KREBS CYCLE

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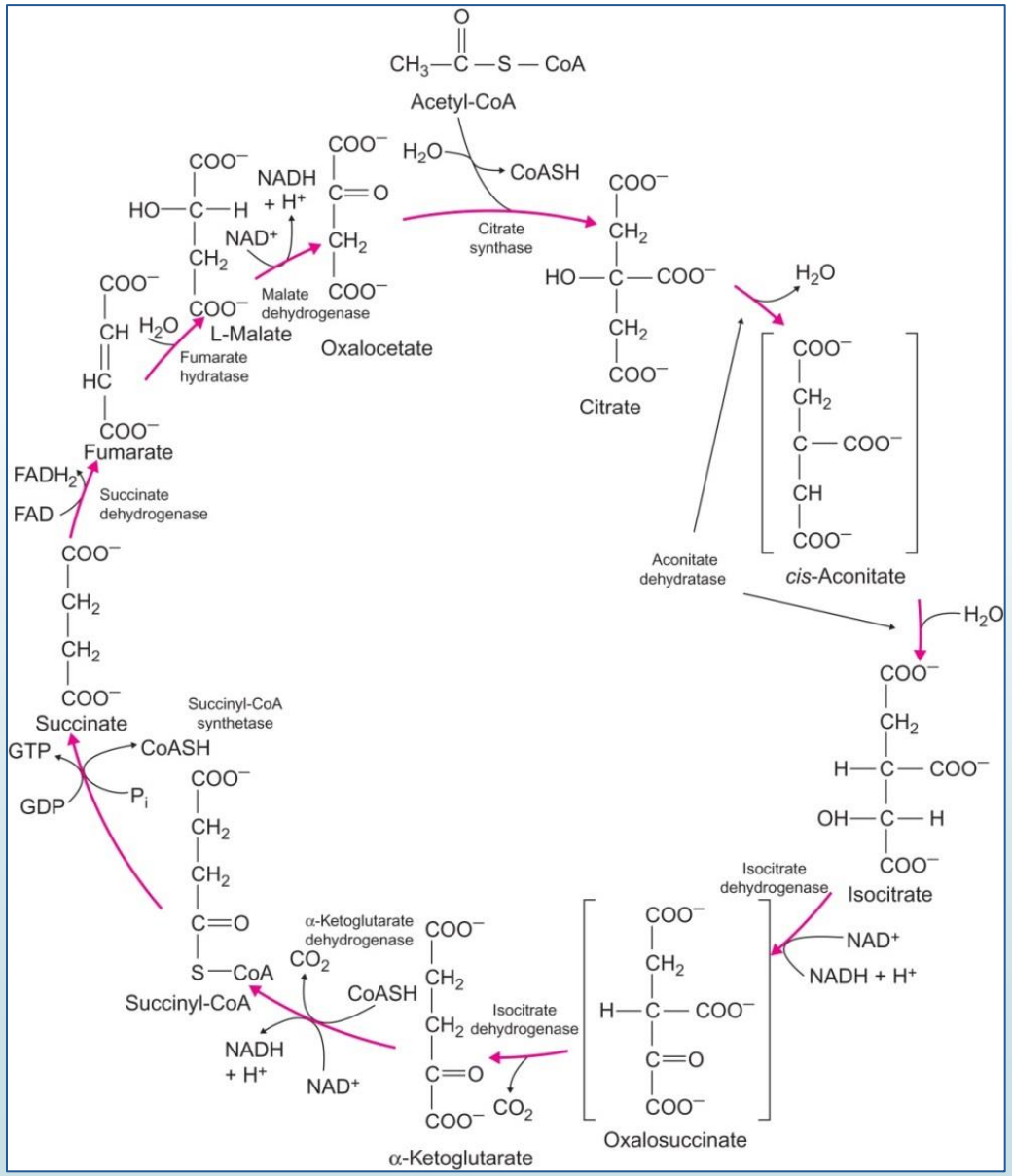
BIOMEDICAL IMPORTANCE

- The tricarboxylic acid cycle (the TCA cycle, also called the Krebs cycle or the citric acid cycle) plays several roles in metabolism.
- The TCA cycle is the final common pathway for the oxidation of carbohydrate, lipid, and protein because glucose, fatty acids, and most amino acids are metabolized to acetyl-CoA or intermediates of the cycle.
- This oxidation provides energy for the production of the majority of ATP in most animals, including humans.
- It also has a central role in gluconeogenesis, lipogenesis, and interconversion of amino acids.
- The cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport, which oxidize the reduced coenzymes produced by the cycle.

REACTIONS OF THE TCA CYCLE

- In the TCA cycle, oxaloacetate is first condensed with an acetyl group from acetyl coenzyme A (CoA), and then is regenerated as the cycle is completed.
- Thus, the entry of one acetyl CoA into one round of the TCA cycle does not lead to the net production or consumption of intermediates.
- Two carbons entering the cycle as acetyl CoA are balanced by two CO_2 exiting.

THE TCA CYCLE



Oxidative decarboxylation of pyruvate

- Pyruvate, the end product of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle.
- This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane.
- Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex, which is a multienzyme complex.
- The pyruvate dehydrogenase complex is not part of the TCA cycle, but is a major source of acetyl CoA which is substrate for the cycle.

Oxidative decarboxylation of pyruvate

- The pyruvate dehydrogenase complex (PDH complex) is a multimolecular aggregate of three enzymes, pyruvate dehydrogenase (PDH or E_1, also called a decarboxylase), dihydrolipoyl transacetylase (E_2), and dihydrolipoyl dehydrogenase(E_3).
- In addition to the enzymes participating in the conversion of pyruvate to acetyl CoA, the complex also contains two tightly bound regulatory enzymes, pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase.
- The PDH complex contains five coenzymes that act as carriers or oxidants for the intermediates of the reactions. E_1 requires thiamine pyrophosphate (TPP), E_2 requires lipoic acid and CoA, and E_3 requires FAD and NAD^+.

Regulation of the PDH complex

- Covalent modification by the two regulatory enzymes that are part of the complex alternately activate and inactivate E_1 (PDH).
- The cyclic AMP-independent PDH kinase phosphorylates and, thereby, inhibits E_1, whereas PDH phosphatase dephosphorylates and activates E_1.
- Pyruvate is a potent inhibitor of PDH kinase.
- Although covalent regulation by the kinase and phosphatase is main, the complex is also subject to product (NADH, acetyl CoA) inhibition.

Synthesis of citrate from acetyl CoA and oxaloacetate

- The condensation of acetyl CoA and oxaloacetate to form citrate (a tricarboxylic acid) is catalyzed by citrate synthase.
- It is inhibited by its product, citrate.
- Substrate availability is another means of regulation for citrate synthase.

Isomerization of citrate

- Citrate is isomerized to isocitrate by aconitase, an Fe-S protein.
- Aconitase is inhibited by fluoroacetate, a compound that is used as a rat poison.

Oxidation and 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.

Oxidative decarboxylation of α-ketoglutarate

- The conversion of a-ketoglutarate to succinyl CoA is catalyzed by the a-ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes.
- The reaction releases the second CO_2 and produces the second NADH of the cycle.
- The coenzymes for the enzyme complex are thiamine pyrophosphate, lipoic acid, FAD, NAD^+, and CoA.

Cleavage of succinyl CoA

- Succinate thiokinase (also called succinyl CoA synthetase) cleaves the high-energy thioester bond of succinyl CoA.
- This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP).
- The generation of GTP by succinate thiokinase is another example of substrate-level phosphorylation.

Oxidation of succinate

- Succinate is oxidized to fumarate by succinate dehydrogenase, as FAD is reduced to $FADH_2$.
- The reaction is inhibited by malonate.
- Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane.
- It functions as Complex II of the electron transport chain.

Hydration of fumarate

- Fumarate is hydrated to malate in a freely reversible reaction catalyzed by fumarase (also called fumarate hydratase).
- Fumarate is also produced by the urea cycle, in purine synthesis, and during catabolism of the amino acids, phenylalanine and tyrosine.

Oxidation of malate

- Malate is oxidized to oxaloacetate by malate dehydrogenase.
- This reaction produces the third and last NADH of the cycle.
- The ΔG^0 of the reaction is positive, but the reaction is driven in the direction of oxaloacetate by the highly exergonic citrate synthase reaction.
- Oxaloacetate is also produced by the transamination of aspartic acid.

ENERGY PRODUCED BY THE TCA CYCLE

- **Ten ATP are formed per turn of the citric acid cycle.**
- As a result of oxidations catalyzed by the dehydrogenases of the citric acid cycle, three molecules of NADH and one of $FADH_2$ are produced for each molecule of acetyl-CoA catabolized in one turn of the cycle.
- These reducing equivalents are transferred to the respiratory chain, where reoxidation of each NADH results in formation of ~2.5 ATP, and of each $FADH_2$ results in formation of ~1.5 ATP.
- In addition, 1 ATP (or GTP) is formed by substrate-level phosphorylation catalyzed by succinate thiokinase.

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 with highly negative ΔG^0: **citrate synthase**, **isocitrate dehydrogenase**, and **a-ketoglutarate dehydrogenase complex**.
- Reducing equivalents needed for oxidative phosphorylation are generated by the pyruvate dehydrogenase complex and the TCA cycle, and both processes are upregulated in response to a surge in ADP.

THE CITRIC ACID CYCLE PLAYS A CRUCIAL ROLE IN METABOLISM

- The citric acid cycle is not only a pathway for oxidation of two carbon units, but it is also a major pathway for
  - interconversion of metabolites arising from **transamination** and **deamination** of amino acids,
  - providing the substrates for **amino acid synthesis** by transamination,
  - providing the substrates for **gluconeogenesis** and **fatty acid synthesis**.
- Because it functions in both oxidative and synthetic processes, it is **amphibolic.**

REFERENCES

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