Citric acid cycle (Krebs cycle)

The citric acid cycle, also known as the Krebs cycle or tricarboxylic acid (TCA) cycle, is the second stage of cellular respiration.

- **Citric acid cycle**: a series of chemical reactions used by all aerobic organisms to generate energy through the oxidization of acetate derived from carbohydrates, fats, and proteins into carbon dioxide

- **Krebs cycle**: a series of enzymatic reactions that occurs in all aerobic organisms; it involves the oxidative metabolism of acetyl units and serves as the main source of cellular energy

The usable energy found in the carbohydrates, proteins, and fats we eat is released mainly through the citric acid cycle. Although the citric acid cycle does not use oxygen directly, it works only when oxygen is present. The first phase of cellular respiration, called glycolysis, takes place in the cytosol of the cell's cytoplasm. The citric acid cycle, however, occurs in the matrix of cell mitochondria. Prior to the beginning of the citric acid cycle, pyruvic acid generated in glycolysis crosses the mitochondrial membrane and is used to form **acetyl coenzyme A (acetyl CoA)**. Acetyl CoA is then used in the first step of the citric acid cycle. Each step in the cycle is catalyzed by a specific enzyme.

REACTIONS OF THE TCA CYCLE

1. Oxidative decarboxylation of pyruvate

The major source of acetyl CoA, the two-carbon substrate for the TCA cycle, is the oxidative decarboxylation of pyruvate. Pyruvate, the end product of aerobic glycolysis, must be transported from the cytosol into the mitochondrion. This is accomplished by a specific transporter that facilitates movement of pyruvate across the inner mitochondrial membrane. Once in the mitochondrial matrix, pyruvate is converted to acetyl CoA by the **pyruvate dehydrogenase complex** (PDH complex), which is a multi enzyme complex.

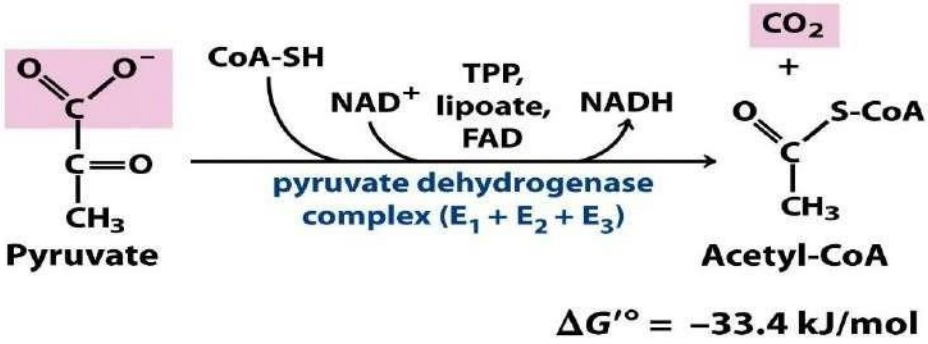

Figure 16-2
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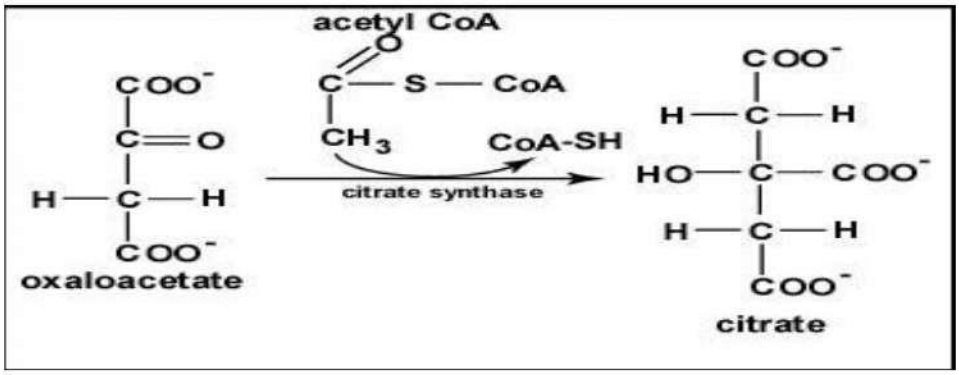
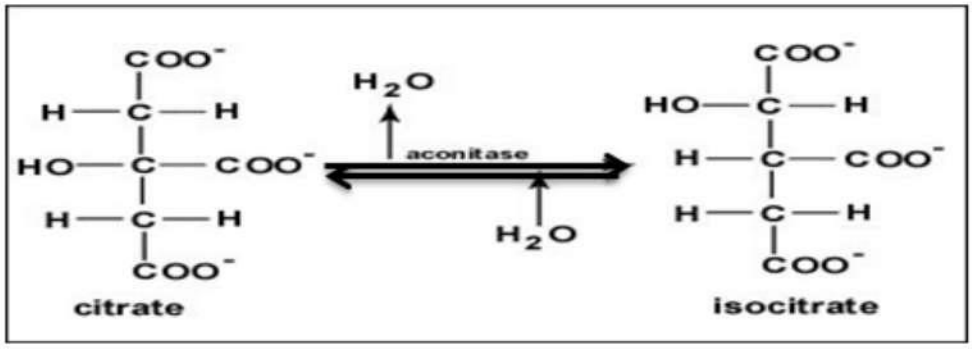
2. Synthesis of citrate from acetyl coenzyme A and oxaloacetate

The first reaction of the cycle is the condensation of acetyl-CoA with oxaloacetate to form citrate, catalyzed by **citrate synthase**.



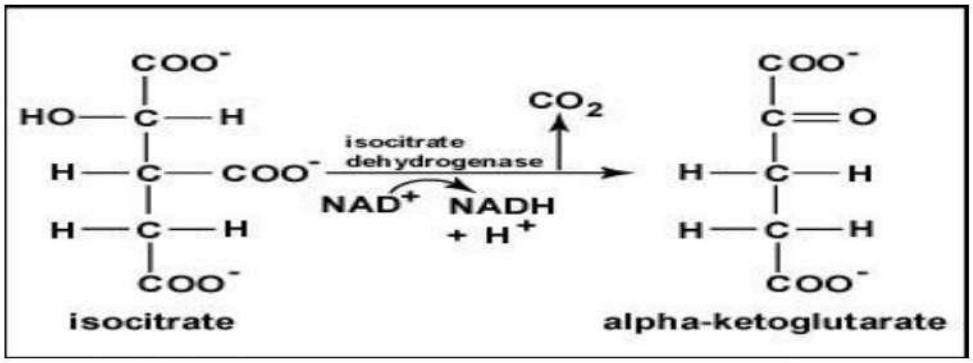
3. Isomerization of citrate

Citrate is isomerized to isocitrate by **aconitase** (aconitate hydratase)



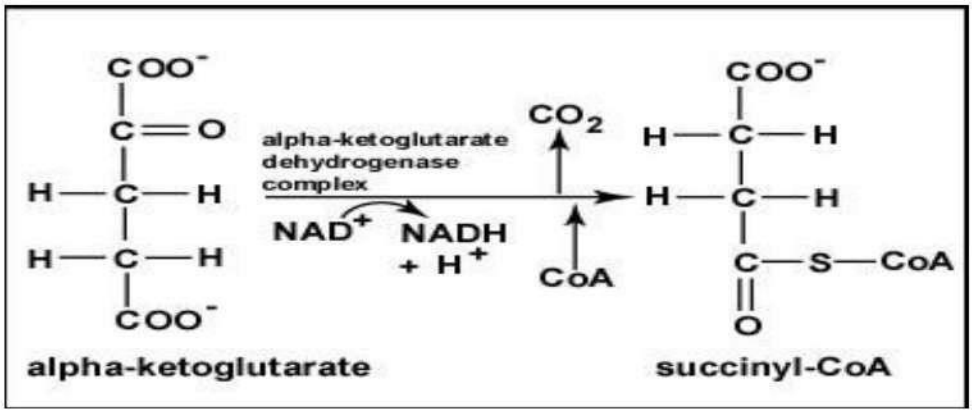
4. Oxidative decarboxylation of isocitrate

Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate, yielding the first of three NADH molecules produced by the cycle and the first release of CO_2 .This is one of the rate-limiting steps of the TCA cycle. The enzyme is allosterically activated by ADP (a low-energy signal) and Ca^{2+} and is inhibited by ATP and NADH, levels of which are elevated when the cell has abundant energy stores.



5. Oxidative decarboxylation of α -ketoglutarate

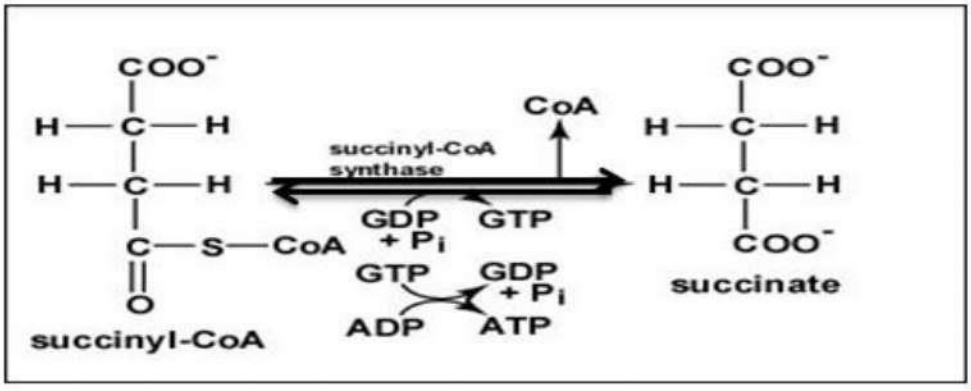
The conversion of α-ketoglutarate to succinyl CoA is catalyzed by the **α-ketoglutarate dehydrogenase complex**,. The reaction releases the second CO_2 and produces the second NADH of the cycle. The coenzymes required are TPP, lipoic acid, FAD , NAD^+, and CoA. α-Ketoglutarate dehydrogenase complex is inhibited by its products, NADH and succinyl CoA, and activated by Ca^{2+}.



6. Cleavage of succinyl coenzyme A

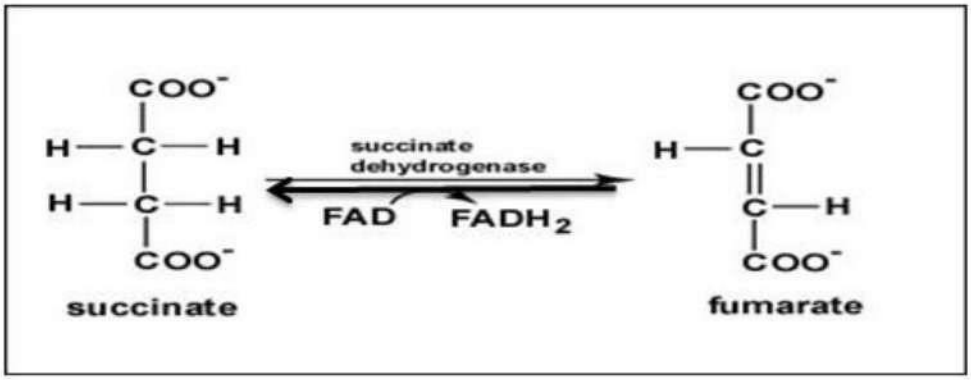
Succinate thiokinase (also called **succinyl CoA synthetase**, named for the reverse reaction) cleaves the high-energy thio ester bond of succinyl CoA . This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction:

GTP + ADP ⟷ GDP + ATP



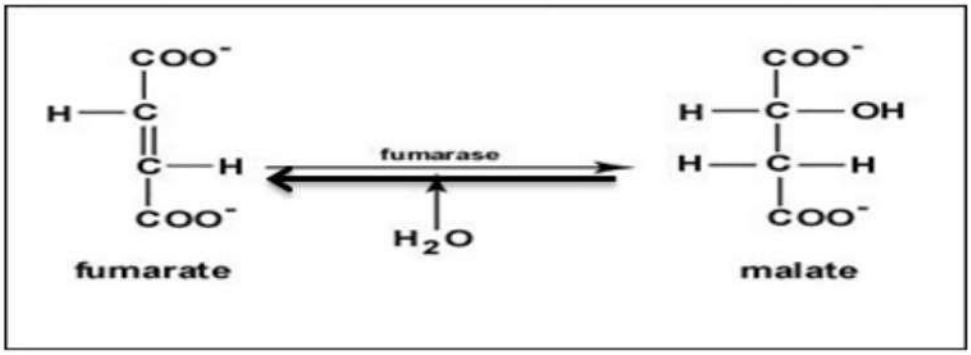
7. Oxidation of succinate

Succinate is oxidized to fumarate by **succinate dehydrogenase**. Flavin adenine dinucleotide (FAD) is reduced and forms $FADH_2$ in the process.



8. Hydration of fumarate

Fumarate is hydrated to malate in a freely reversible reaction catalyzed by **fumarase** .A water molecule is added and bonds between the carbons in fumarate are rearranged forming malate.



9. Oxidation of malate

Malate is oxidized to oxaloacetate by malate dehydrogenase . This reaction produces the third and final NADH of the cycle.

The Net Equation

acetyl CoA + 3 NAD^+ + FAD + ADP + HPO_4^{-2} ——————> 2 CO_2 + CoA + 3 NADH + $FADH_2$ + ATP

ENERGY PRODUCED BY THE TCA CYCLE

Two carbon atoms enter the cycle as acetyl CoA and leave as CO_2.The cycle does not involve net consumption or production of oxaloacetate or of any other intermediate. Four pairs of electrons are transferred during one turn of the cycle: three pairs of electrons reducing three NAD^+ to NADH and one pair reducing FAD to $FADH_2$. Oxidation of one NADH by the electron transport chain leads to formation of approximately three ATP, whereas oxidation of $FADH_2$ yields approximately two ATP .

- one NADH generates 3 ATP molecules
- one $FADH_2$ generates 2 ATP molecules
- one ATP is generated during step 5

Thus 12 ATP are generated for each turn of the cycle. (how many ATPs are generated for each molecule of glucose?)

Energy producing reaction	Number of ATP produced
3 NADH ⟶ 3 NAD^+	9
$FADH_2$ ⟶ FAD	2
GDP + P_i ⟶ GTP	1
	12 ATP/acetyl CoA oxidized

(Fg 1.3) Number of ATP molecules produced from the oxidation of one molecule of acetyl CoA (using both substrate-level and oxidative phosphorylation).

Regulation of the TCA cycle

The TCA cycle is controlled by the regulation of several enzyme activities. The most important of these regulated enzymes are those that catalyze reactions:

Citrate synthase, Isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase complex.

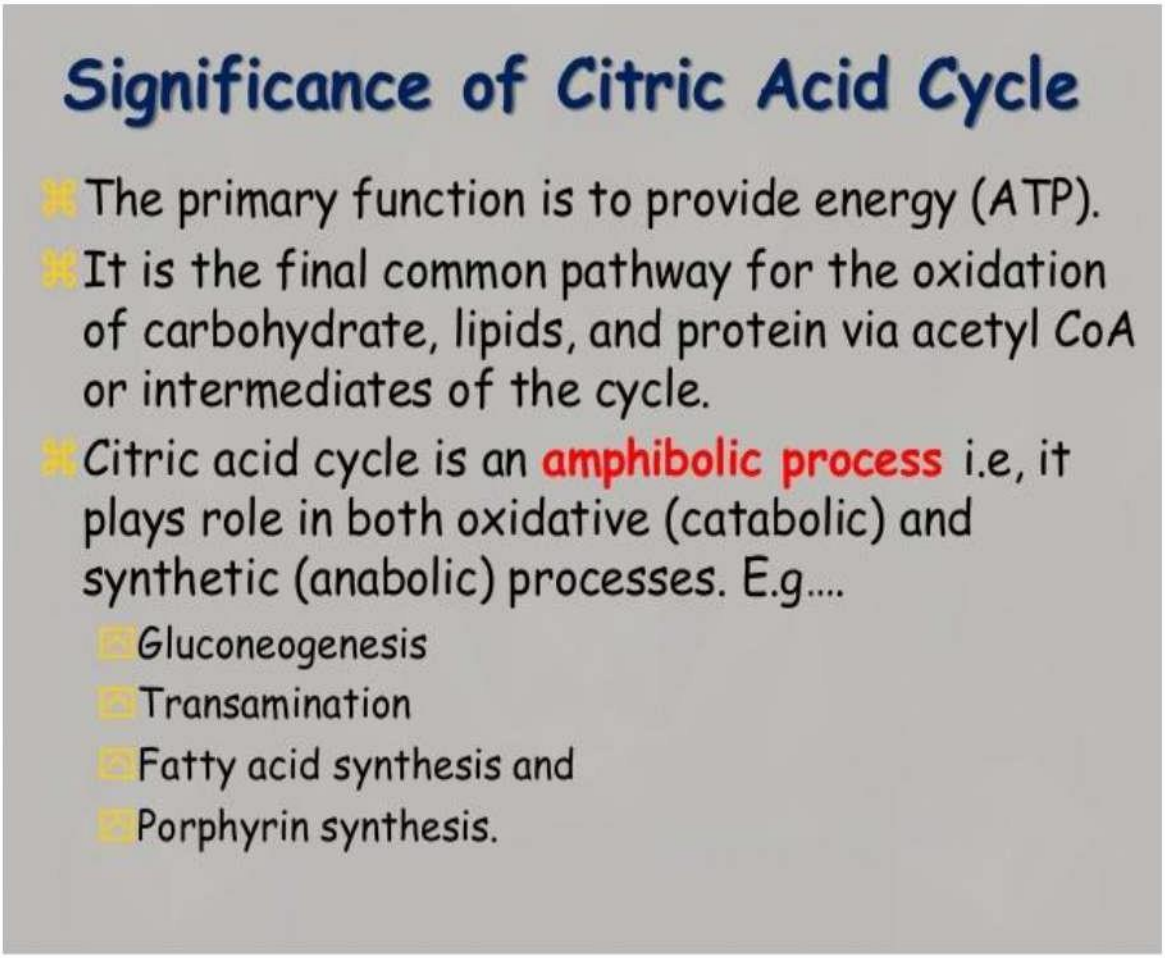
Reducing equivalents needed for oxidative phosphorylation are generated by the pyruvate dehydrogenase complex and the TCA cycle, and both processes are up regulated in response to a rise in ADP.

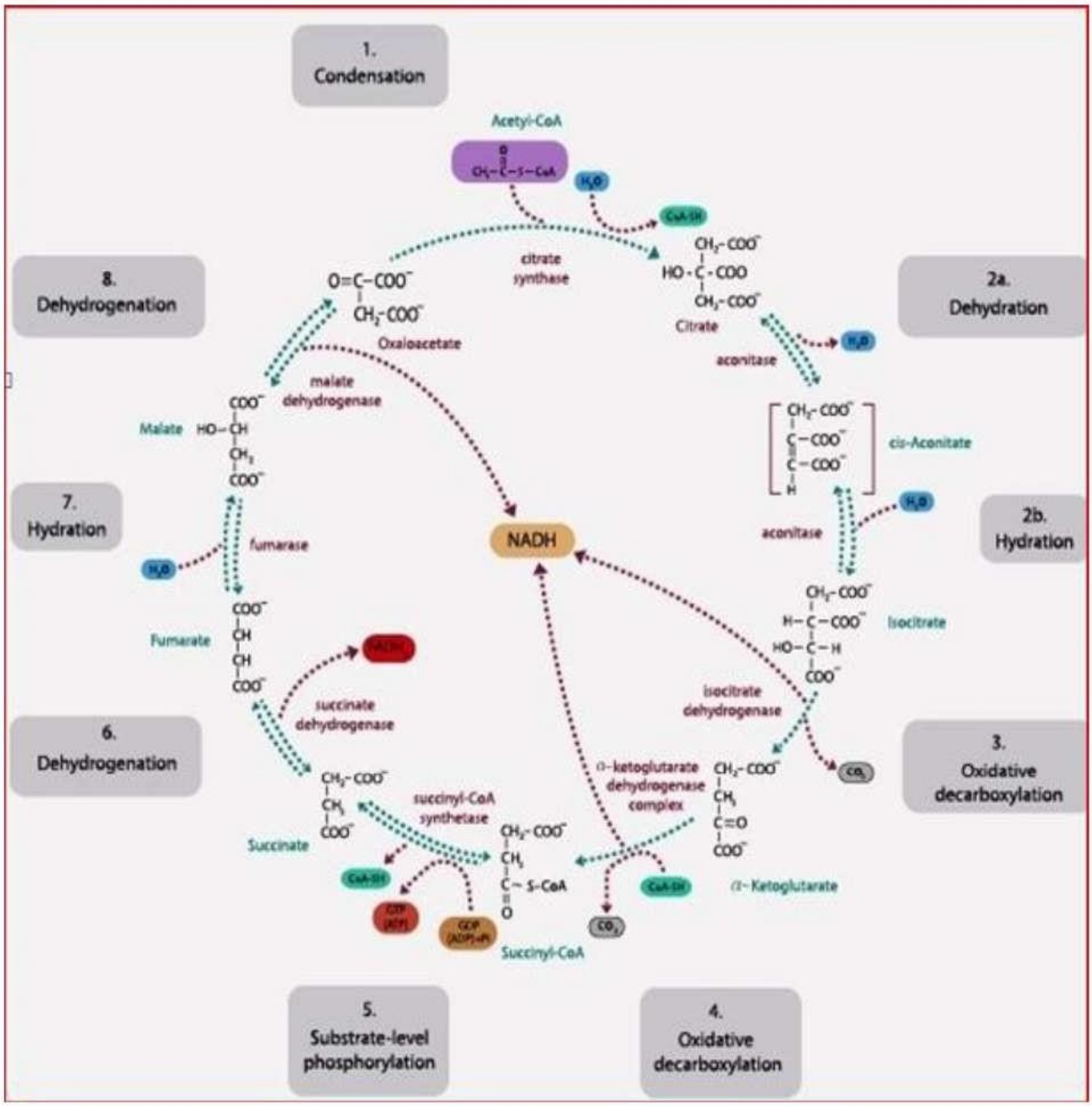
Regulatory factors of the TCA cycle:

1) $NADH/NAD^+$ ratio – respiratory control

2) ATP/ADP (AMP) ratio – energetic control

3) Availability of substrates for the TCA cycle – substrate control




1.
Condensation
Acetyl-CoA
citrate
synthase
2a.
Dehydration
Citrate
aconitase
cis-Aconitate
2b.
Hydration
aconitase
Isocitrate
isocitrate
dehydrogenase
3.
Oxidative
decarboxylation
α-ketoglutarate
dehydrogenase
complex
α-Ketoglutarate
4.
Oxidative
decarboxylation
5.
Substrate-level
phosphorylation
succinyl-CoA
synthetase
Succinyl-CoA
Succinate
6.
Dehydrogenation
succinate
dehydrogenase
Fumarate
7.
Hydration
fumarase
Malate
8.
Dehydrogenation
malate
dehydrogenase
Oxaloacetate
NADH