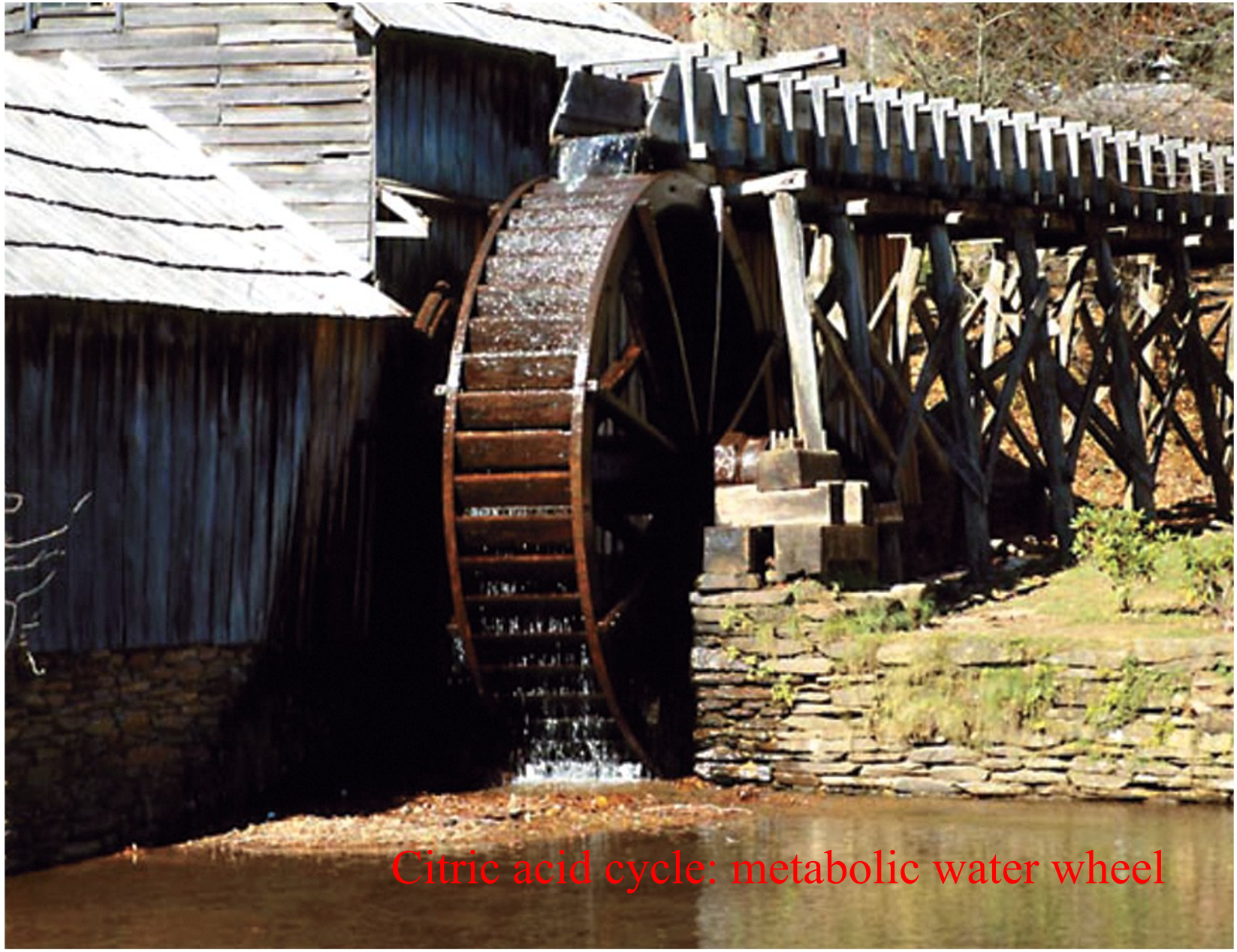
Chapter 4-I:
Citric Acid Cycle



Chapter 16 Opener Fundamentals of Biochemistry, 2/e

Overview of metabolic fuel metabolism

Citric acid cycle (CAC)

Not merely an oxidation of pyruvate to CO_2

A central pathway for recovering energy from several metabolic fuels

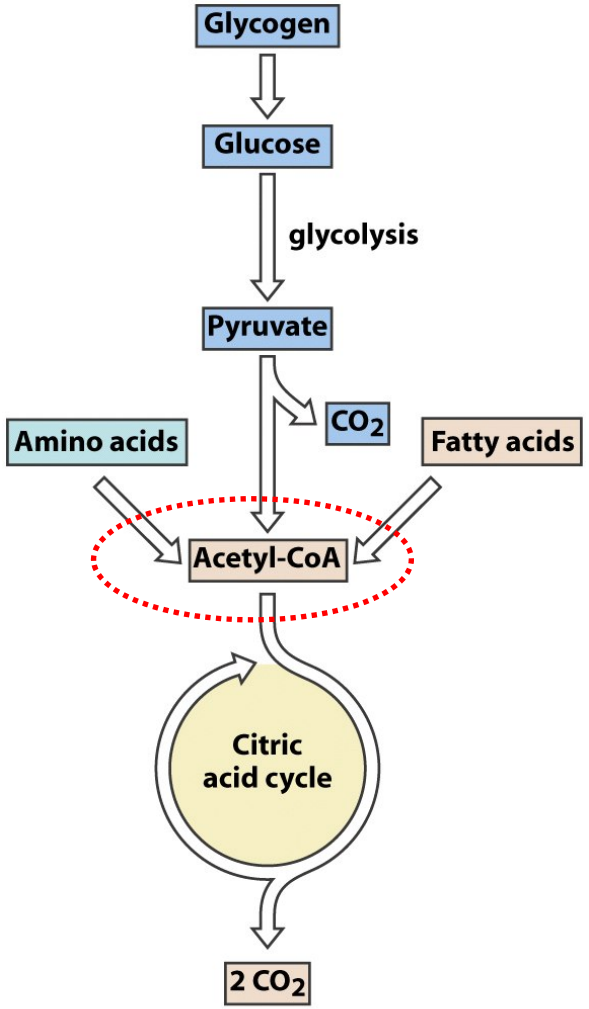


Figure 16-1 Fundamentals of Biochemistry, 2/e

Overview of CAC

8 reactions oxidizing acetyl-CoA to 2 CO_2
Generation of 3 NADH, 1 FADH2, 1 ATP(GTP)

1930s Hans Krebs linked the already known compounds

Plant products

Citric acid: citrus fruit
Aconitate: monkshood
Succinate: amber
Fumarate: Fumaria
Malate: apple

General features

Circular pathway: TCA cycle
Net reaction
Mitochondrial location
Amphibolic pathway: provides biosynthetic intermediates

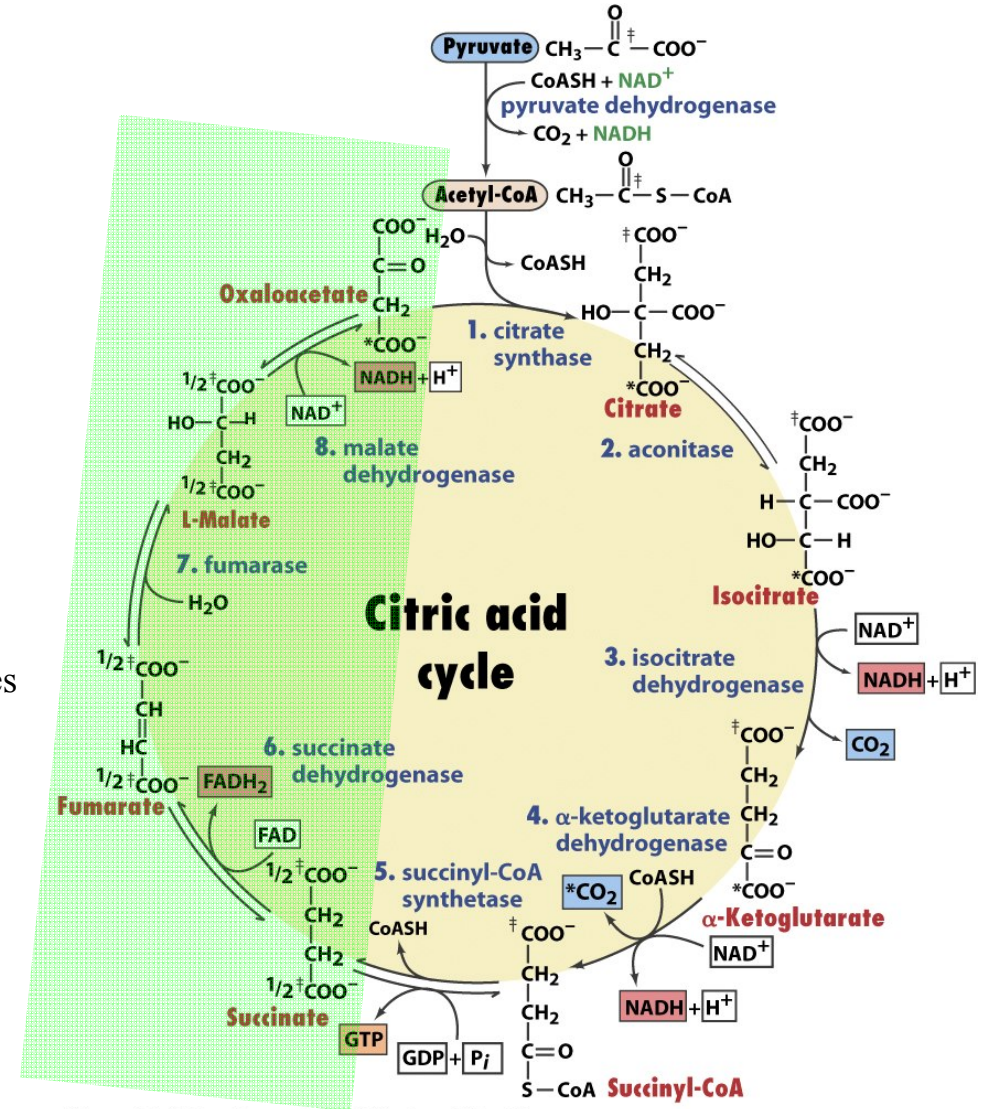


Figure 16-2 Fundamentals of Biochemistry, 2/e

Synthesis of Acetyl-CoA

Pyruvate to acetyl-CoA (high-E compound)
Transport to mitochondria via pyruvate-H^+ symport

Pyruvate dehydrogenase multienzyme complex (PDH)
Noncovalently associated enzymes
E. coli enzyme

- Pyruvae dehydrogenase (E1): 24 subunits
- Dihydrolipoyl transacetylase (E2): 24 subunits
- Dihydrolipoyl dehydrogenase (E3): 12 subunits

Multienzyme complex: evolution of catalytic efficiency

1. Overcome diffusion
2. Minimize side reactions
3. Coordinate control

EM of *E. coli* pyruvate dehydrogenase complex

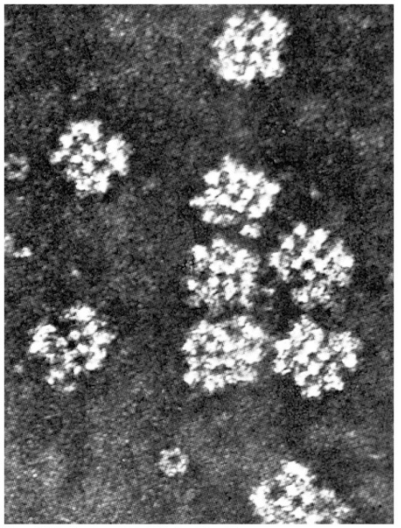


Figure 16-3a Fundamentals of Biochemistry, 2/e

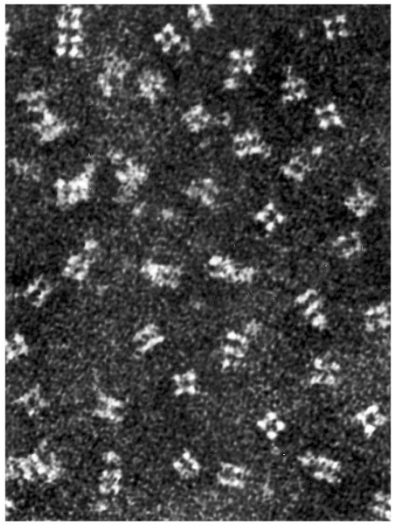


Figure 16-3b Fundamentals of Biochemistry, 2/e

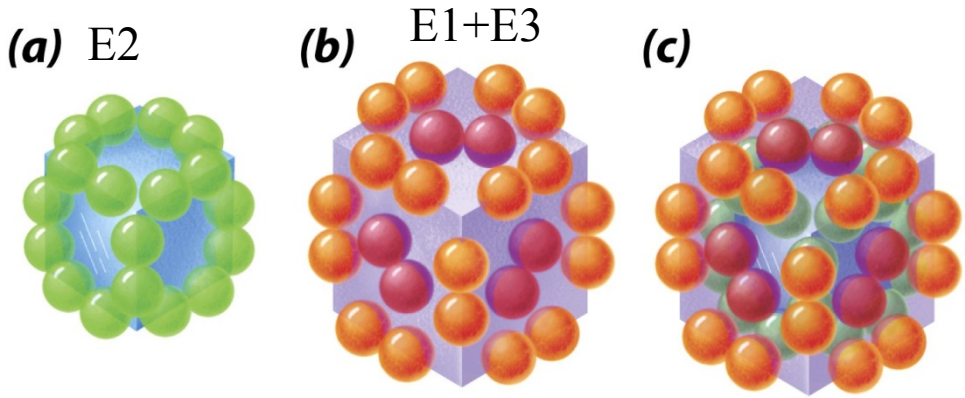


Figure 16-4 Fundamentals of Biochemistry, 2/e

Different complexity of PDH in different species

E2 core: 24 subunits (8 trimers)

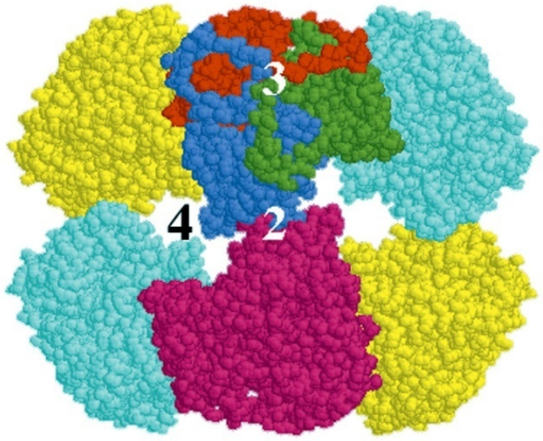


Figure 16-5a Fundamentals of Biochemistry, 2/e

E2 core: 60 subunits (20 trimers)

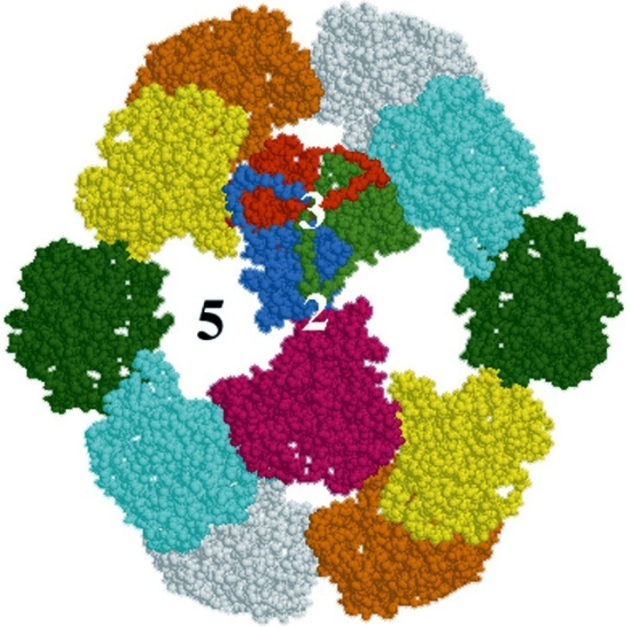


Figure 16-5b Fundamentals of Biochemistry, 2/e

The reactions of PDH

Pyruvate + CoA + NAD^+ → acetyl-CoA + CO_2 + NADH + H^+

5 different coenzymes

- TPP
- Lipoic acid
- CoA
- FAD
- NAD^+

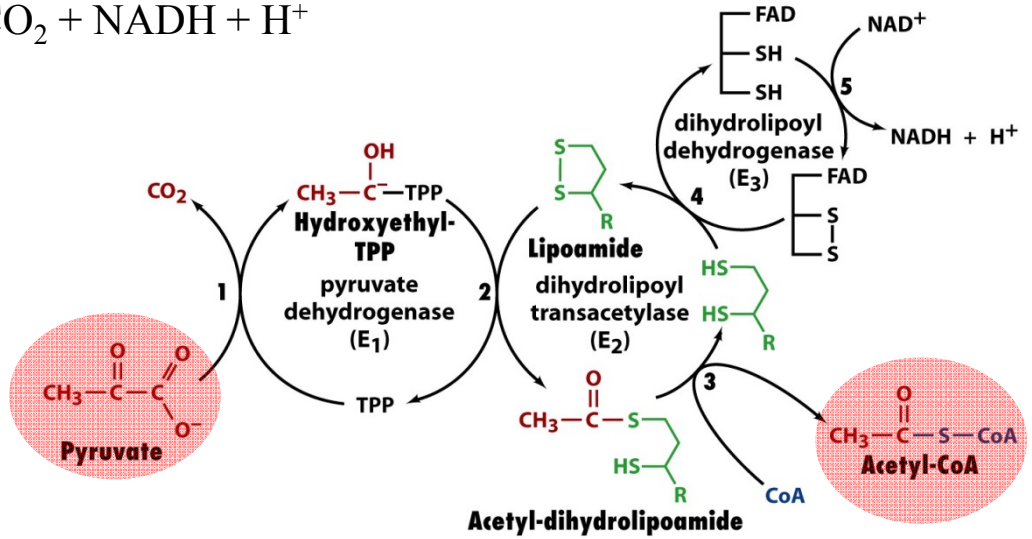


Figure 16-6 Fundamentals of Biochemistry, 2/e
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Table 16-1 The Coenzymes and Prosthetic Groups of Pyruvate Dehydrogenase

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 lipoamide
Flavin adenine dinucleotide (FAD)	Bound to E_3	Reduced by lipoamide
Nicotinamide adenine dinucleotide (NAD^+)	Substrate for E_3	Reduced by $FADH_2$

Table 16-1 Fundamentals of Biochemistry, 2/e
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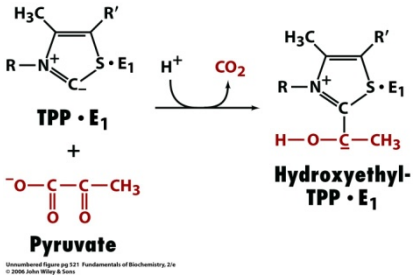
The sequence of reactions

1



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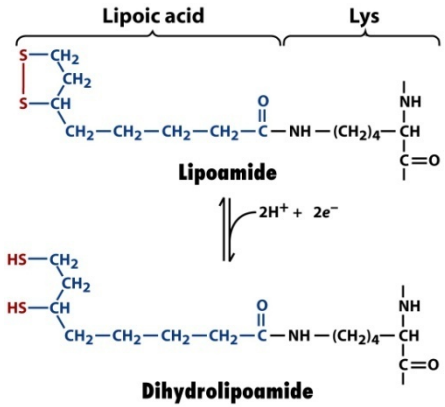

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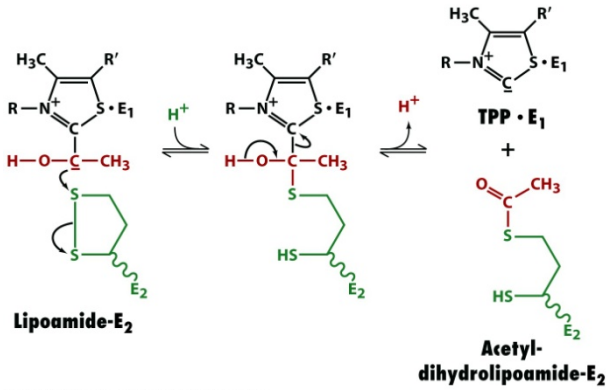

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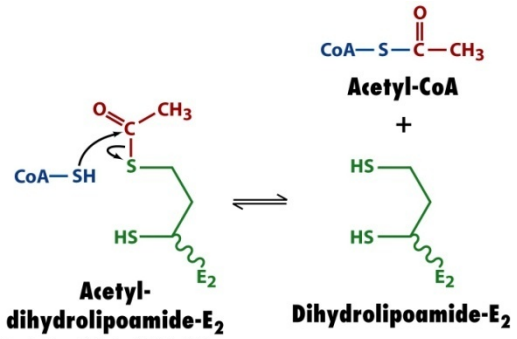

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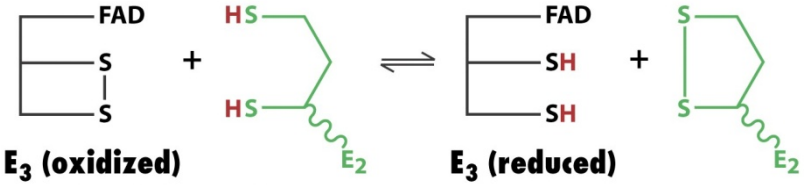

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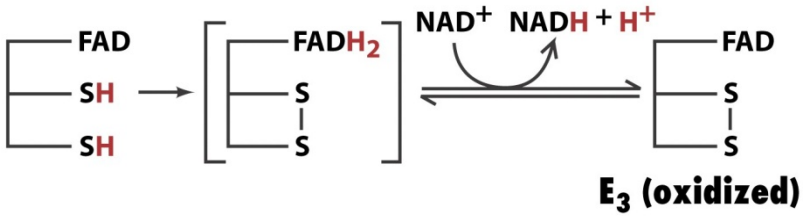


The active site of dihydrolipoamide dehydrogenase

Redox-active disulfide bond

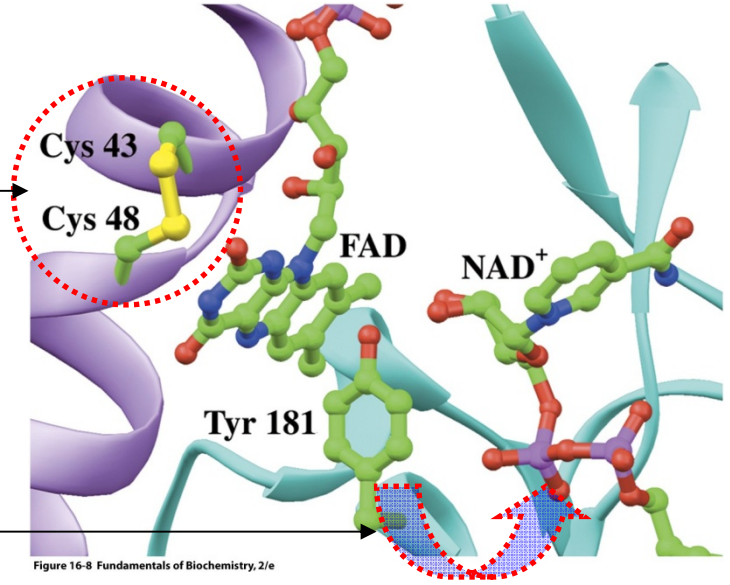


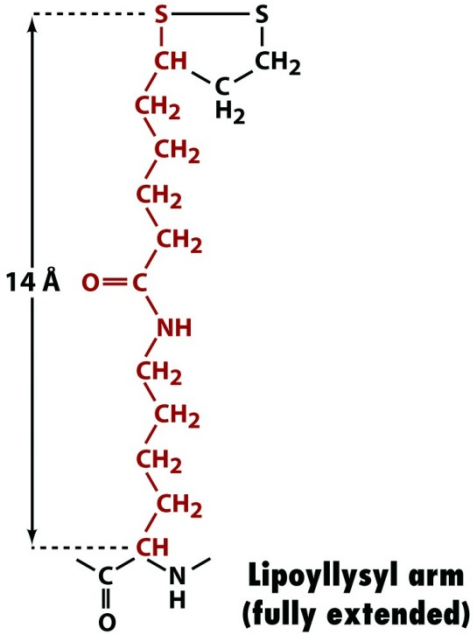
Figure 16-8 Fundamentals of Biochemistry, 2/e

Blocking NAD^+ binding site to shield flavin from solution

Check reduction potential of FAD & NAD^+

The lipoamide group of E2

Channeling of reaction intermediates between E2 (inside) and E1+E3 (outside)
Swing action from E1 to the E2 active site, and then to E3
Flexibility of the bound domain



Unnumbered figure pg 524 Fundamentals of Biochemistry, 2/e

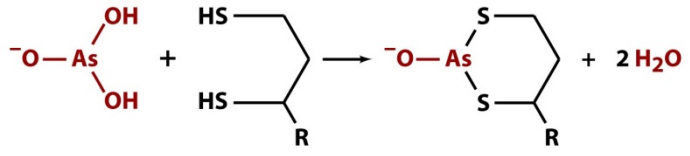
Arsenic poisoning

Arsenite

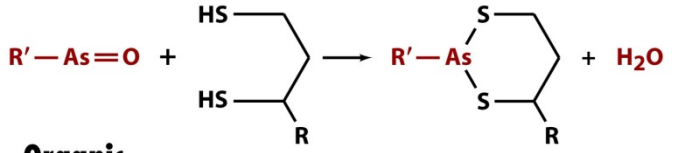
Organic arsenicals

Binding to -SH of lipoamide

Inactivation of PDH & α-ketoglutarate dehydrogenase



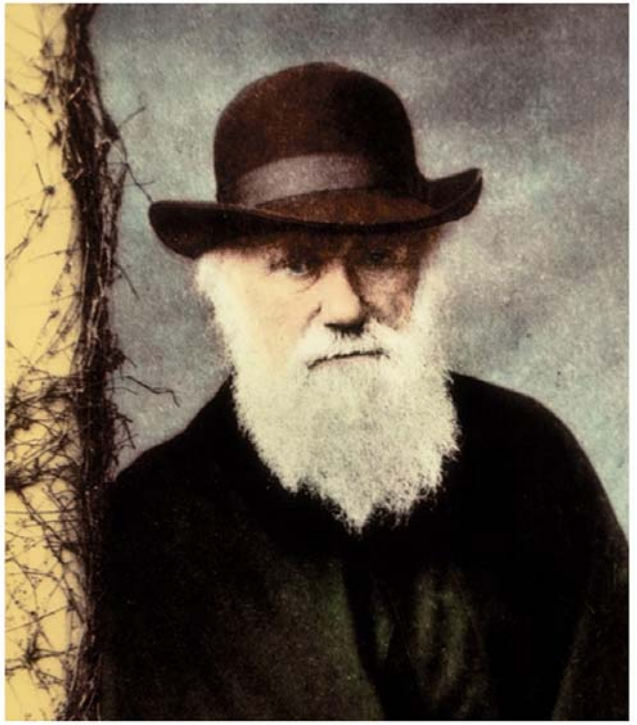
Arsenite **Dihydro-lipoamide**



Organic arsenical

Box 16-2 figure 1 Fundamentals of Biochemistry, 2/e

Box 16-2 figure 2 Fundamentals of Biochemistry, 2/e



Box 16-2 figure 3 Fundamentals of Biochemistry, 2/e

Enzymes of the citric acid cycle

8 enzymes

Citrate synthase
Aconitase
NAD^+-dependent isocitrate dehydrogenase: CO_2 release
α-ketoglutarate dehydrogenase: CO_2 release
Succinyl-CoA synthetase: GTP generation

complete oxidation of acetyl-CoA

Succinate dehydrogenase
Fumarase
Malate dehydrogenase

Regeneration of oxaloacetate

Citrate synthase

Condensation of acetyl-CoA and oxaloacetate

Ordered sequential reaction

oxaloacetate followed by acetyl-CoA

Large conformational changes

sealing the oxaloacetate binding site

generating acetyl-CoA binding site

open

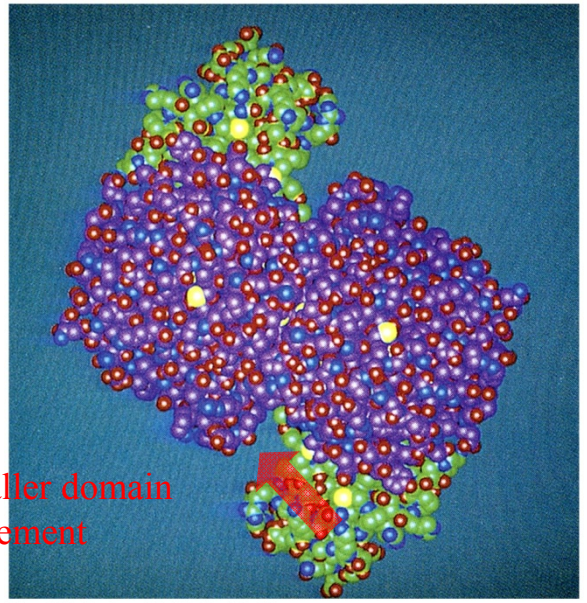


Figure 16-9a Fundamentals of Biochemistry, 2/e

Reaction mechanism

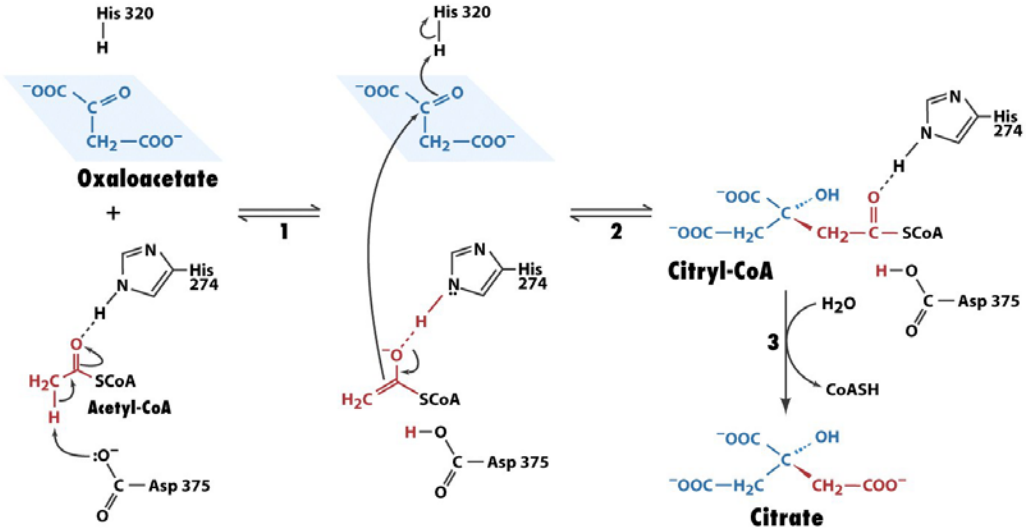


Figure 16-10 Fundamentals of Biochemistry, 2/e
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closed

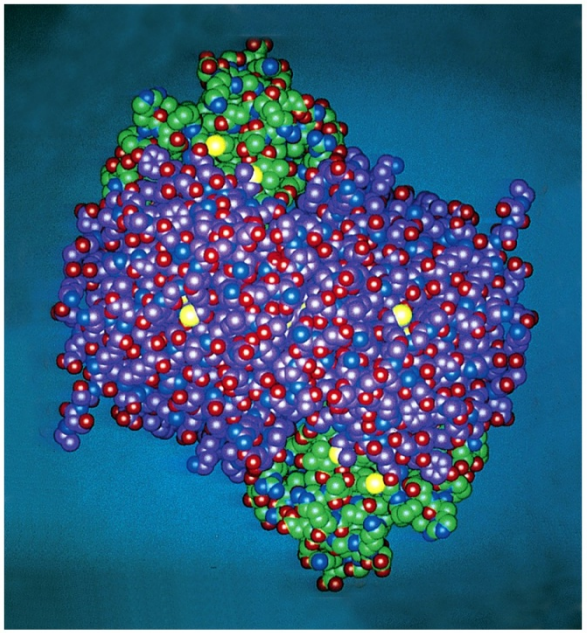


Figure 16-9b Fundamentals of Biochemistry, 2/e

Aconitase

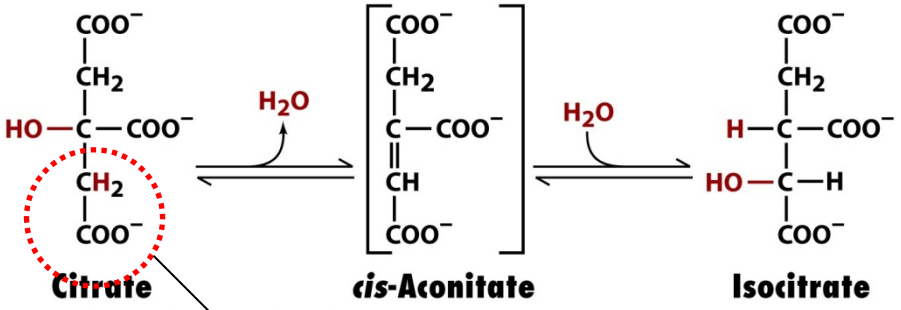
Reversible isomerization of citrate and isocitrate with cis-aconitate as an intermediate

Dehydration and rehydration

Citrate: pro-chiral compound

Isocitrate: chiral compound

[4Fe-4S] iron-sulfur cluster: (normally involve in redox reactions)



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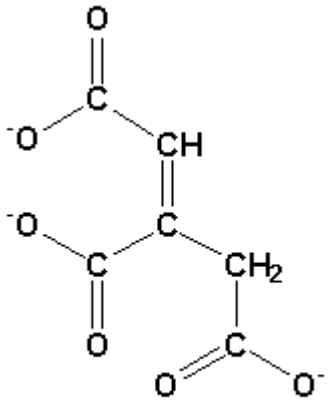
pro-R arm

4 possible stereoisomers



Cis-aconitate

NAD^+-dependent isocitrate dehydrogenase

There is a mammalian isozyme that depends on $NADP^+$ (cytosolic and unrelated to citric acid cycle)
Oxidative decarboxylation (similar to phosphogluconate dehydrogenase in PPP)

- oxidation of secondary alcohol to ketone
- decarboxylation of β-carboxyl group
- polarization of carbonyl group by Mn^{2+} (or Mg^{2+})
- first CO_2 generation

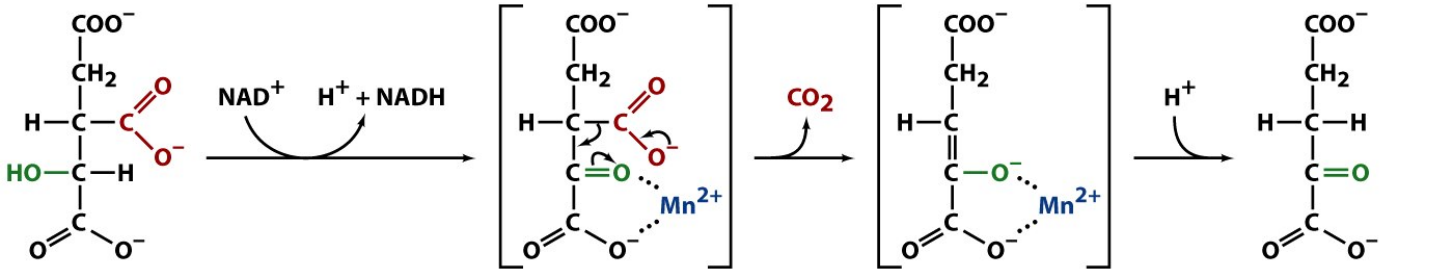


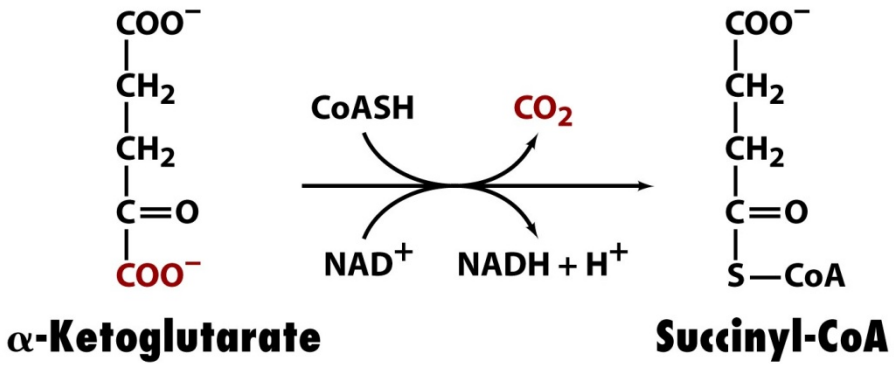
Figure 16-11 Fundamentals of Biochemistry, 2/e

Transient (β-keto acid is unstable)

α-ketoglutarate dehydrogenase

Oxidative decarboxylation of α-keto acid
Second CO_2 generation
Chemically resemble the reaction by PDH

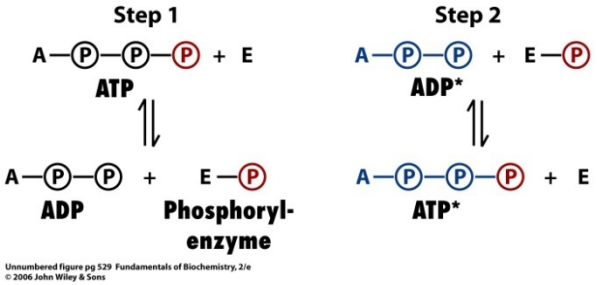
Multienzyme complex
- α-ketoglutarate dehydrogenase (E1)
- Dihydrolipoyl transsuccinylase (E2)
- Dihydrolipoyl dehydrogenase (E3): identical to the E3 of PDH



Unnumbered figure pg 528 Fundamentals of Biochemistry, 2/e

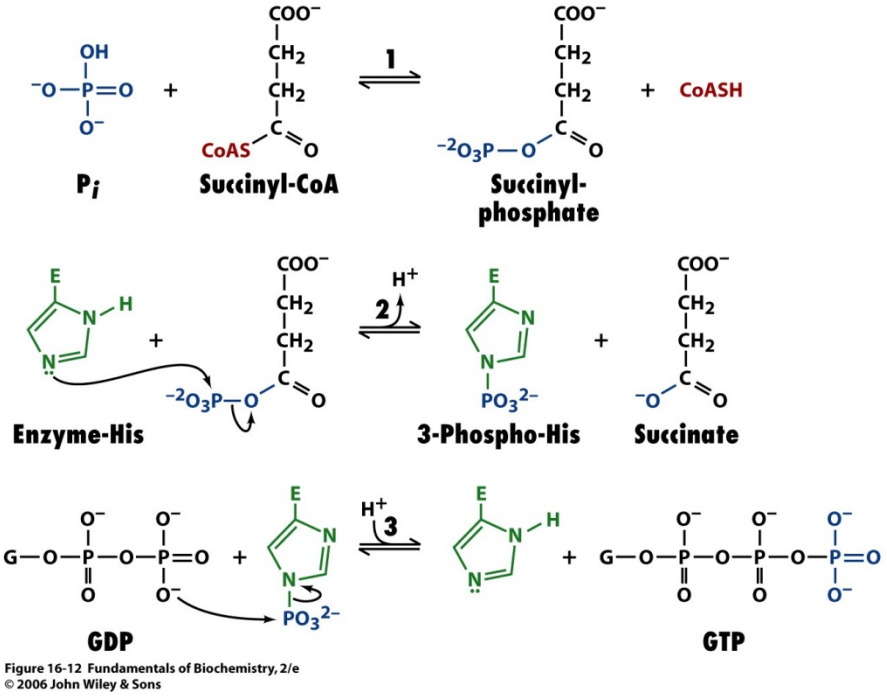
Succinyl-CoA synthetase

Succinate thiokinase
Generation of GTP (high-E succinyl-CoA):
substrate level phosphorylation
How?
Phosphoryl enzyme intermediate: passing of a hot potato



Isotope exchange reaction

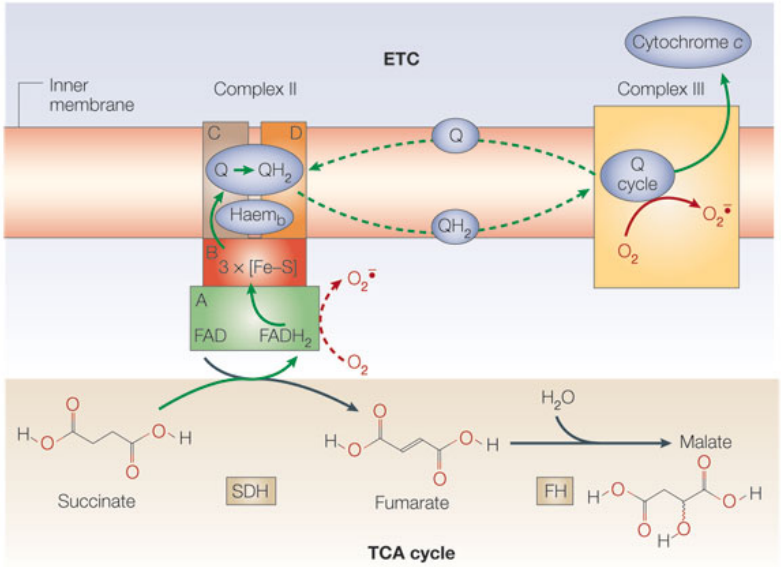
One acetyl equivalent is completely oxidized to two CO_2
Generation of two NADH + one GTP



Succinate dehydrogenase

Stereospecific dehydrogenation of succinate (alkane) to fumarate (alkene)
Inhibited by malonate
FAD prosthetic group

Only membrane bound enzyme
to funnel electrons to ETS



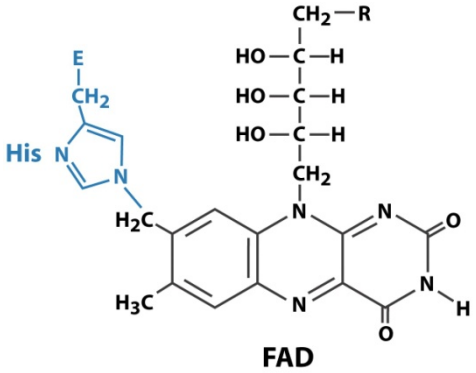


Figure 16-13 Fundamentals of Biochemistry, 2/e
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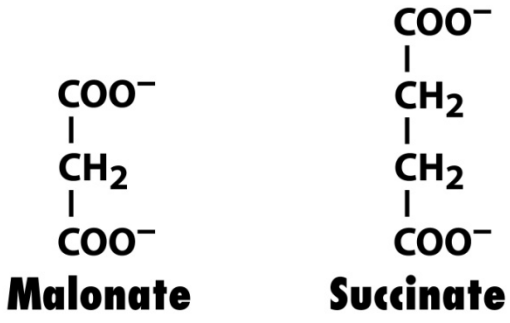

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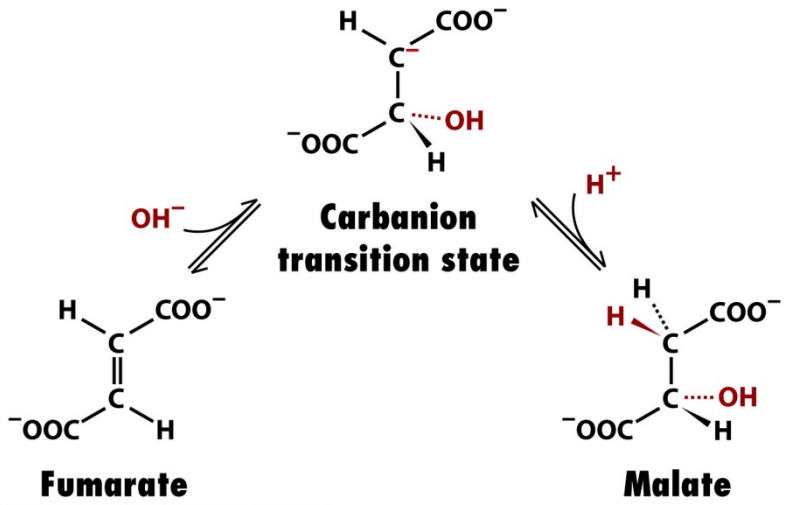
Fumarase (fumarate hydratase)

Hydration of the double bond to form malate via carbanion transition state



Malate dehydrogenase

Regeneration of oxaloacetate

The same catalytic reaction with ADH & LDH