

Citric Acid Cycle: Central Role in Catabolism

- Stage II of catabolism involves the conversion of carbohydrates, fats and aminoacids into acetylCoA
- In aerobic organisms, citric acid cycle makes up the final stage of catabolism when acetyl CoA is completely oxidized to CO_2 .
- Also called Krebs cycle or tricarboxylic acid (TCA) cycle.
- It is a central integrative pathway that harvests chemical energy from biological fuel in the form of electrons in NADH and FADH_2 (oxidation is loss of electrons).
- NADH and FADH_2 transfer electrons via the electron transport chain to final electron acceptor, O_2 , to form H_2O .

Entry of Pyruvate into the TCA cycle

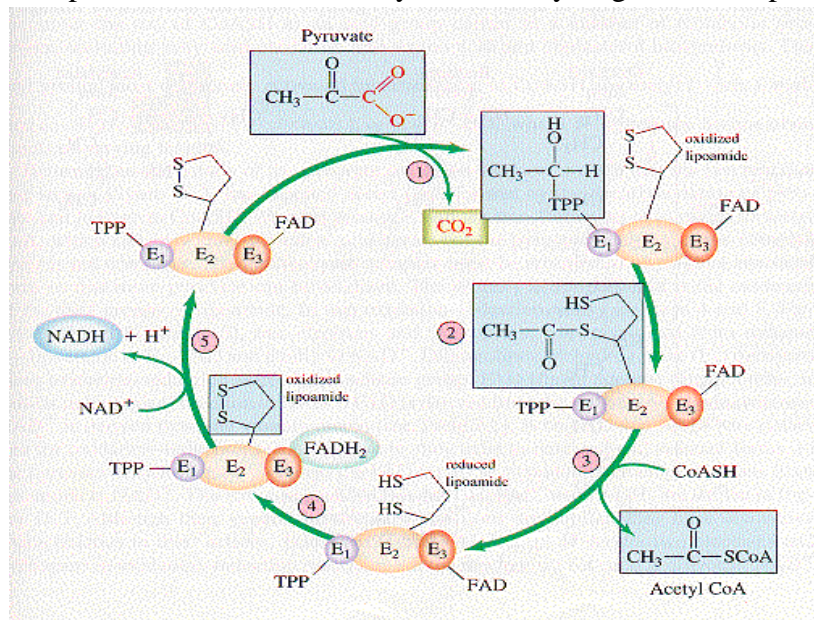
- Pyruvate is formed in the cytosol as a product of glycolysis
- For entry into the TCA cycle, it has to be converted to Acetyl CoA.
- Oxidation of pyruvate to acetyl CoA is catalyzed by the pyruvate dehydrogenase complex in the mitochondria
- Mitochondria consist of inner and outer membranes and the matrix
- Enzymes of the PDH complex and the TCA cycle (except succinate dehydrogenase) are in the matrix
- **Pyruvate translocase** is an antiporter present in the inner mitochondrial membrane that allows entry of a molecule of pyruvate in exchange for a hydroxide ion.

The Pyruvate Dehydrogenase (PDH) complex

- The PDH complex consists of 3 enzymes. They are: **pyruvate dehydrogenase (E1)**, **dihydrolipoyl transacetylase (E2)** and **dihydrolipoyl dehydrogenase (E3)**.
- It has 5 cofactors: CoASH, NAD⁺, lipoamide, TPP and FAD. CoASH and NAD⁺ participate stoichiometrically in the reaction, the other 3 cofactors have catalytic functions. TPP is the cofactor for E1, lipoamide and CoASH are the cofactors for E2 and FAD and NAD⁺ are the cofactors of E3.
- The PDH reaction is irreversible. The overall reaction is:

$$\text{CH}_3\text{-CO-COO}^- + \text{CoASH} + \text{NAD}^+ \rightarrow \text{CH}_3\text{-CO-SCoA} + \text{CO}_2 + \text{NADH}$$
- This is called as activation of pyruvate. In the TCA cycle, intermediates are activated by formation of high energy thioester bonds.
- One carbon of pyruvate is oxidized to CO₂ and one NADH is formed by the PDH reaction.

Step-wise Reactions of the Pyruvate Dehydrogenase Complex



From: Concepts in Biochemistry by Rodney Boyer

Step-wise reactions of PDH

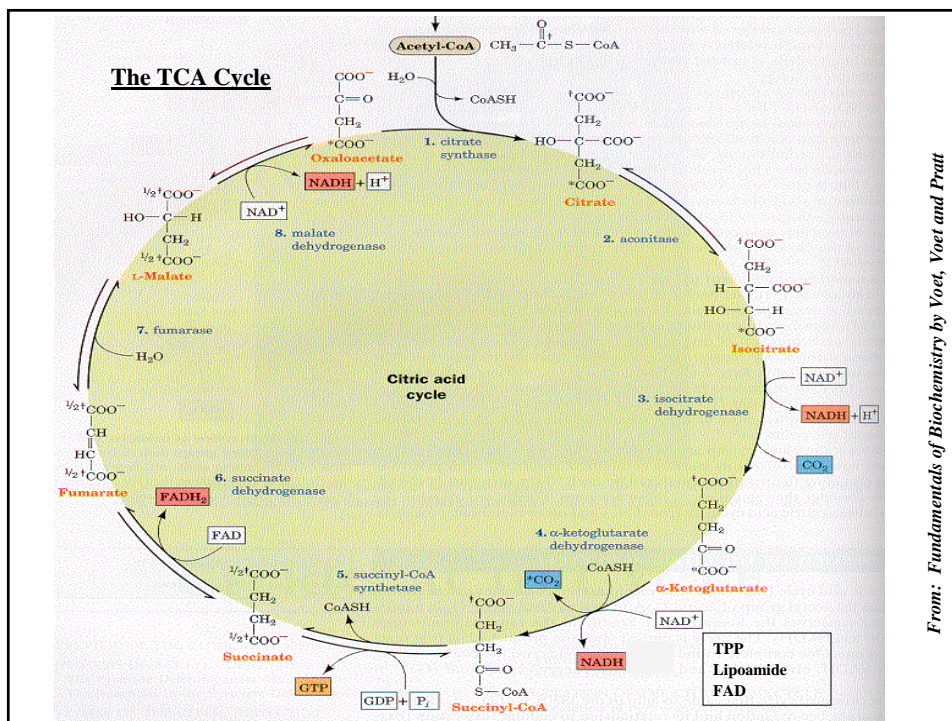
- Step 1. decarboxylation of pyruvate, catalyzed by E1, pyruvate dehydrogenase, cofactor TPP. (TPP, the coenzyme form of vitamin B1 facilitates decarboxylation reactions). Pyruvate gets complexed with TPP and loses a CO_2 to become an alcohol.
- Step 2. The substrate gets transferred from TPP to lipoamide forming a thioester linkage. In the process, the $-\text{C}-\text{OH}$ is converted to $-\text{C}=\text{O}$. This is catalyzed by E1.
- Step 3. Catalyzed by E2. CoASH, cofactor of E2, participates in a group transfer reaction and removes the 2-carbon unit as acetylSCoA, leaving reduced lipoamide attached to E2.
- Step 4. Catalyzed by E3. FAD, cofactor of E3 gets reduced to FADH_2 and lipoamide reverts back to its oxidized form.
- Step 5. Catalyzed by E3. Hydrogens from FADH_2 are transferred to NAD^+ to generate $\text{NADH} + \text{H}^+$. This resets the PDH complex for the next cycle

Regulation of PDH

- The PDH step is irreversible; as a result, animals are not able to synthesize glucose from acetyl CoA (fat)
- PDH is regulated allosterically and by covalent phosphorylation.
- PDH is inhibited by acetyl CoA (E2, dihydrolipoyl transacetylase) and NADH (E3, dihydrolipoyl dehydrogenase)
- Covalent phosphorylation of PDH turns off its activity, dephosphorylation results in activation. High ATP, NADH and acetylCoA stimulate PDH kinase
- Insulin, NAD^+ and ADP stimulate PDH phosphatase

The TCA cycle (Overview)

- It is a cyclic pathway. All intermediates are derivatives of citric acid, a tricarboxylic acid
- Total of 8 steps. All except 3 are reversible
- Acetyl CoA, a 2 carbon molecule enters the cycle by condensation with the 4- carbon oxaloacetate. Each of the two carbons of acetylCoA are oxidized and removed as CO₂ in two separate reactions. Oxaloacetate is regenerated
- 3 molecules of NAD⁺ are reduced to 3 molecules of NADH + H⁺
- one molecule of FAD is reduced to FADH₂
- one molecule of GDP is phosphorylated to GTP. This is called substrate level phosphorylation (as opposed to oxidative phosphorylation).



Step-wise reactions of the TCA cycle

1. Aldol condensation of oxaloacetate with acetylCoA followed by hydrolysis of CoASH to give citrate.
Enzyme: citrate synthase. This is the first irreversible step
2. Dehydration followed by hydration leads to interchange of -H and -OH and isomerization of citrate to isocitrate.
Enzyme called aconitase because of formation of intermediate called cis-aconitate.
3. Oxidative decarboxylation of isocitrate is catalyzed by isocitrate dehydrogenase to form α -keto-glutarate. This is the second irreversible step. NAD^+ is reduced to NADH and one C is removed as CO_2 .

TCA cycle reactions (continued)

4. This is the second oxidative decarboxylation and 3rd irreversible step catalyzed by α -ketoglutarate dehydrogenase. The enzyme is a complex similar to PDH and the coenzymes TPP, lipoamide and FAD are required. CO_2 is removed, NADH is formed from NAD^+ and a thioester bond is formed with CoASH to form succinylCoA.
5. Hydrolysis of the thioester of Succinyl CoA releases ~31 kJ/mol which is captured for the synthesis of GTP. The enzyme is succinyl CoA synthetase.
6. Succinate is oxidized to fumarate by succinate dehydrogenase. A double bond is introduced between the 2 central carbons and FAD is reduced to FADH_2 . Succinate dehydrogenase is an electron carrier of the ETC and is the only enzyme of the TCA cycle present on the inner mitochondrial membrane rather than in the matrix.

TCA cycle reactions (continued)

7. Fumarate is hydrated to malate by the enzyme fumarase
 8. In the final step, malate is oxidized to oxaloacetate with the coupled reduction of NAD^+ to NADH by the enzyme malate dehydrogenase
- Overall reaction: $\text{AcetylCoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{Pi} + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + \text{CoASH} + 3\text{NADH} + 2\text{H}^+ + \text{FADH}_2 + \text{GTP}$
 - Recent evidence suggests that enzymes of the TCA cycle may be physically associated with each other to facilitate handing of substrates from one enzyme to the next.

Regulation of the TCA cycle

- The TCA cycle is regulated allosterically at the 3 irreversible steps: citrate synthase, isocitrate dehydrogenase and α -ketoglutarate dehydrogenase.

<u>Enzyme</u>	<u>+ Effectors</u>	<u>- Effectors</u>
citrate synthase:	ADP	ATP, NADH, Citrate, Succinyl CoA
isocitrate DH:	ADP	ATP, NADH
α -ketoglutarate DH:		ATP, NADH, Succinyl CoA

Anabolic role of TCA cycle

- Intermediates of the TCA cycle serve as precursors for biosynthesis of biomolecules
- Many aminoacids are synthesized starting with transamination of α -ketoglutarate
- Porphyrins and heme are synthesized from succinyl CoA
- Oxaloacetate is another α -keto acid and its transamination leads to aspartate and other amino acid biosynthesis.
- Oxaloacetate is also the precursor of purines and pyrimidines via aspartate
- Fatty acids and sterols are synthesized from citrate

Anaplerotic reactions

- Since the TCA cycle intermediates are used for anabolism, their concentration varies according to the needs of the cell.
- Reactions that replenish the TCA cycle intermediates are called as anaplerotic reactions.
- Oxaloacetate can be considered as a primary substrate of the TCA cycle. It is replenished from pyruvate by the gluconeogenic enzyme pyruvate carboxylase:
$$\text{Pyruvate} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O} \rightarrow \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$
- Pyruvate carboxylase is activated in the presence of acetyl CoA
- Pyruvate can also replenish malate