Lecture 7

Krebs cycle (citric acid cycle)

Carbohydrate metabolism

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Krebs cycle

- The Krebs cycle, also known as the tricarboxylic acid cycle (TCA), or citric acid cycle was first recognized in 1937 by the man for whom it is named, German biochemist Hans Adolph Krebs

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Historical perspective

- 1937: Krebs: Enzymatic conversion of Pyruvate + Oxaloacetate to citrate and CO_2 Discovered the cycle of these reactions and found it to be a major pathway for pyruvate oxidation in muscle.

Hans Krebs, 1900–1981

The Krebs Cycle

- Occurs in the matrix of the mitochondrion
- Aerobic phase (requires oxygen)
- **2-carbon acetyl CoA** joins with a **4-carbon compound** to form a **6- carbon compound** called **Citric acid**

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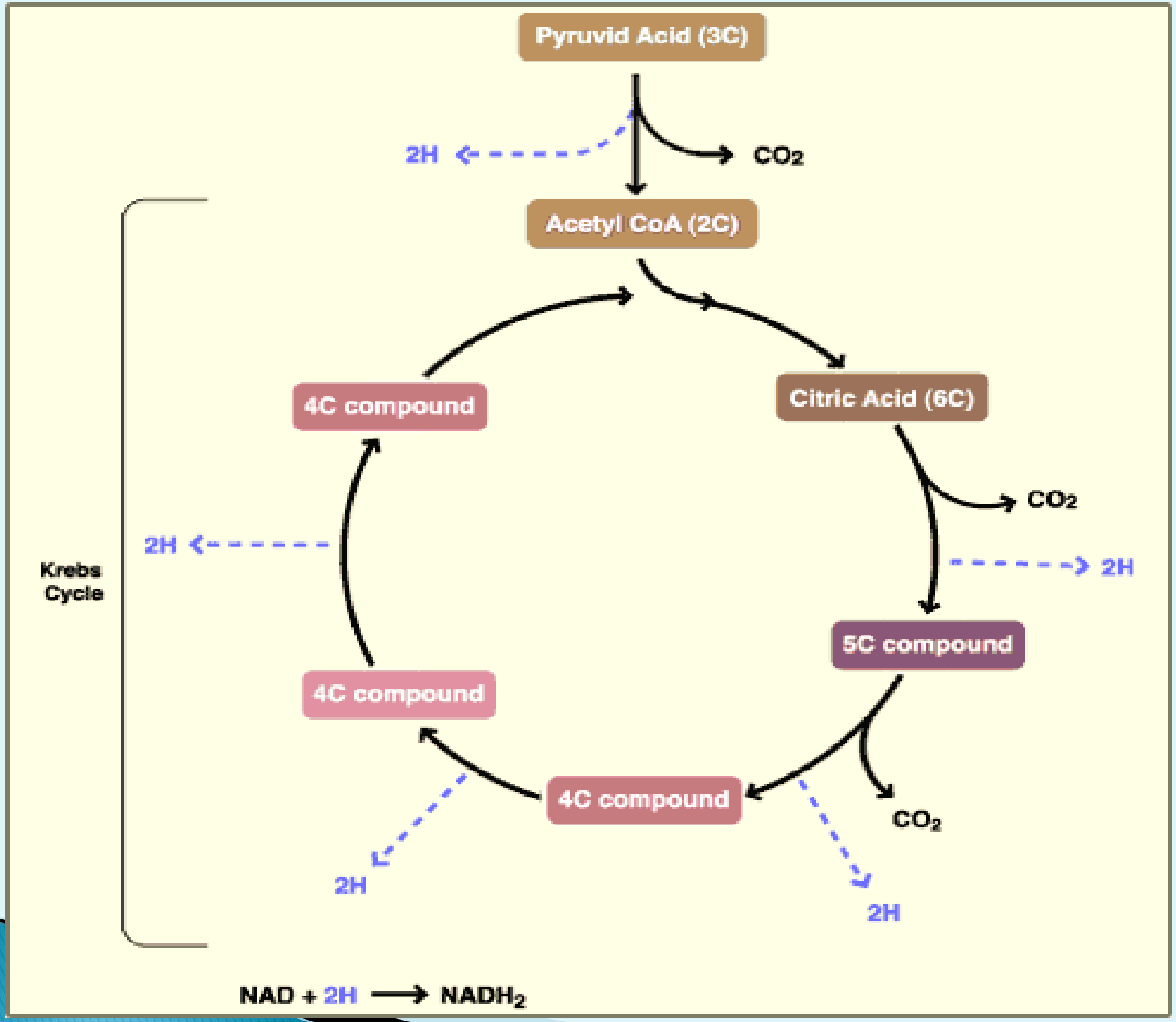
- Citric acid (6C) is gradually converted back to the 4-carbon compound
  -ready to start the cycle once more

- The carbons removed are released as CO_2
  -enzymes controlling this process called **decarboxylases**

- The hydrogens, which are removed, join with NAD to form NADH
  -enzymes controlling the release of hydrogen are called **dehydrogenases**


Pyruvid Acid (3C)
2H
CO2
Acetyl CoA (2C)
Citric Acid (6C)
CO2
2H
5C compound
CO2
4C compound
2H
2H
4C compound
4C compound
2H
Krebs Cycle
NAD + 2H → NADH2

- Transfer of hydrogen from each $NADH_2$ along system
  -produces 3 ATP
  -process called **oxidative phosphorylation**

- Complete oxidation of glucose yields **38 ATP**
  -2 during glycolysis
  -36 during oxidative phosphorylation

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Diagram of TCA

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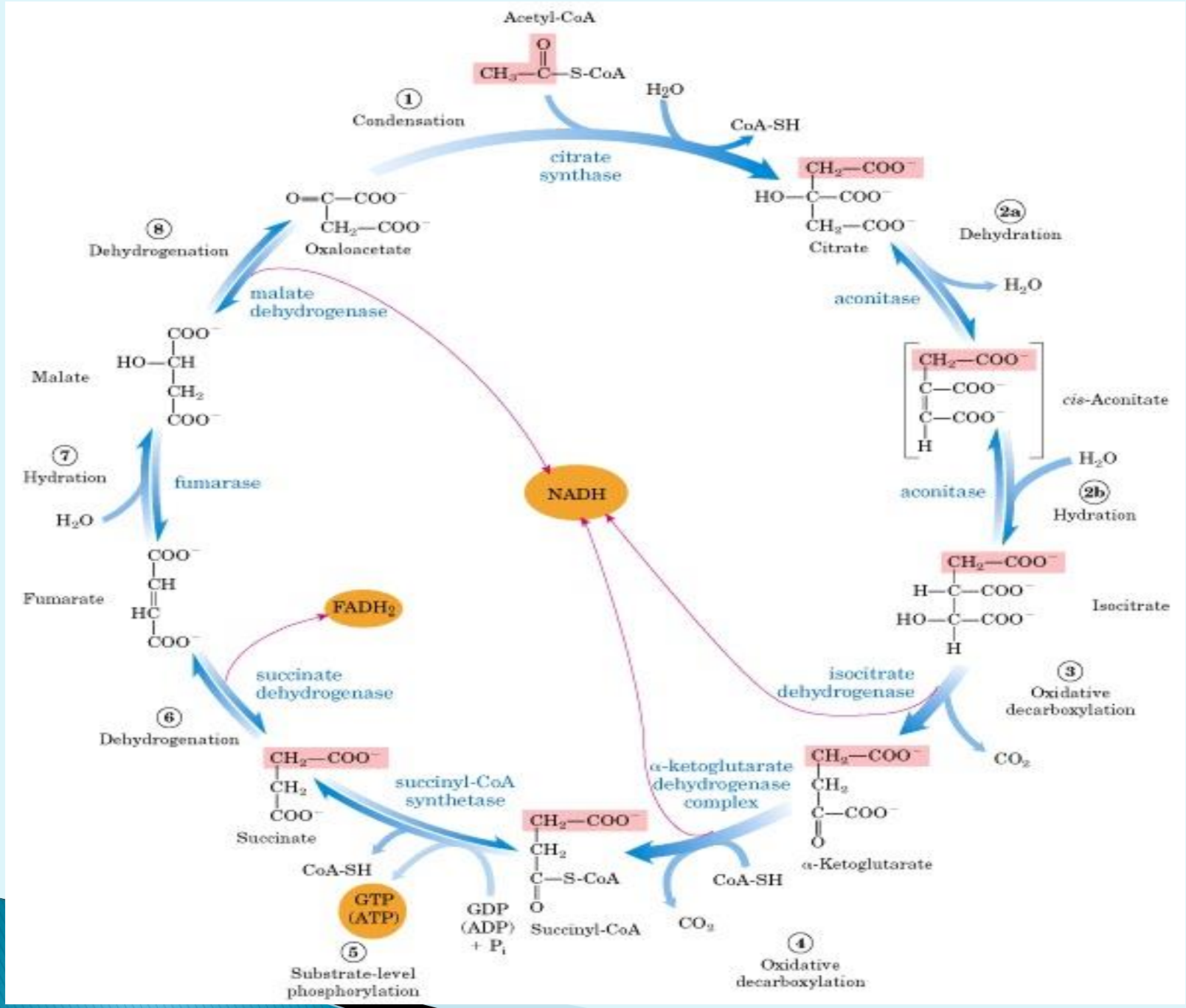
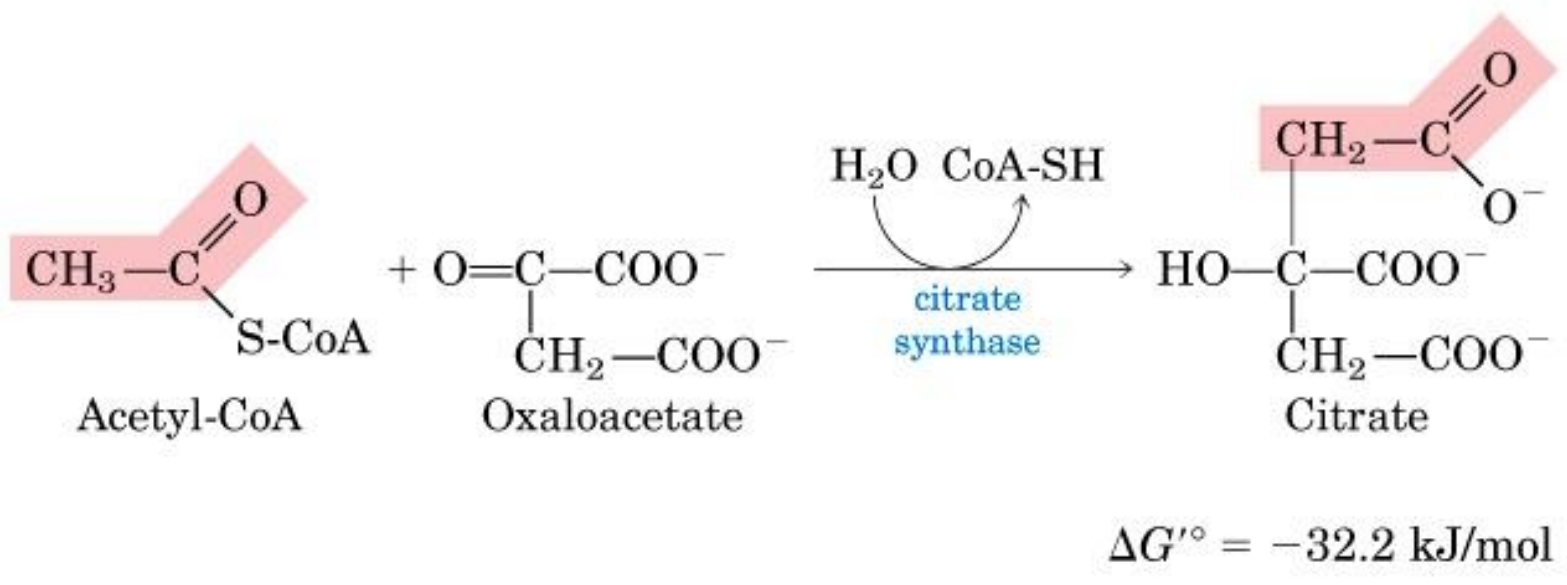

Acetyl-CoA
CH3—C(=O)—S-CoA
1
Condensation
H2O
CoA-SH
citrate synthase
CH2—COO⁻
HO—C—COO⁻
CH2—COO⁻
Citrate
2a
Dehydration
H2O
aconitase
CH2—COO⁻
C—COO⁻
C—COO⁻
H
cis-Aconitate
H2O
2b
Hydration
aconitase
CH2—COO⁻
H—C—COO⁻
HO—C—COO⁻
H
Isocitrate
isocitrate dehydrogenase
3
Oxidative decarboxylation
CO2
CH2—COO⁻
CH2
C—COO⁻
O
α-Ketoglutarate
α-ketoglutarate dehydrogenase complex
CO2
CH2—COO⁻
CH2
C—S-CoA
O
Succinyl-CoA
4
Oxidative decarboxylation
CoA-SH
succinyl-CoA synthetase
GTP (ATP)
GDP (ADP) + Pi
5
Substrate-level phosphorylation
CH2—COO⁻
CH2
COO⁻
Succinate
6
Dehydrogenation
succinate dehydrogenase
FADH2
COO⁻
CH
HC
COO⁻
Fumarate
7
Hydration
H2O
fumarase
COO⁻
HO—CH
CH2
COO⁻
Malate
8
Dehydrogenation
malate dehydrogenase
O=C—COO⁻
CH2—COO⁻
Oxaloacetate
NADH

Reactions of Citric Acid Cycle

Reactions of Citric Acid Cycle

1. Citrate synthase: Formation of Citroyl CoA intermediate.
2. Binding of Oxaloacetate to the enzyme results in conformational change which facilitates the binding of the next substrate, the acetyl Coenzyme A. There is a further conformational change which leads to formation of products. This mechanism of reaction is referred as induced fit model.



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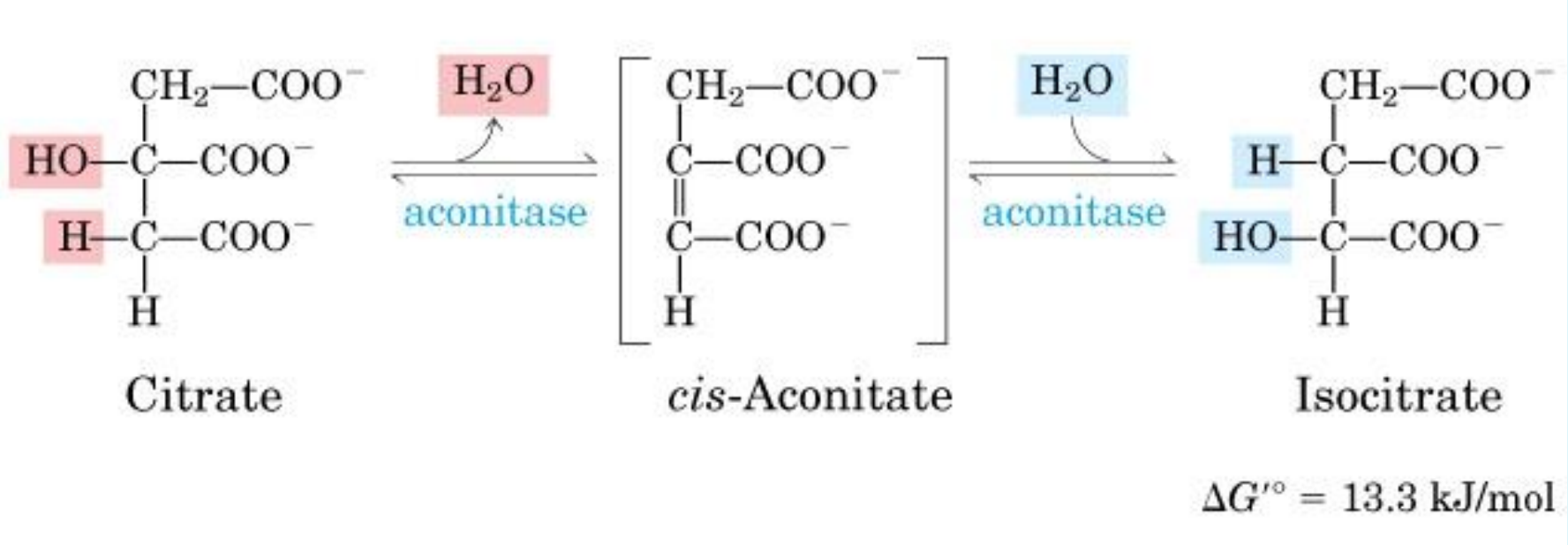
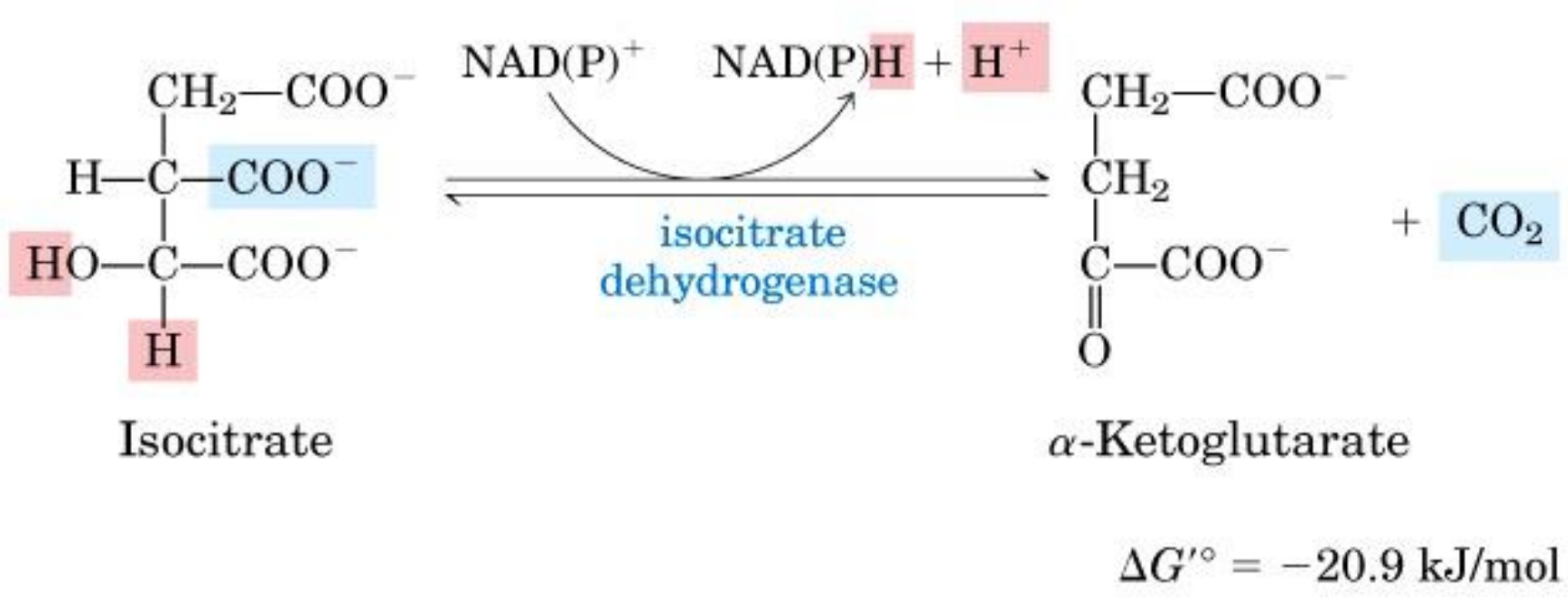
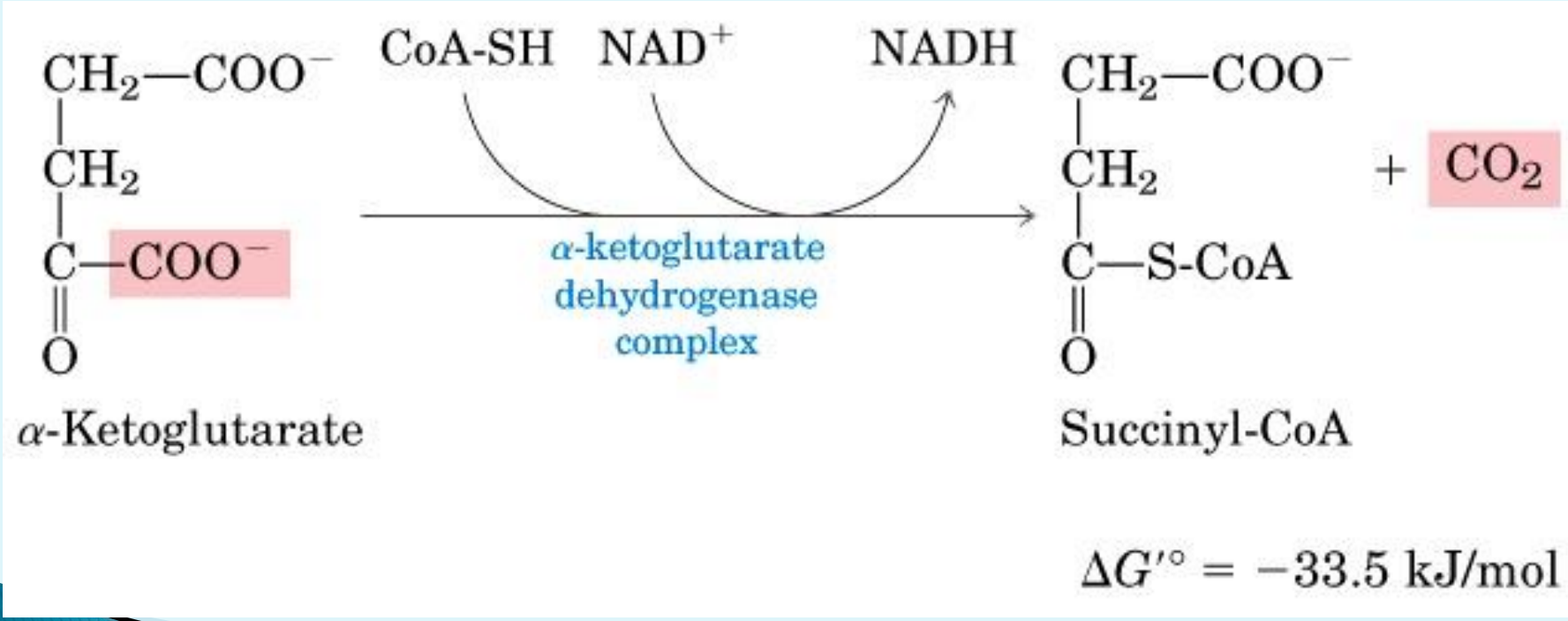
2. **Aconitase**: This enzyme catalyses the isomerization reaction by removing and then adding back the water (H and OH) to cis-aconitate in at different positions. Isocitrate is consumed rapidly by the next step thus deriving the reaction in forward direction.



3. Isocitrate dehydrogenase: There are two isoforms of this enzyme, one uses NAD^+ and other uses $NADP^+$ as electron acceptor.



4. α-Ketoglutarate dehydrogenase: This is a complex of different enzymatic activities similar to the pyruvate dyhdogenase complex. It has the same mechanism of reaction with E1, E2 and E3 enzyme units. NAD+ is an electron acceptor.



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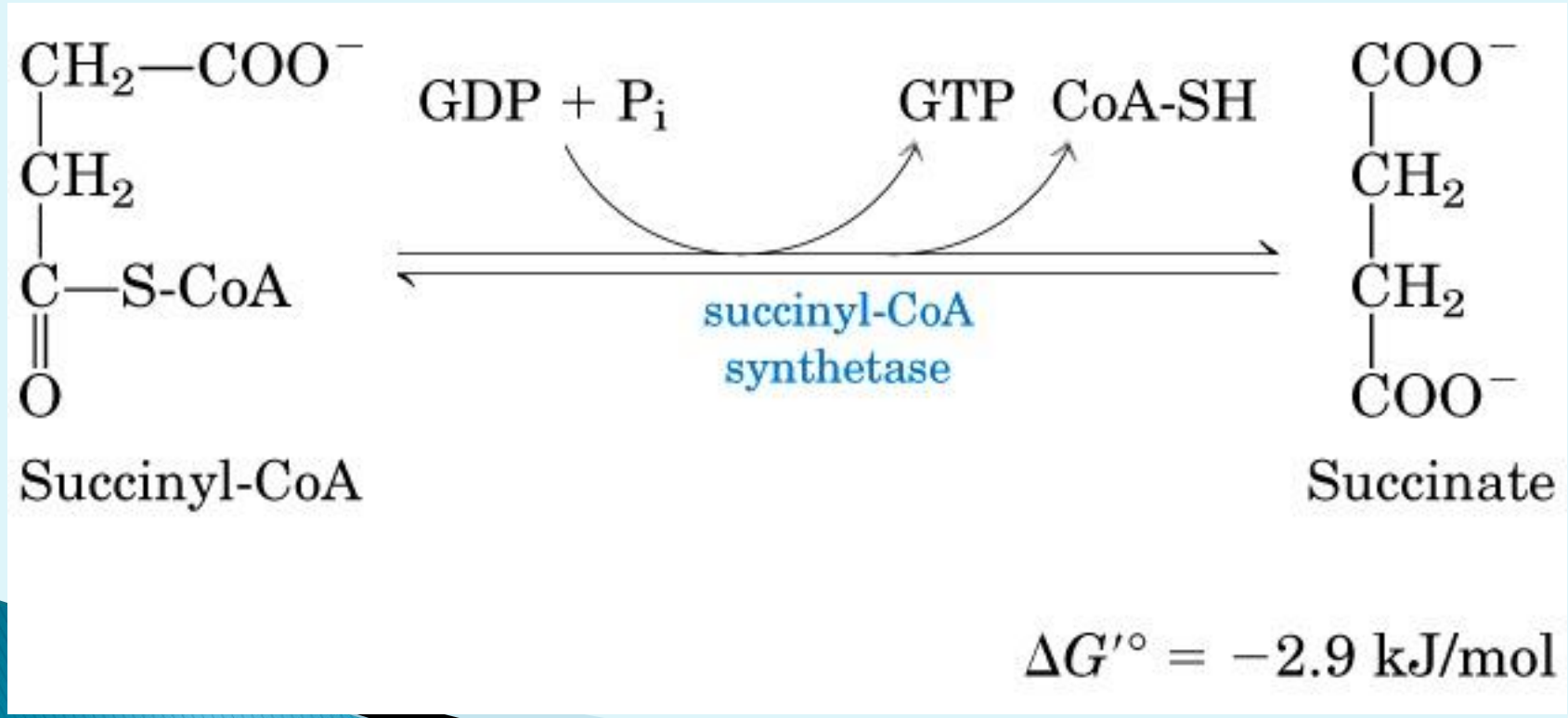
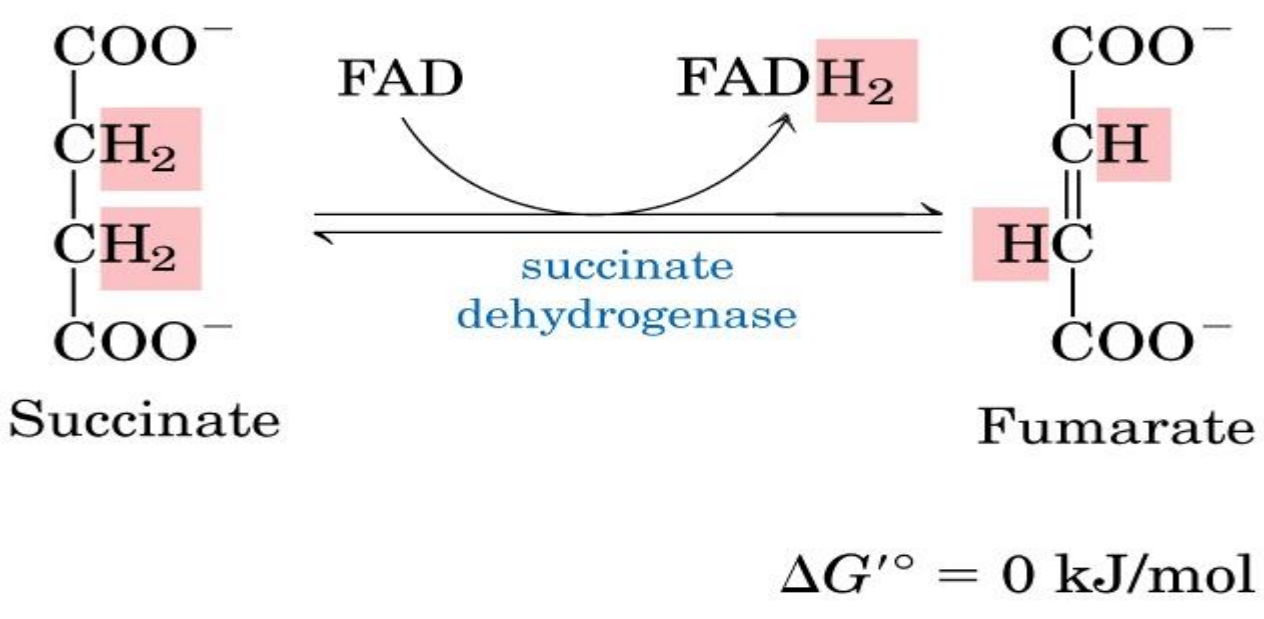
5. Succinyl CoA synthatse: Sccinyl CoA, like Acetyl CoA has a thioester bond with very negative free energy of hydrolysis. In this reaction, the hydrolysis of the thioester bond leads to the formation of phosphoester bond with inorganic phosphate. This phosphate is transferred to Histidine residue of the enzyme and this high energy, unstable phosphate is finally transferred to GDP resulting in the generation of GTP.

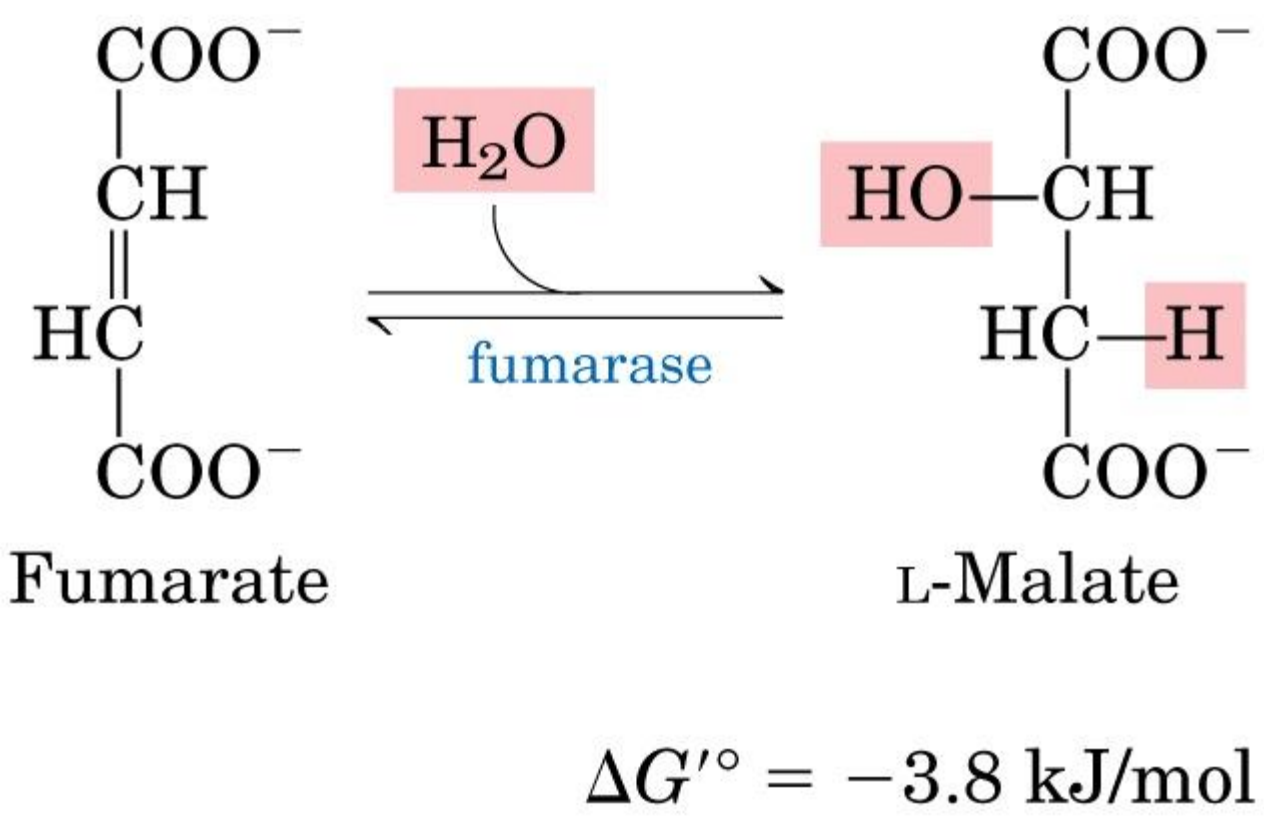


6. Succinate Dehydrogenase: Oxidation of succinate to fumarate. This is the only citric acid cycle enzyme that is tightly bound to the inner mitochondrial membrane. It is an FAD dependent enzyme.

Malonate has similar structure to Succinate, and it competitively inhibits SDH.



7. Fumarase: Hydration of Fumarate to malate: It is a highly stereospecific enzyme. Cis-Maleate (the cis form of fumarate is not recognized by this enzyme.



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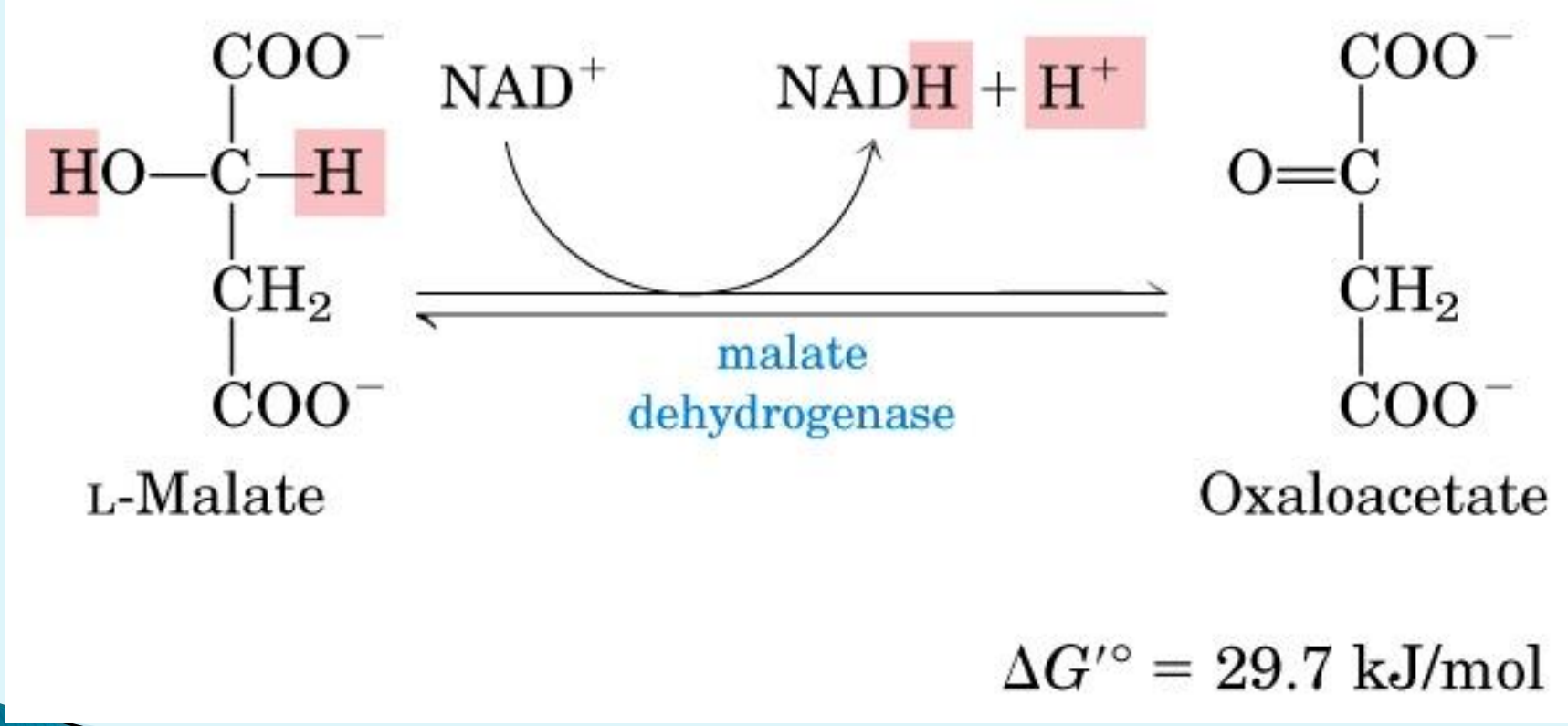
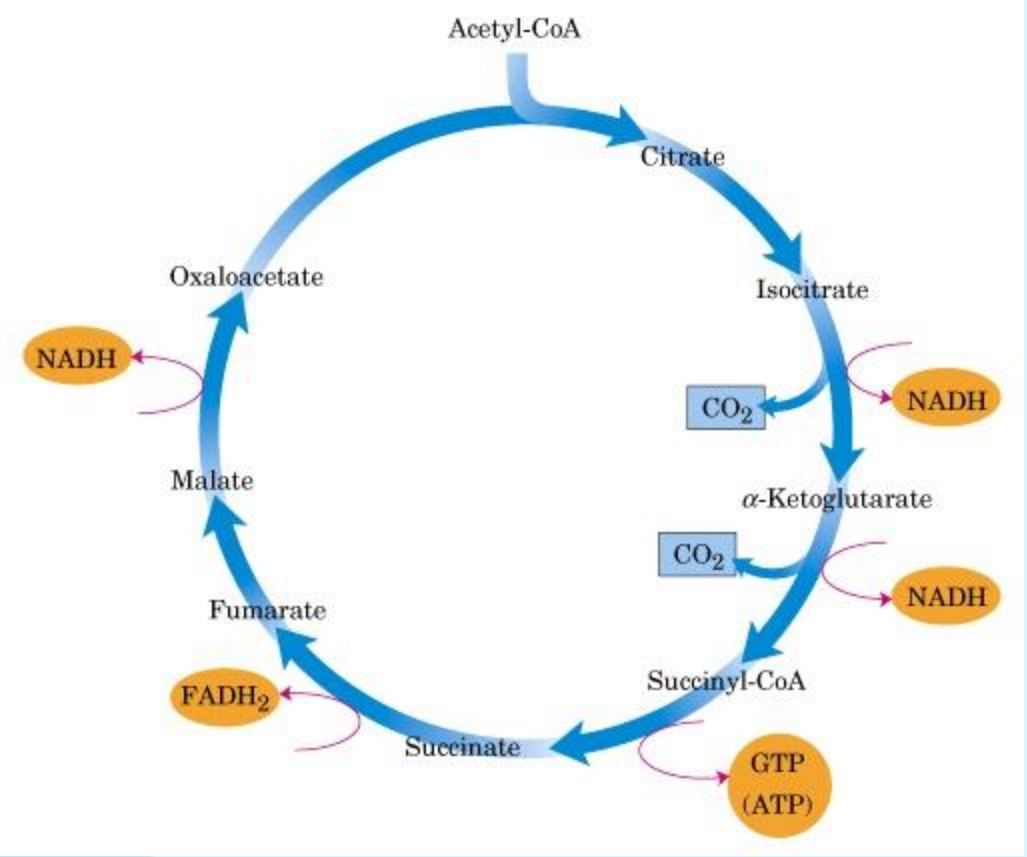
8. L-Malate dehydrogenase: Oxidation of malate to oxaloacetate: It is an NAD^+dependent enzyme. Reaction is pulled in forward direction by the next reaction (citrate synthase reaction) as the oxaloacetate is depleted at a very fast rate.



Conservation of energy of oxidation in the krebs cycle :

The two carbon acetyl group generated in PDC reaction enter the krebs cycle , and two molecules of CO2 are released in on cycle. Thus there is complete oxidation of two carbons during one cycle. Although the two carbons which enter the cycle become the part of oxaloacetate, and are released as CO2 only in the third round of the cycle. The energy released due to this oxidation is conserved in the reduction of 3 NAD+, 1 FAD molecule and synthesis of one GTP molecule which is converted to ATP.



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Efficiency of Biochemical engine in Living Systems:

Oxidation of one glucose yields 2840 kJ/mole energy

Energy obtained by biological engine: 32ATP X 30.5 kJ/Mol = 976 kJ/mol

Thus 34% efficiency is obtained if calculations are done using standard conditions. But if concentrations in the cellular condition are taken in account, the efficiency is close to 65%.

In the cytoplasm			
Glycolysis:		2 ATP	→ 2 ATP
In the mitochondria			
From glycolysis:	2 NADH	→ 6 ATP	→ 6 ATP*
From respiration:			
Pyruvic acid → acetyl CoA:	1 NADH	→ 3 ATP	(× 2) → 6 ATP
Krebs cycle:		1 ATP	
	3 NADH	→ 9 ATP	(× 2) → 24 ATP
	1 $FADH_2$	→ 2 ATP	
Total:			38 ATP

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Thank you