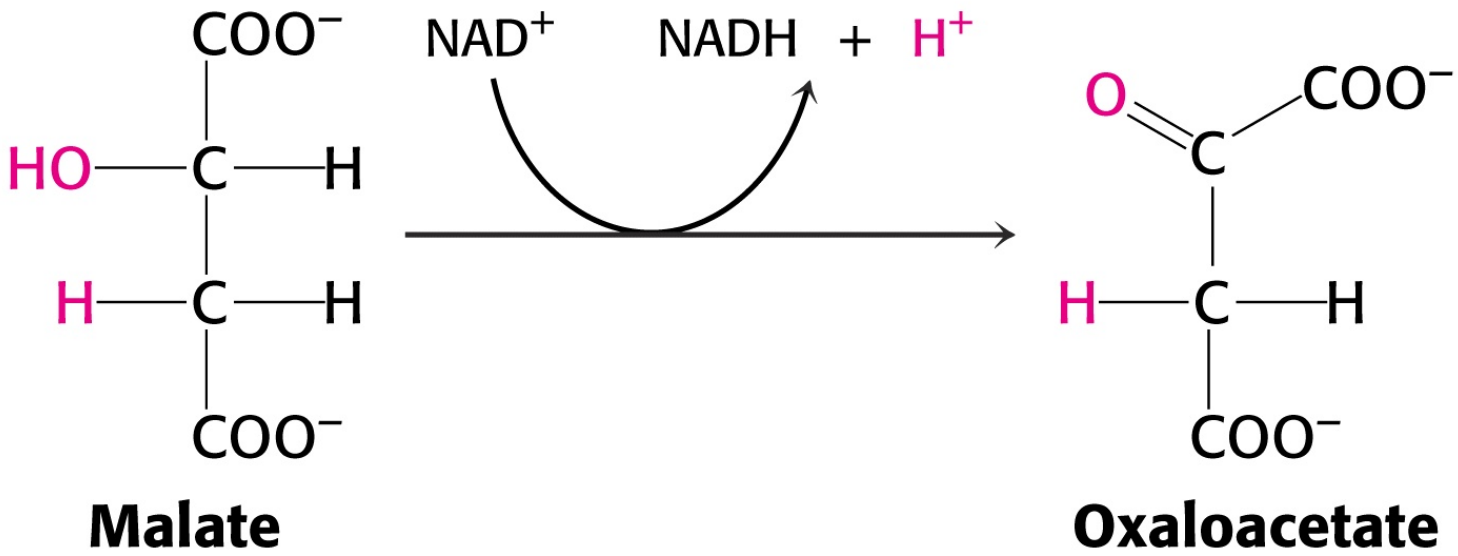
- The enzyme is stereo-selective.
- Only trans double bond is hydrated, producing only L-malate.


⁻OH
COO⁻
H
⁻OOC
H
H⁺
Fumarate
⁻OOC
H
OH
H
COO⁻
H
L-Malate



8. Oxidation of malate to oxaloacetate

$\Delta G = 7.1$ kcal/mol



- Reduction of Malate to Oxaloacetate allows one to close the citric acid cycle pathway.
- Since the reaction is endergonic, the concentration of oxaloacetate is kept very low.
- Furthermore the reaction catalyzed by citrate synthase is highly exergonic and drives the cycle.

TABLE 17.2 Citric acid cycle

Step	Reaction	Enzyme	Prosthetic group	Type*	$\Delta G°'$ kcal mol^{-1}	$\Delta G°'$ kJ mol^{-1}
1	Acetyl CoA + oxaloacetate + H_2O $\longrightarrow$ citrate + CoA + H^+	Citrate synthase		a	−7.5	−31.4
2a	Citrate $\rightleftharpoons$ *cis*-aconitate + H_2O	Aconitase	Fe–S	b	+2.0	+8.4
2b	*cis*-Aconitate + H_2O $\rightleftharpoons$ isocitrate	Aconitase	Fe–S	c	−0.5	−2.1
3	Isocitrate + NAD^+ $\rightleftharpoons$ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase		d + e	−2.0	−8.4
4	α-Ketoglutarate + NAD^+ + CoA $\rightleftharpoons$ succinyl CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	Lipoic acid, FAD, TPP	d + e	−7.2	−30.1
5	Succinyl CoA + P_i + GDP $\rightleftharpoons$ succinate + GTP + CoA	Succinyl CoA synthetase		f	−0.8	−3.3
6	Succinate + FAD (enzyme-bound) $\rightleftharpoons$ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	FAD, Fe–S	e	~0	0
7	Fumarate + H_2O $\rightleftharpoons$ L-malate	Fumarase		c	−0.9	−3.8
8	L-Malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+	Malate dehydrogenase		e	+7.1	+29.7

*Reaction type: (a) condensation; (b) dehydration; (c) hydration; (d) decarboxylation; (e) oxidation; (f) substrate-level phosphorylation.

Sum = -9.8 kcal/mol

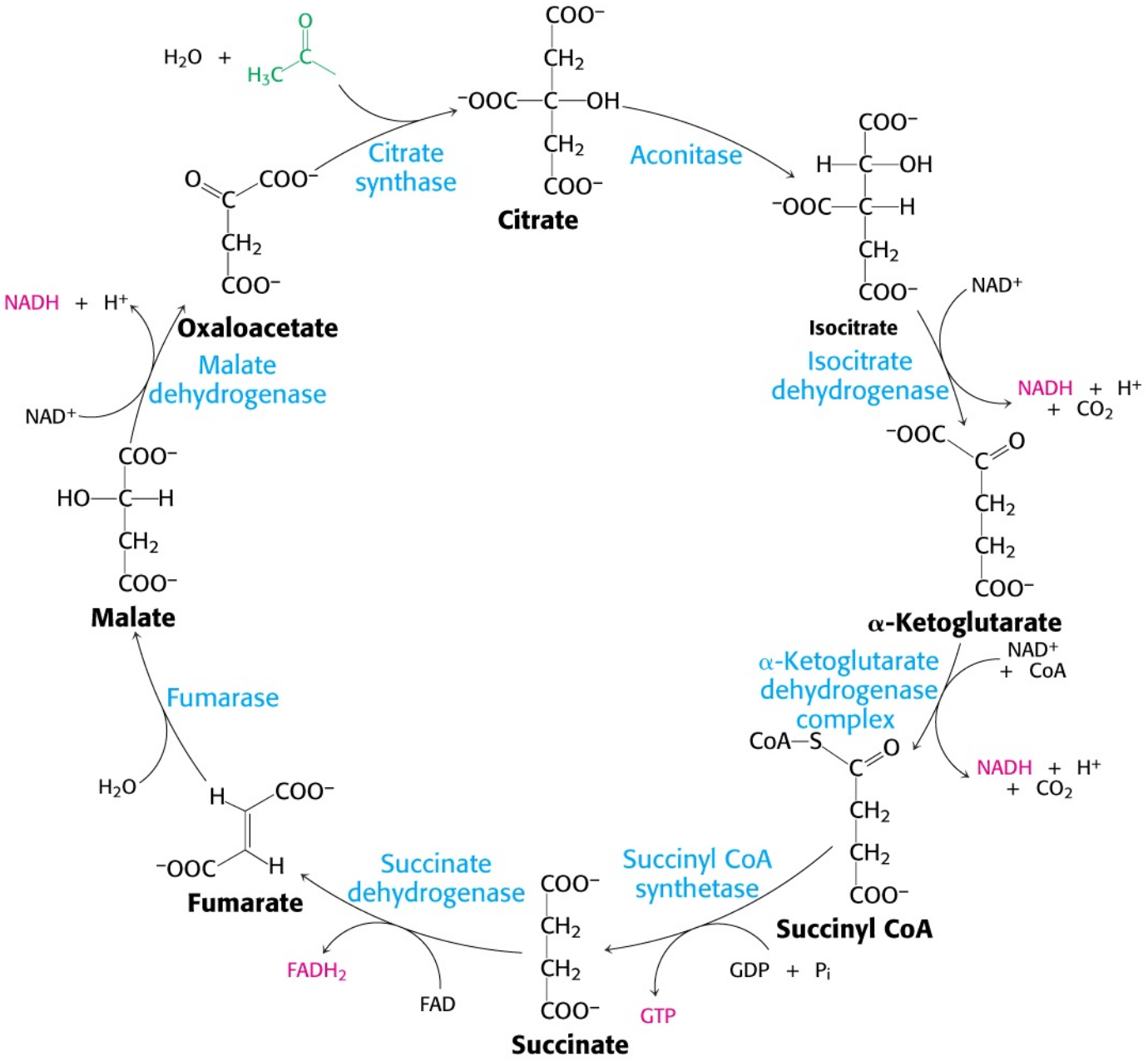

Citrate synthase
Citrate
Aconitase
Isocitrate
Isocitrate dehydrogenase
α-Ketoglutarate
α-Ketoglutarate dehydrogenase complex
Succinyl CoA
Succinyl CoA synthetase
Succinate
Succinate dehydrogenase
Fumarase
Fumarate
Malate dehydrogenase
Malate
Oxaloacetate

table 16–1

Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

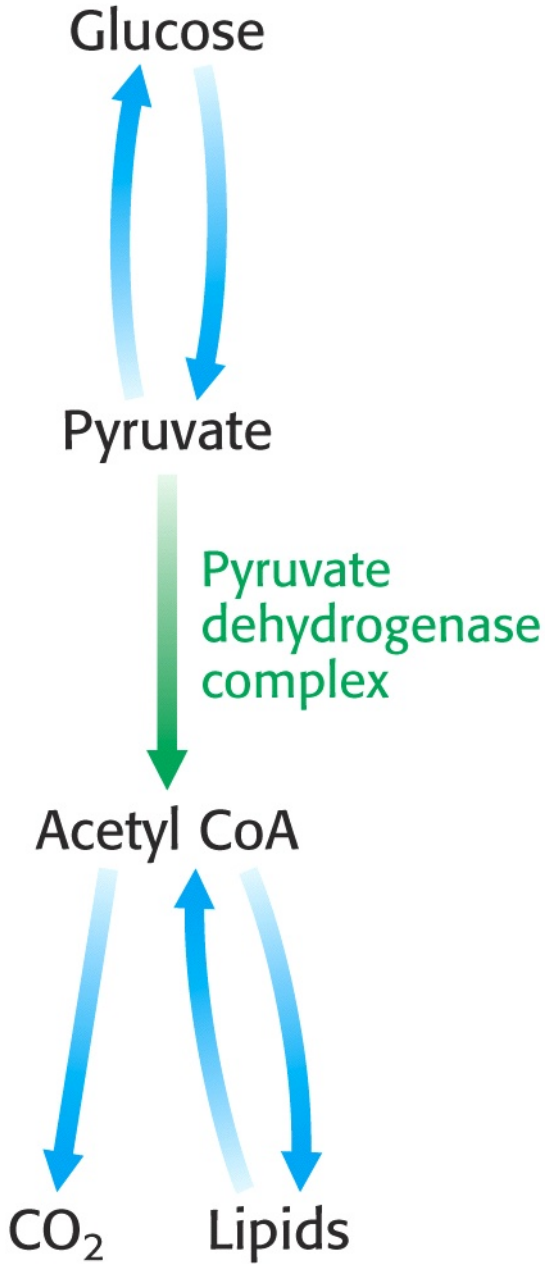
Reaction	Number of ATP or reduced coenzymes directly formed	Number of ATP ultimately formed*
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3–5
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

$$\Delta G^{0'} = 32 \times 30.5 = 976\text{kJ/mol}$$

$$= 35\% \text{ of } 2840\text{kJ/mol}$$

$$\Delta G \cong 65\%$$


Glucose
Pyruvate
Pyruvate dehydrogenase complex
Acetyl CoA
CO_2
Lipids

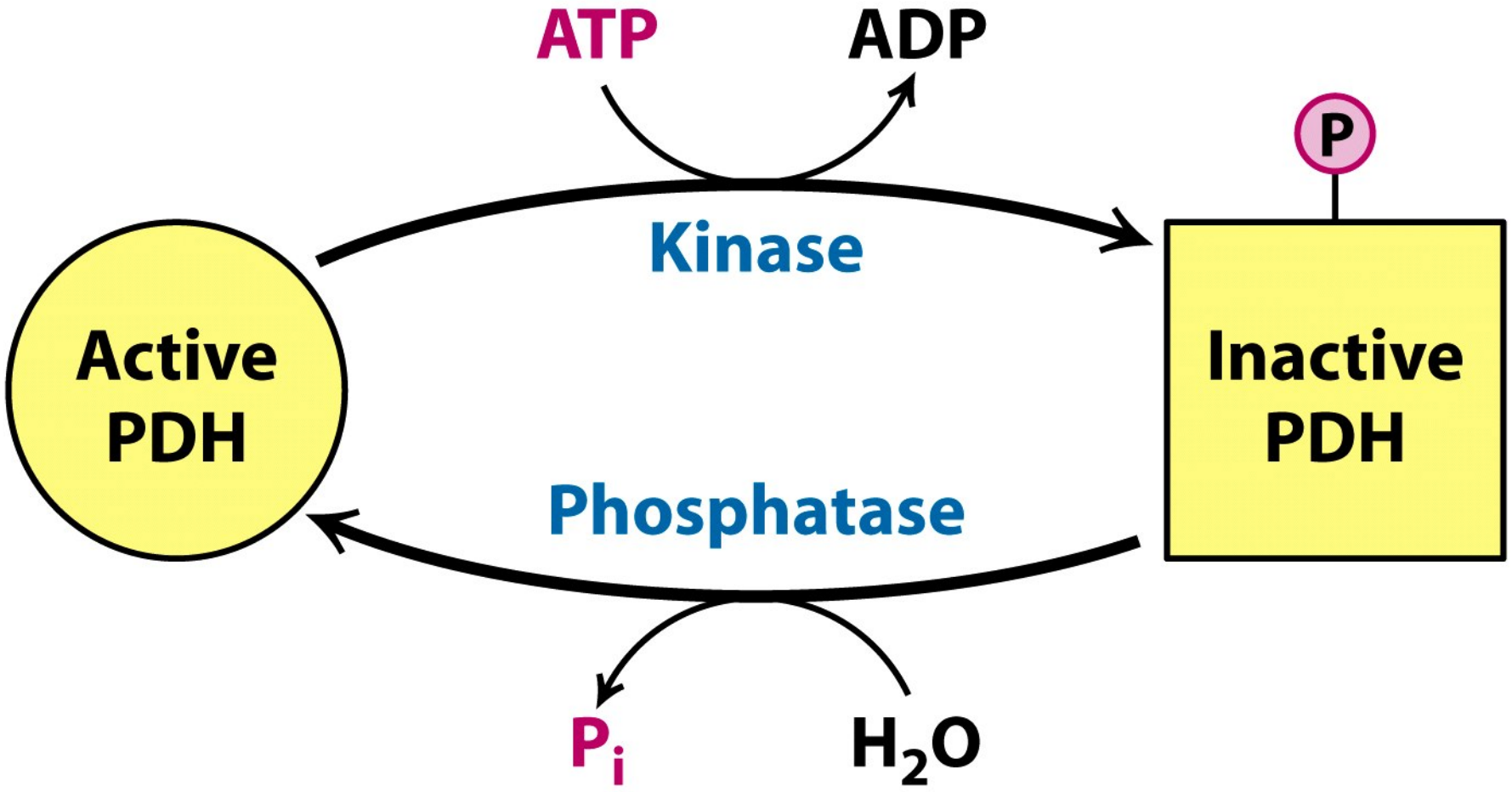


Figure 17-17
Biochemistry, Sixth Edition

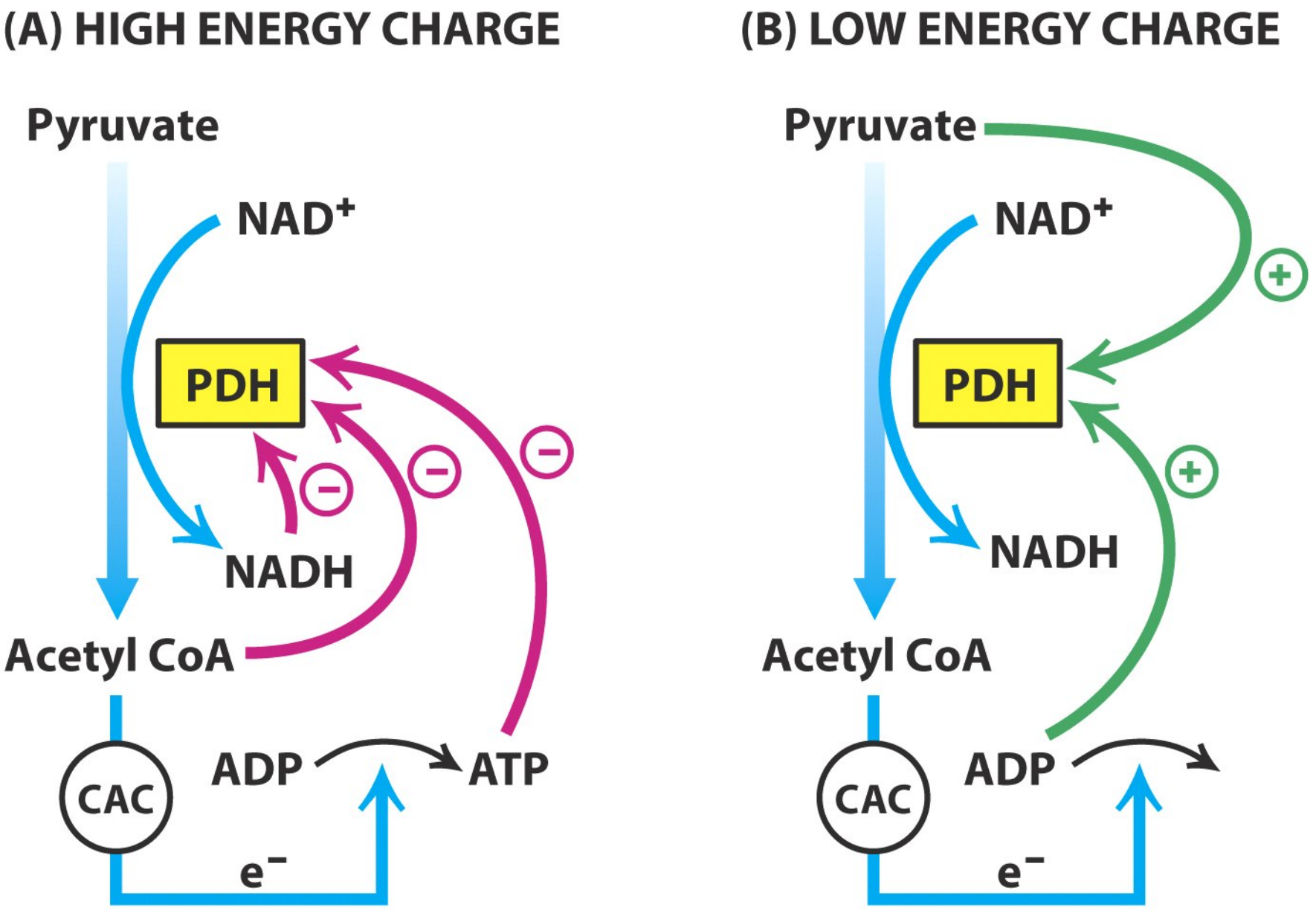
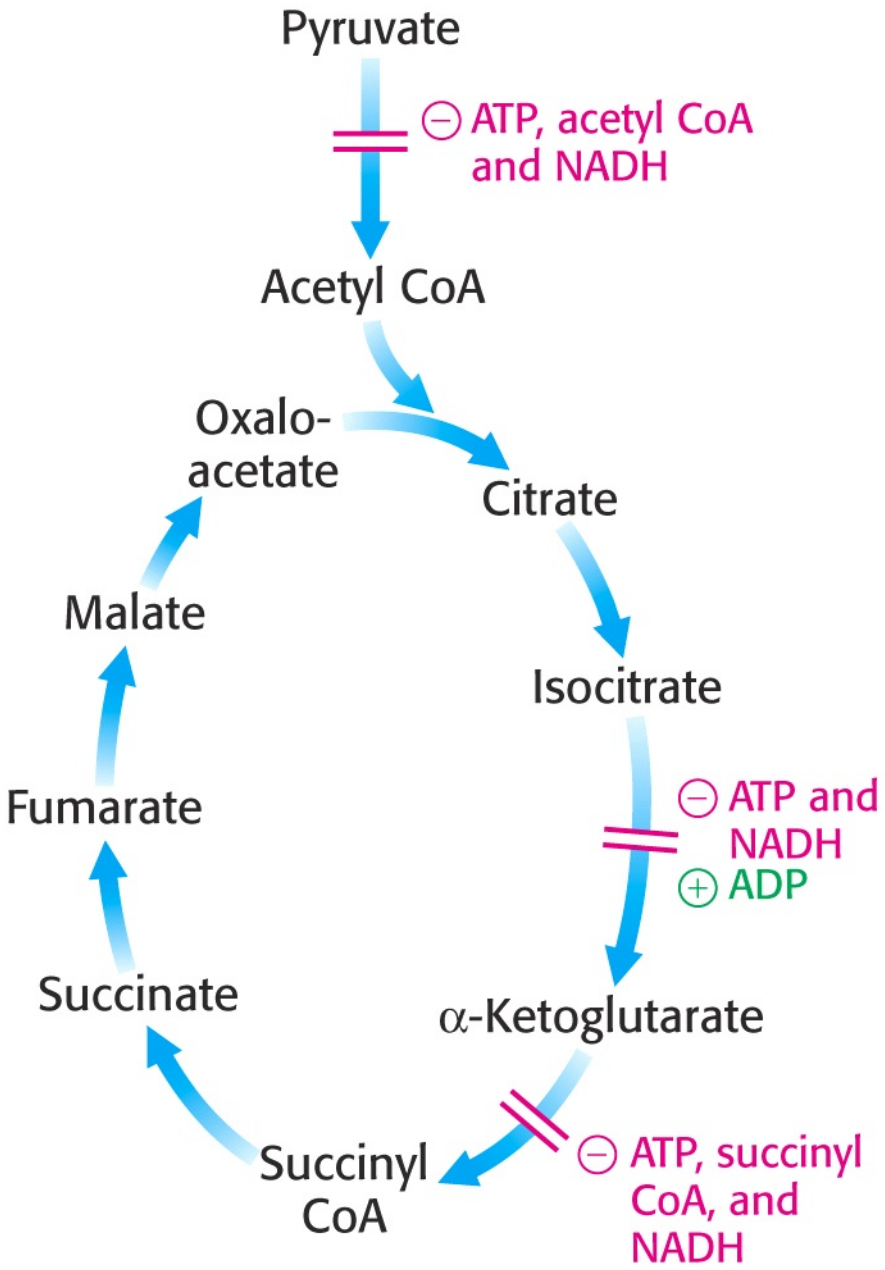
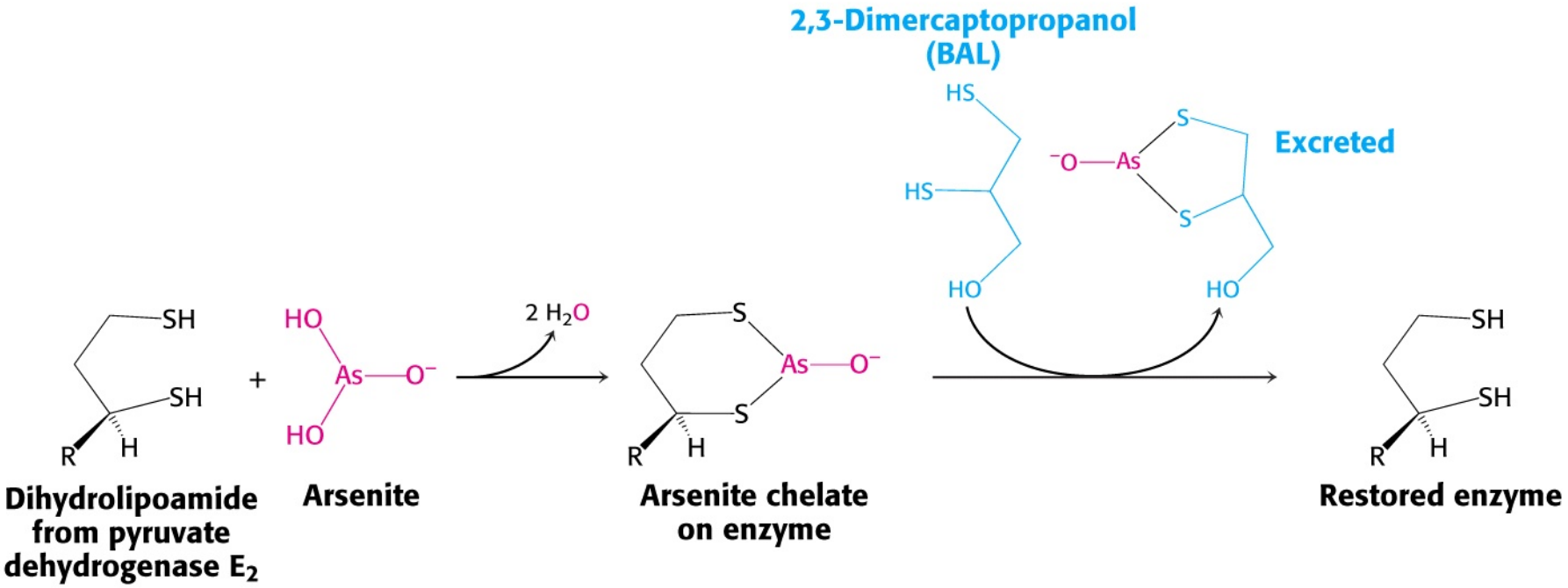
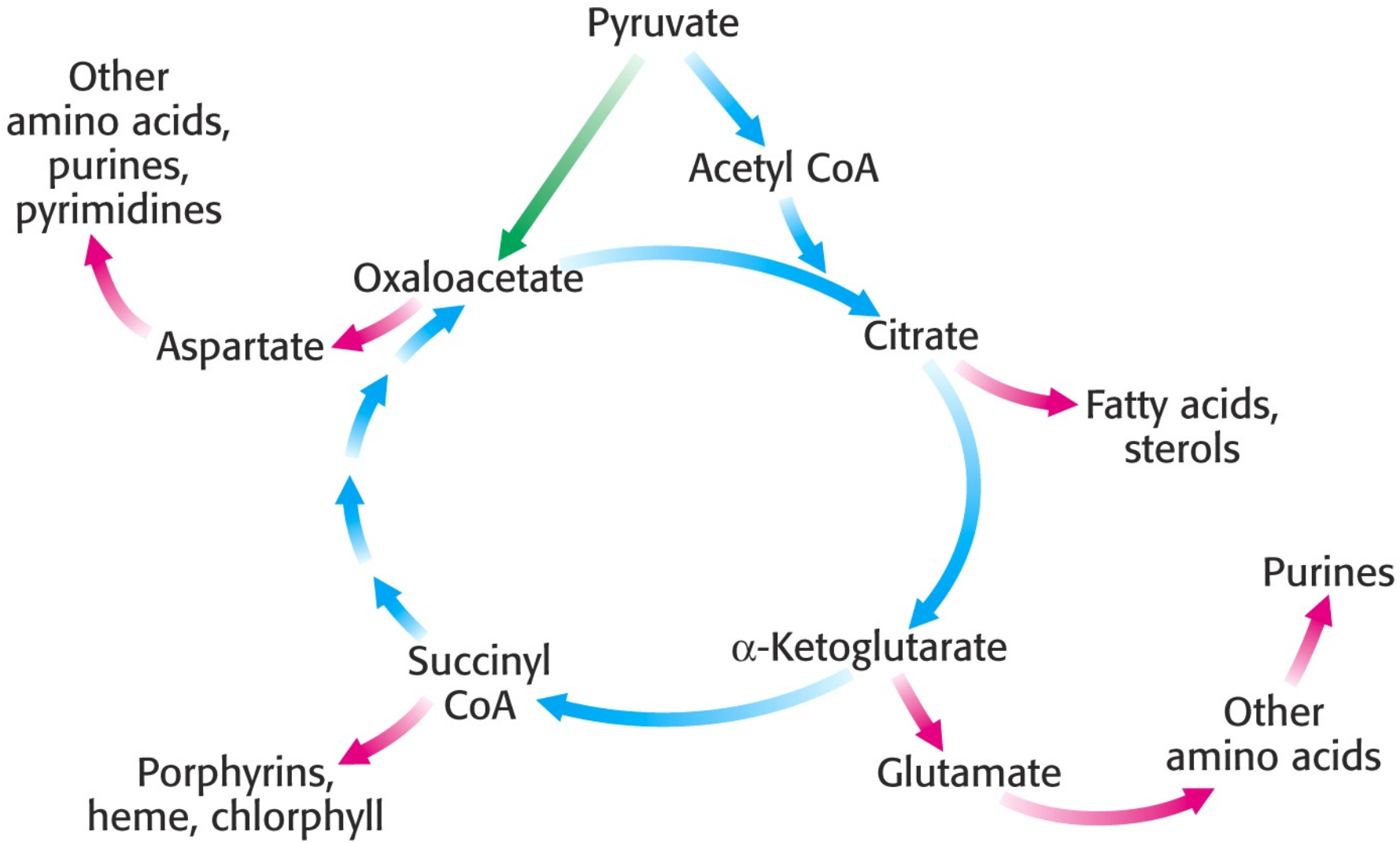


Figure 17-18
Biochemistry, Sixth Edition


Pyruvate
⊖ ATP, acetyl CoA and NADH
Acetyl CoA
Oxalo-acetate
Citrate
Isocitrate
⊖ ATP and NADH
⊕ ADP
α-Ketoglutarate
⊖ ATP, succinyl CoA, and NADH
Succinyl CoA
Succinate
Fumarate
Malate

Arsenite poisning

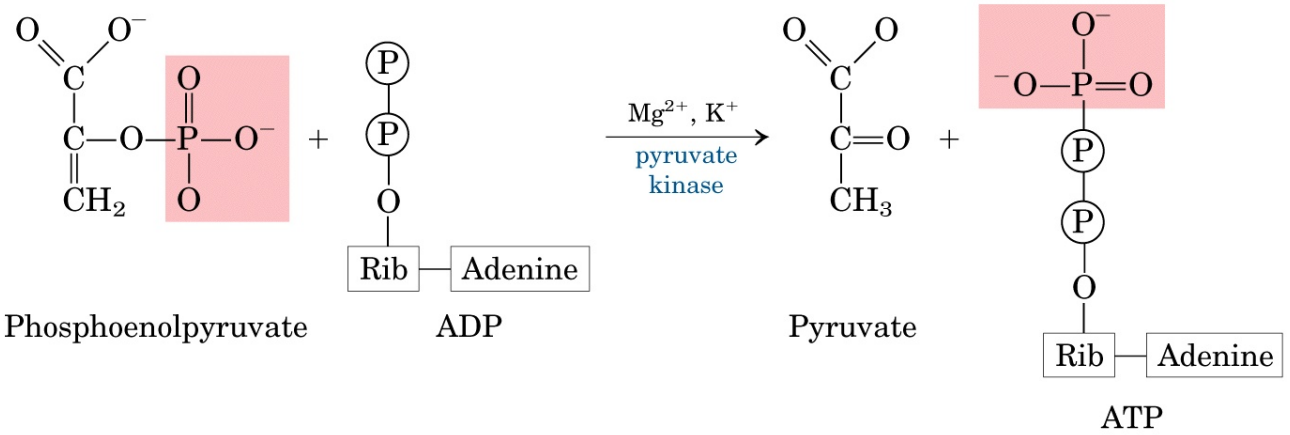



Pyruvate
Other amino acids, purines, pyrimidines
Acetyl CoA
Oxaloacetate
Aspartate
Citrate
Fatty acids, sterols
Purines
α-Ketoglutarate
Succinyl CoA
Other amino acids
Glutamate
Porphyrins, heme, chlorphyll

Pyruvate carboxylase

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \rightleftharpoons \text{oxaloacetate} + \text{ADP} + \text{Pi} + 2H^+$$

Making sugar from pyruvate is difficult due to the essentially irreversible reaction.



$\Delta G'^{\circ} = -31.4$ kJ/mol

So how can we make sugar if all we have is fat that is degraded to Acetyl-CoA?

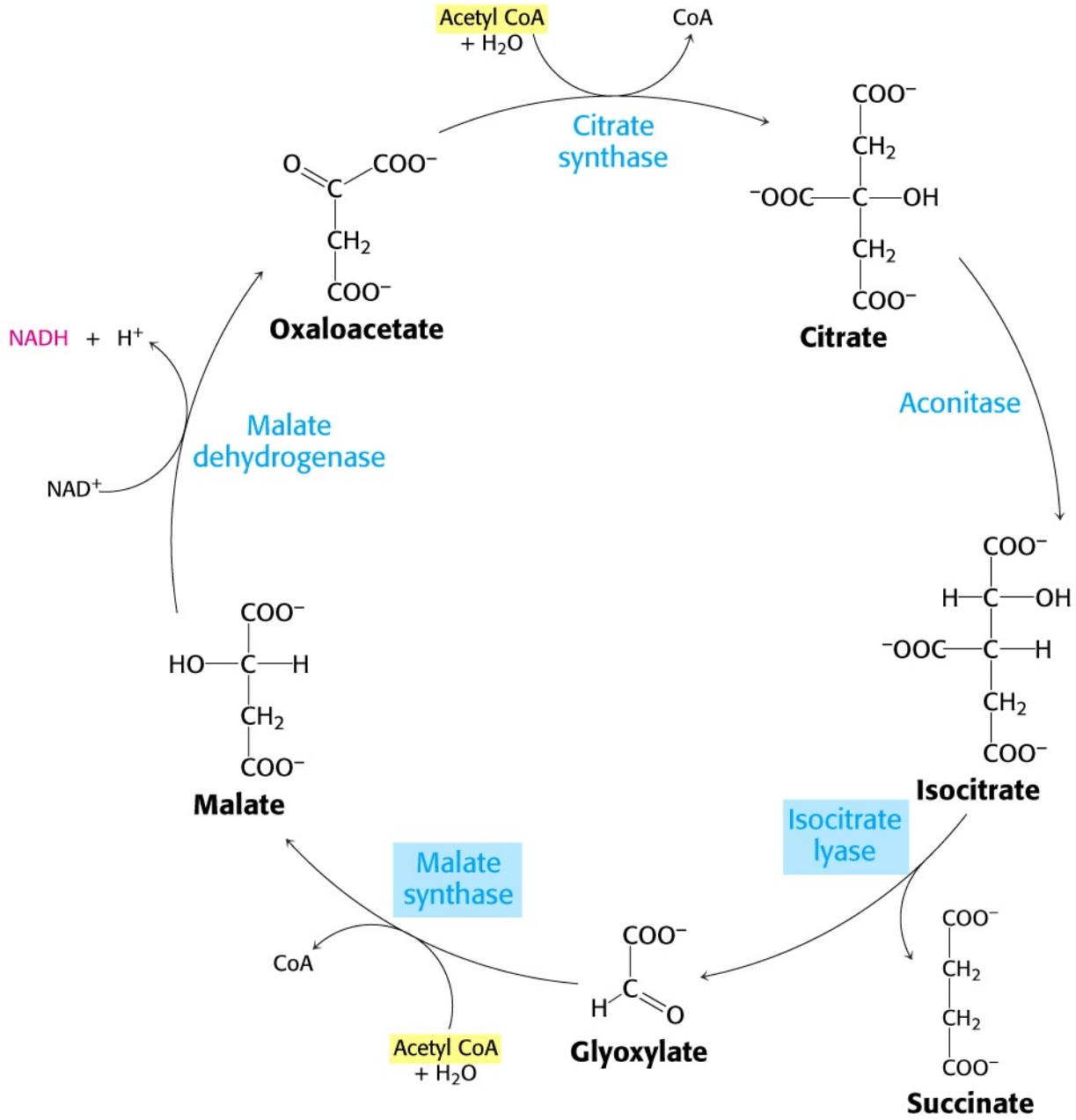
The reversible anaplerotic* reaction in the Krebs cycle is not the answer!

There is no net accumulation of oxaloacetate (or any other intermediate in the Krebs cycle). Both carbon atoms of acetate are released as CO_2.

The answer is the glyoxylate cycle discovered by Hans Kornberg!

This does not take place in vertebrates.

Anaplerotic is of Greek origin, meaning to fill up


Acetyl CoA
+ H2O
CoA
Citrate synthase
Oxaloacetate
Citrate
Aconitase
Isocitrate
Isocitrate lyase
Succinate
Glyoxylate
Malate synthase
CoA
Acetyl CoA
+ H2O
Malate
Malate dehydrogenase
NADH + H+
NAD+