M.Sc. Botany
Semester-II (2018-20)
MBOTCC-7: Physiology & Biochemistry

Unit –V
CITRIC ACID CYCLE

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CITRIC ACID CYCLE

Cellular respiration occurs in three major stages. In the first,organic fuel molecules—glucose, fatty acids, and some amino acids—are oxidized to yield two-carbon fragments in the form of the acetyl group of acetyl-coenzyme A (acetyl-CoA). In the second stage, the acetyl groups are oxidized to CO_2 in the citric acid cycle, and much of the energy of these oxidations is conserved in the reduced electron carriers NADH and $FADH_2$. In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons (H^+) and electrons. The electrons are transferred to O_2 via a series of electron-carrying molecules known as the respiratory chain, resulting in the formation of water (H2O).

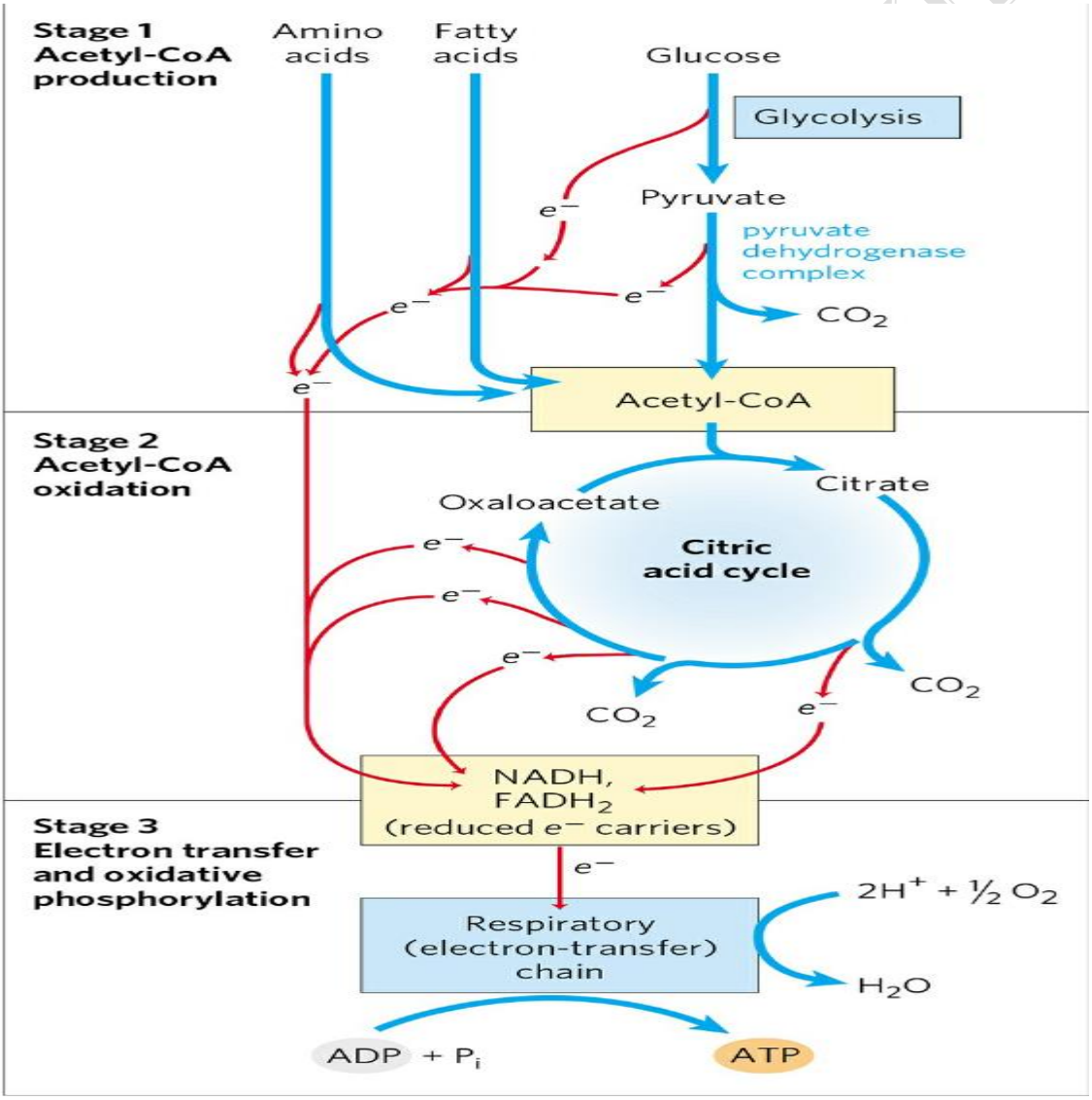


Fig: Catabolism of proteins, fats, and carbohydrates in the three stages of cellular respiration. Stage 1: oxidation of fatty acids, glucose, andsome amino acids yields acetyl-CoA.

Stage 2: oxidation of acetyl groups in the citric acid cycle includes four steps in which electrons are abstracted. Stage 3:electrons carried by NADH and $FADH_2$ are funneled into a chain of mitochondrial (or, in bacteria, plasma membrane-bound) electron carriers—the respiratory chain—ultimately reducing O_2 to H_2O. This electron flow drives the production of ATP.

Production of Acetyl-CoA (Activated Acetate)

- Pyruvate, the product of glycolysis, is transported into the mitochondrial matrix by the mitochondrial pyruvate carrier.
- Pyruvate is converted to acetyl-CoA, the starting material for the citric acid cycle, by the pyruvate dehydrogenase complex. The combined dehydrogenation and decarboxylation of pyruvate to the acetyl group of acetyl-CoA requires the sequential action of three different enzymes and five different coenzymes or prosthetic groups— thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), coenzyme A (CoA, sometimes denoted CoA-SH, to emphasize the role of the —SH group), nicotinamide adenine dinucleotide (NAD), and lipoate. Four different vitamins required in human nutrition are vital components of this system: thiamine (in TPP), riboflavin (in FAD), niacin (in NAD), and pantothenate (in CoA)
- The PDH complex is composed of multiple copies of three enzymes: pyruvate dehydrogenase, E1 (with its bound cofactor TPP); dihydrolipoyltransacetylase, E2 (with its covalently bound lipoyl group); and dihydrolipoyl dehydrogenase, E3 (with its cofactors FAD and NAD).
- E1 catalyzes first the decarboxylation of pyruvate, producing hydroxyethyl- TPP, and then the oxidation of the hydroxyethyl group to an acetyl group.The electrons from this oxidation reduce the disulfide . of lipoate bound to E2, and the acetyl group is transferred into thioester linkage with one –SH group of reduced lipoate.
- E2 catalyzes the transfer of the acetyl group to coenzyme A, forming acetyl-CoA.
- E3 catalyzes the regeneration of the disulfide (oxidized) form of lipoate; electrons pass first to FAD, then to NAD^+.
- The long lipoyllysyl arm swings from the active site of E1 to E2 to E3, tethering the intermediates to the enzyme complex to allow substrate channeling.
- The organization of the PDH complex is very similar to that of the enzyme complexes that catalyze the oxidation of α-ketoglutarate and the branched chainα-keto acids.

The overall reaction catalyzed by the pyruvate dehydrogenase complex is an **oxidative decarboxylation**, an irreversible oxidation process in which thecarboxyl group is removed from pyruvate as a molecule of CO2 and the two remaining carbons become the acetyl group of acetyl-CoA . The NADH formed in this reaction gives up a hydride ion ($:H^-$) to the respiratory chain , which carries the two electrons to oxygen or, in anaerobic microorganisms, to an alternative electron acceptor such as nitrate or sulfate. The transfer of electrons from NADH to oxygen ultimately generates 2.5 molecules of ATP per pair of electrons.

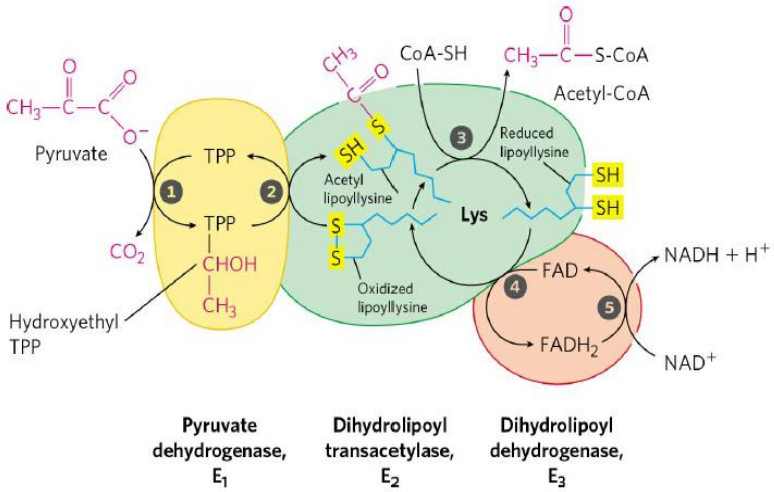
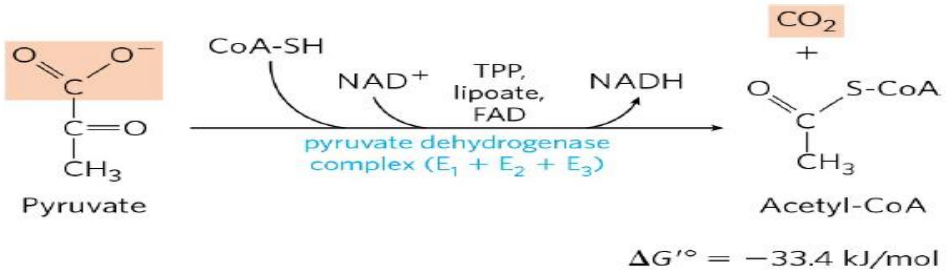


Fig: **Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex.** The fate of pyruvate is traced in red. In step 1 pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E1) and is decarboxylated to the hydroxyethyl derivative. Pyruvate dehydrogenase also carries out step 2 , the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group of the core enzyme, dihydrolipoyl transacetylase (E2), to form the acetyl thioester of the reduced lipoyl group. Step 3 is a transesterification in which the —SH group of CoA replaces the —SH group of E2 to yield acetyl-CoA and the fully reduced (dithiol) form of the lipoyl group. In step 4 dihydrolipoyl dehydrogenase (E3) promotes transfer of two hydrogen atoms from the reduced lipoyl groups of E2 to the FAD prosthetic group of E3, restoring the oxidized form of the lipoyllysyl group of E2. In step 5 the reduced FADH2 of E3 transfers a hydride ion to NAD+, forming NADH. The enzyme complex is now ready for another catalytic cycle.



Reactions of the Citric Acid Cycle

Eugene Kennedy and Albert Lehninger showed in 1948 that, in eukaryotes, the entire set of reactions of the citric acid cycle takes place in mitochondria. Isolated mitochondria were found to contain not only all the enzymes and coenzymes required for the citric acid cycle, but also all the enzymes and proteins necessary for the last stage of respiration—electron transfer and ATP synthesis by oxidative phosphorylation. In most bacteria, the enzymes of the citric acid cycle are in the cytosol, and the plasma membrane plays a role analogous to that of the inner mitochondrial membrane in ATP synthesis

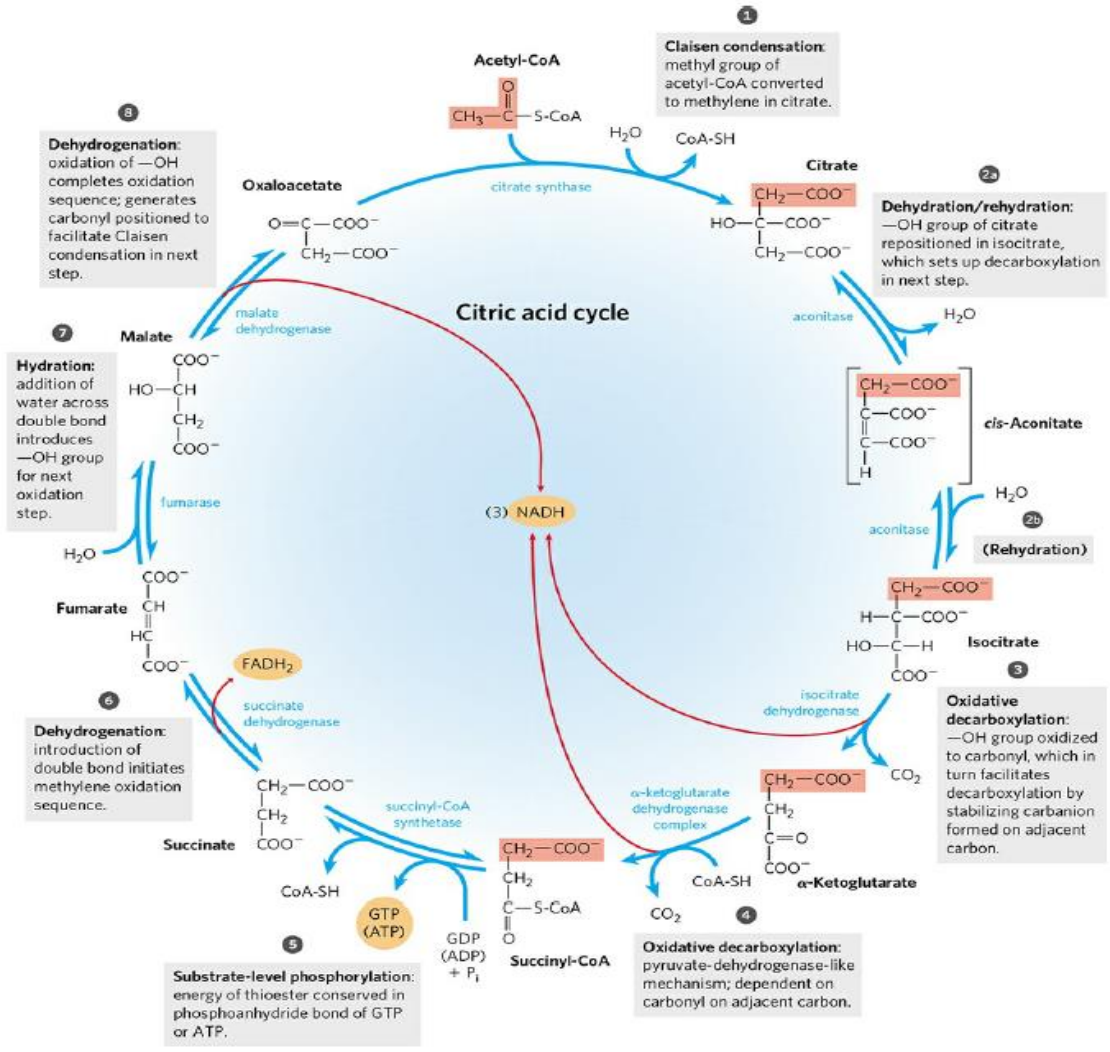
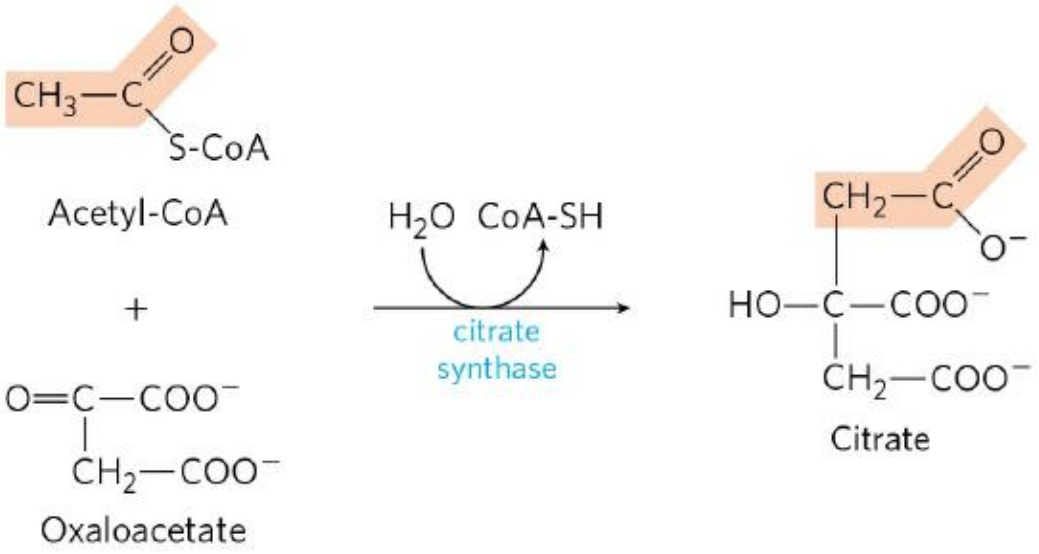


FIG: Reactions of the citric acid cycle. The carbon atoms shaded in pink are those derived from the acetate of acetyl-CoA in the first turn of the cycle; these are *not* the carbons released as CO_2 in the first turn. Note that in succinate and fumarate, the two-

carbon group derived from acetate can no longer be specifically denoted; because succinate and fumarate are symmetric molecules, C-1 and C-2 are indistinguishable from C-4 and C-3. The number beside each reaction step corresponds to a numbered heading on . The red arrows show where energy is conserved by electron transfer to FAD or NAD^+, forming $FADH_2$ or NADH + H^+. Steps 1,3, and 4 are essentially irreversible in the cell; all other steps are reversible. The nucleoside triphosphate product of step 5 may be either ATP or GTP, depending on which succinyl-CoA synthetase isozyme is the catalyst.

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

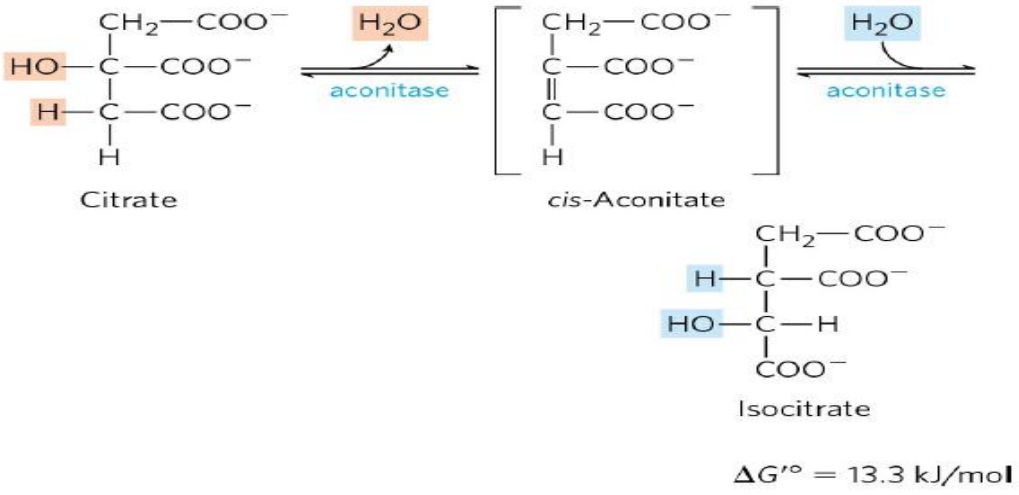


$\Delta G'^{\circ} = -32.2$ kJ/mol

In this reaction, the methyl carbon of the acetyl group is joined to the carbonyl group (C-2) of oxaloacetate. Citroyl-CoA is a transient intermediate formed on the active site of the enzyme . It rapidly undergoes hydrolysis to free CoA and citrate, which are released from the active site. The hydrolysis of this high-energy thioester intermediate makes the forward reaction highly exergonic.

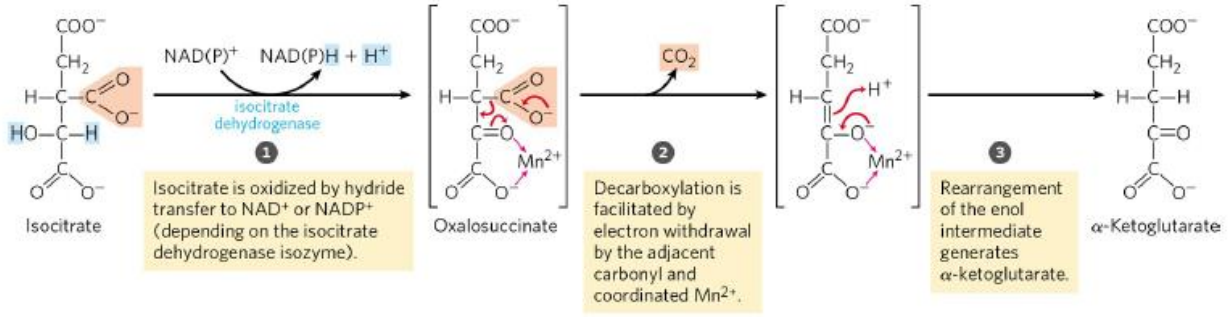
2. **Formation of Isocitrate via *cis*-Aconitate** The enzyme **aconitase** (more formally, **aconitate hydratase**) catalyzes the reversible transformation of citrate to **isocitrate**, through the intermediary formation of the tricarboxylic acid ***cis* aconitate**, which normally does not dissociate from the active site.

Aconitase can promote the reversible addition of H_2O to the double bond of enzyme-bound *cis*-aconitate in two different ways, one leading to citrate and the other to isocitrate:



Although the equilibrium mixture at pH 7.4 and 25 °C contains less than 10% isocitrate, in the cell the reaction is pulled to the right because isocitrate is immediately consumed in the next step of the cycle, lowering its steady-state concentration. Aconitase contains an **iron-sulfur center** , which acts both in the binding of the substrate at the active site and in the catalytic addition or removal of H_2O.

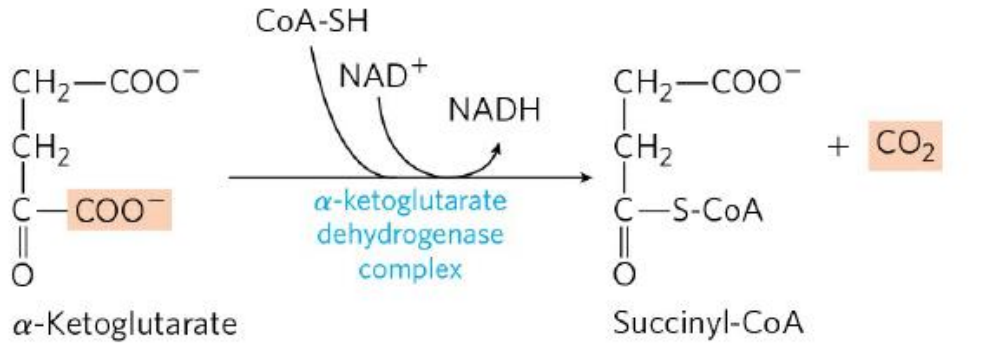
3. **Oxidation of Isocitrate to *α*-Ketoglutarate and CO_2** In the next step, **isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form ***α*-ketoglutarate**.



Mn^{2+} in the active site interacts with the carbonyl group of the intermediate oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to *α*-ketoglutarate. Mn^{2+} also stabilizes the enol formed transiently by decarboxylation. There are two different forms of isocitrate dehydrogenase in all cells, one requiring NAD^+ as electron acceptor and the other requiring $NADP^+$.

4. **Oxidation of α-Ketoglutarate to Succinyl-CoA and CO_2** The next step is another oxidative decarboxylation, in which α-ketoglutarate is converted to

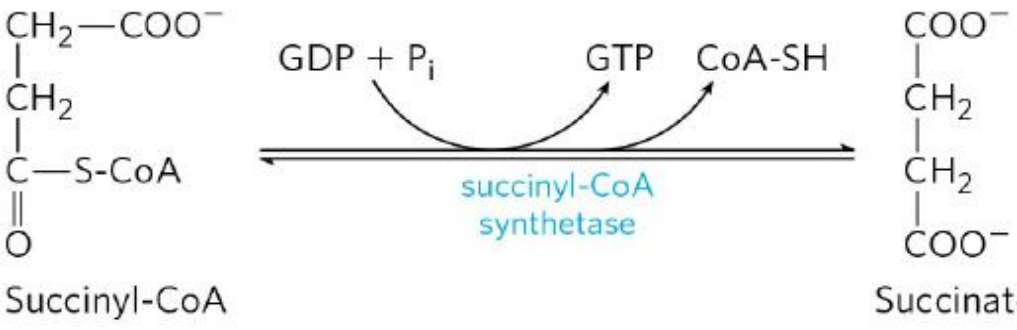
succinyl-CoA and CO_2 by the action of the ***α*-ketoglutarate dehydrogenase complex**; NAD^+ serves as electron acceptor and CoA as the carrier of the succinyl group. The energy of oxidation of *α*-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA:



$\Delta G'^\circ = -33.5$ kJ/mol

The *α*-ketoglutarate dehydrogenase complex closely resembles the PDH complex in both structure and function. It includes three enzymes, homologous to E1, E2, and E3 of the PDH complex, as well as enzyme-bound TPP, bound lipoate, FAD, NAD, and coenzyme A. Both complexes are certainly derived from a common evolutionary ancestor.

5. **Conversion of Succinyl-CoA to Succinate** Succinyl-CoA, like acetyl- CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis ($\Delta G'^\circ \approx -36$ kJ/mol). In the next step of the citric acid cycle, energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP, with a net $\Delta G'^\circ$ of only −2.9 kJ/mol. **Succinate** is formed in the process:



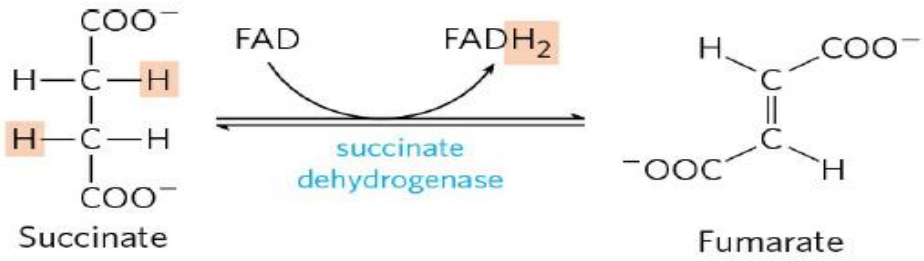
$\Delta G'^\circ = -2.9$ kJ/mol

The enzyme that catalyzes this reversible reaction is called **succinyl-CoA synthetase** or **succinic thiokinase**; both names indicate the participation of a nucleoside triphosphate in the reaction. The formation of ATP (or GTP) at the expense of the energy released by the oxidative decarboxylation of α-ketoglutarate is a substrate-level phosphorylation, The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, in a reversible reaction catalyzed by **nucleoside diphosphate kinase.**

$$\mathbf{GTP + ADP \rightleftharpoons GDP + ATP} \qquad \mathbf{\Delta G'^{\circ} = 0KJ/mol}$$

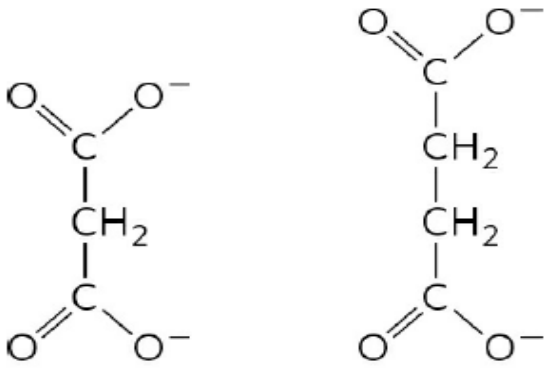
Thus the net result of the activity of either isozyme of succinyl-CoA synthetase is the conservation of energy as ATP. There is no change in free energy for the nucleoside diphosphate kinase reaction; ATP and GTP are energetically equivalent.

6. **Oxidation of Succinate to Fumarate** The succinate formed from succinyl-CoA is oxidized to **fumarate** by the flavoprotein **succinate dehydrogenase**:

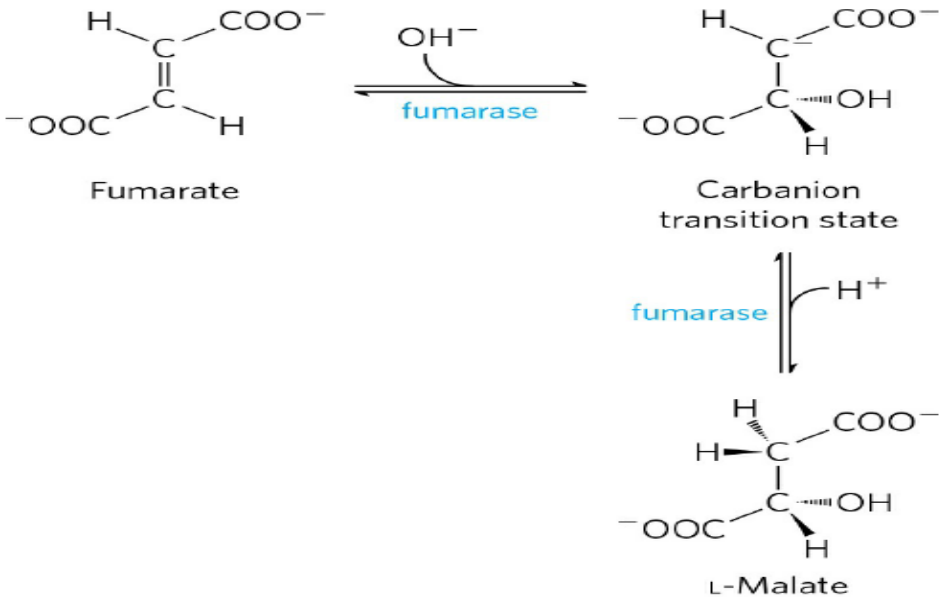


$\Delta G'^{\circ} = 0$ kJ/mol

In eukaryotes, succinate dehydrogenase is an integral protein of the mitochondrial inner membrane; in bacteria, of the plasma membrane. The enzyme contains three different iron-sulfur clusters and one molecule of covalently bound FAD. Malonate, an analog of succinate not normally present in cells, is a strong competitive inhibitor of succinate dehydrogenase, and its addition to mitochondria in the laboratory blocks the activity of the citric acid cycle.



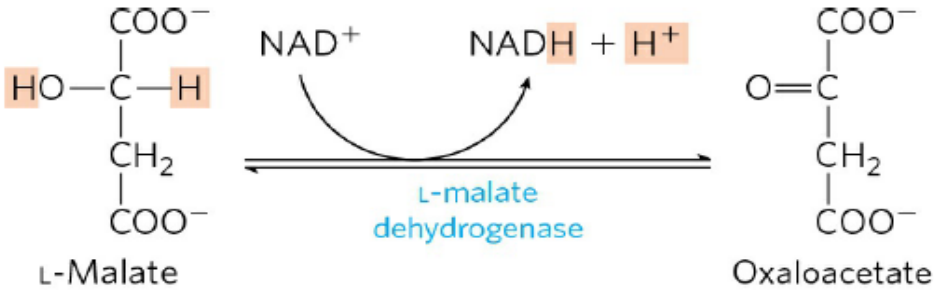
7. **Hydration of Fumarate to Malate** The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase** (formally, **fumarate hydratase**). The transition state in this reaction is a carbanion:



$\Delta G'^{\circ} = -3.8$ kJ/mol

This enzyme is highly stereospecific; it catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate. In the reverse direction (from L-malate to fumarate), fumarase is equallystereospecific: D-malate is not a substrate.

8. In the last reaction of the citric acid cycle, **L-malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate, coupled to the reduction of NAD^+ to NADH:



$\Delta G'^{\circ} = 29.7$ kJ/mol

The equilibrium of this reaction lies far to the left under standard thermodynamic conditions, but in intact cells oxaloacetate is continually removed by the highly exergonic citrate synthase reaction . This keeps the concentration of oxaloacetate in the cell extremely low ($<10^{-6}$ M), pulling the malate dehydrogenase reaction toward the formation of oxaloacetate.

TABLE 16-1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed[a]
Glucose → glucose 6-phosphate	–1 ATP	–1
Fructose 6-phosphate	–1 ATP	–1

→ fructose 1,6-bisphosphate		
2 Glyceraldehyde 3-phosphate → 2 1,3-bisphosphoglycerate	2 NADH	3 or 5[b]
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

[a]This is calculated as 2.5 ATP per NADH and 1.5 ATP per FADH2. A negative value indicates consumption.

bThis number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix;

For each acetyl-CoA oxidized by the citric acid cycle, the energy gain consists of three molecules of NADH, one FADH2, and one nucleoside triphosphate (either ATP or GTP). Besides acetyl-CoA, any compound that gives rise to a four- or five-carbon intermediate of the citric acid cycle—for example, the breakdown products of many amino acids—can

be oxidized by the cycle. The citric acid cycle is amphibolic, serving in both catabolism and anabolism; cycle intermediates can be drawn off and used as the starting material for a variety of biosynthetic products.

Vertebrates cannot synthesize glucose from acetate or from the fatty acids that give rise to acetyl-CoA. When intermediates are shunted from the citric acid cycle to other pathways, they are replenished by several anaplerotic reactions, which produce four-carbon intermediates by carboxylation of three-carbon compounds; these reactions are catalyzed by pyruvate carboxylase, PEP carboxykinase, PEP carboxylase, and malic enzyme.