

Chapter 16 - Citric Acid Cycle

TCA (tricarboxylic acid cycle) Citric acid cycle and Krebs cycle. Named after Sir Hans Krebs, Nobel Laureate. He worked as an assistant professor for Otto Warburg (Nobel Prize 1931) and his position terminated 1933 and at Sir Fredrick Gowland Hopkin's (Nobel prize 1929) request he left Germany to hold a Rockefeller Studentship at the School of Biochemistry, Cambridge. In 1953 he earned the Nobel Laureate in Medicine for his discovery of the citric acid cycle

Discovery of the cycle

- Discovered by observing the reduction of compounds in muscle tissue. Certain key molecules (succinate, oxaloacetate) served as catalysts in O₂ consumption and oxidative metabolism of glucose and pyruvate.
- Szent-Gyorgyi determined the catalytic affect of small amounts of future TCA intermediates
- Knoop (also key in fatty acid metabolism) the formation of citrate from OAA and Pyruvate
- Krebs found a cycle of reforming catalytic amount of oxaloacetate

The Krebs cycle is a central pathway for recovering energy from three major metabolites: carbohydrates, fatty acids, and amino acids.

Most enter the cycle through Acetyl~CoA. The two carbons entered at this step are lost as CO₂ (the reason you breath out CO₂). The carbon atoms that enter by A-CoA leave after the second turn of the cycle.

Synthesis of the TCA

- Reactions take place in mitochondria - thus transport of reactants and products are important
- Overall reaction involves the entry of a 2 carbon compound (acetyl CoA) into the cycle with the loss of 2 CO₂ and formation of 3 NADH, FADH₂ and GTP or ATP.



- No net change in the concentration of the 4 carbon compound oxaloacetate.
- The carbons lost as CO₂ are from previous A-CoAs not from the reactant A-Co

Think of why this is a cycle vs. pathway - not because it is written that way.

- Oxaloacetate - only a small amount is needed - catalytic role
- Anapleurotic - "filling up" cycle can be used as entry and exit for production of other essential metabolites

The TCA

- Takes place in mitochondria in the matrix
- Like glycolysis pathway, the TCA is highly regulated
- Sources of Acetyl CoA (another cross road metabolite)
 - glycolysis - via PDH
 - β oxidation of fatty acids
 - selected amino acids

Getting there - don't forget PC!

Pyruvate Dehydrogenase (**PDH**) - Entry of glucose metabolites into cycle is through formation of acetyl-CoA by oxidative decarboxylation of pyruvate

- In eukaryotes, all of the TCA enzymes and the PDH are found in the mitochondria. Either in the inner compartment or the matrix of the mitochondrion.
- Pyruvate is made in the cytosol and transported by a H⁺ / pyruvate symporter.

PDH Exists as large multiunit complex

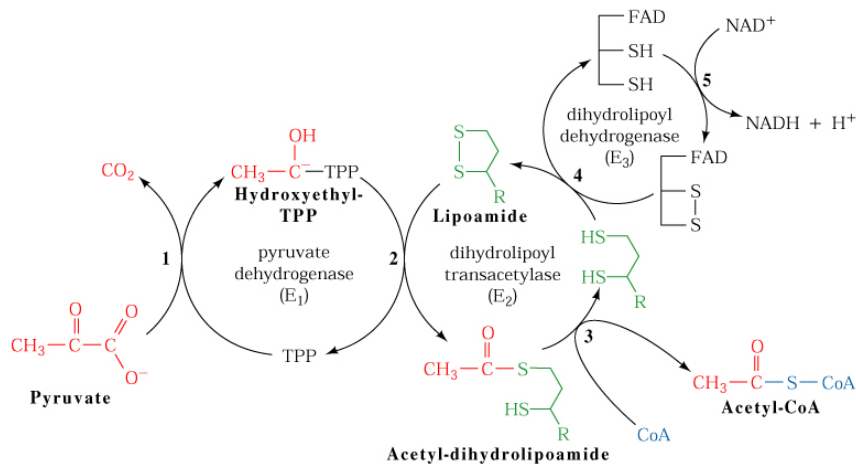
- Coenzymes - Vitamin B1- thiamine pyrophosphate (TPP), pantoic acid (CoA), riboflavin (FAD), Niacin (NAD⁺) and lipoamide
- (3 different subunits)

E₁ Pyruvate Dehydrogenase - 24 copies / E₂ Dihydrolipoyl Transacetylase - 24 copies / E₃ Dihydrolipoyl Dehydrogenase - 12 copies

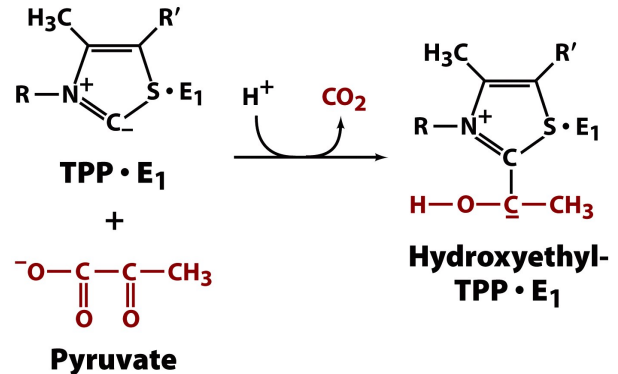
- increases local concentration of substrate for each subunit

- multi-enz complexes allows little chance for diffusion and side reactions and direct transfer of substrate from E₁ to E₂ to E₃

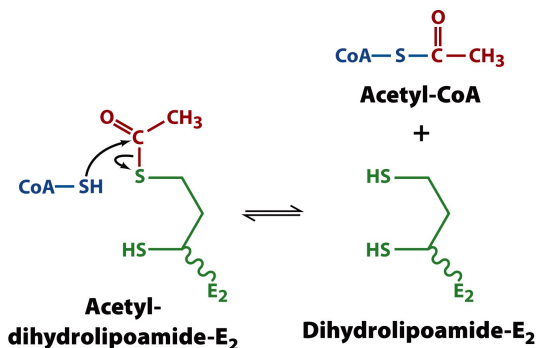
The three enzymes of the PDH



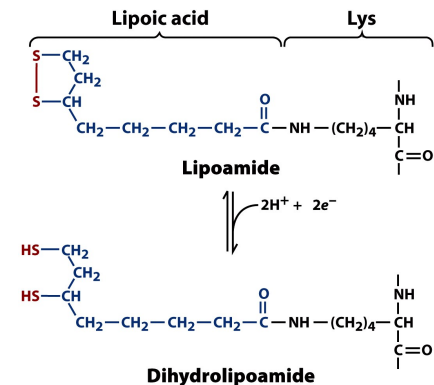
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5 catalytic steps, each with different coenzymes.

1 E₁-Decarboxylation of pyruvate - (condensation with TPP)

- TPP adds to carbonyl carbon
- carbanion intermediate- necessary to attack negative charged C=O carbon resonance-stabilized by ring of thiamine pyrophosphate

2 E₂ transfer from TPP to lipoamide (amide linkage)

- 2 lipoamides involved
- act as transfer/carrier arm for acetyl group *** active site studies show distortion for disulfide bond to form...
- disulfide (oxidized form) is converted to the mercapto (reduced) form

3 E₂ transfer of acetyl group to CoA

4 E₃ Dihydrolipoyl dehydrogenase

- oxidizes the amide group of E₂ by the reduction of the Cys-Cys disulfide bond of E₃

5 E₃ is reoxidized by NAD⁺ in a transient reduction involving FAD (bound to the enzyme). This prepares the enzyme for another round, and produces reduced NADH

Reaction of the cycle

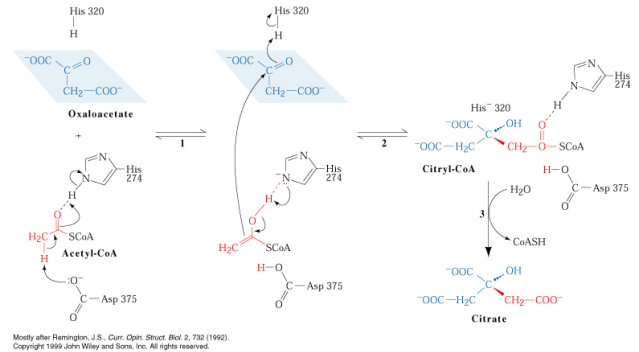
Citrate Synthase (CS) - catalyzes the condensation of acetyl-CoA and OAA in a highly exergonic fashion.

There is a substantial conformational change in the enzyme when substrate binds. “hides” water from the active site and then forms A-CoA binding site. - ordered sequential reaction

Citrate Synthase (CS)

- aldol condensation of Acetyl CoA and oxaloacetate
- involves two His and one Asp
- ordered reaction leading to tertiary changes
- induced fit caused by OAA binding forms reactive site

- order of binding helps stop Acetyl CoA hydrolysis
- two neutral His involved in catalysis
- His donates H to C=O oxygen of Acetyl CoA and OAA
- Asp is the proton acceptor
- loss of acetyl CoA CH₃ hydrogen to Asp
- condensation forms CitrylCoA



Aconitase - catalyzes the isomerization of citrate to isocitrate via stereospecific dehydration and rehydration. (a two step reaction).

- citrate -> isocitrate
- isomerization reaction
- 2 steps removal and addition of water
- Iron required - not heme
- iron-sulfur protein - bound by cys residues

Isocitrate Dehydrogenase (IDH) - catalyzes the oxidative decarboxylation of isocitrate to produce an α-ketoglutarate and the first loss of CO₂ – remember that to get from citrate to OAA, you need to lose two carbons. This step also produces NADH.

- isocitrate -> [oxalosuccinate] -> α-ketoglutarate
- first oxidative conversion
- 2 steps - all on same enzyme
 - 1 - oxidation of alcohol to ketone NAD⁺ -> NADH (reduced) worth 3 ATPs
 - 2 - β decarboxylation

Isocitrate Dehydrogenase (IDH) - catalyzes the oxidative decarboxylation of isocitrate to produce an α -ketoglutarate and the first loss of CO_2 – remember that to get from citrate to OAA, you need to lose two carbons. This step also produces NADH.

- isocitrate \rightarrow [oxalosuccinate] \rightarrow α -ketoglutarate
- first oxidative conversion
- 2 steps - all on same enzyme
 - 1 - oxidation of alcohol to ketone
NAD⁺ \rightarrow NADH (reduced) worth 3 ATPs
 - 2 - β decarboxylation

Alpha Ketoglutarate Dehydrogenase (α KGDH) - another enzyme multienzyme complex similar to the PDH complex. α KGDH catalyzes the second oxidative decarboxylation. Note that the two carbons released as CO_2 in this round of the cycle are not the carbons that entered the cycle as acetyl-CoA

- generates CO_2 , NADH and succinyl CoA
- analogous to PDH
- includes the same E_3 complex

Succinate Thiokinase (STK) - AKA Succinyl-CoA synthetase – The GTP is easily converted to ATP by NDPK. In some tissues and specific species this is an ATP specific enzyme

- succinyl CoA + GDP \rightarrow succinate + GTP + CoASH
- substrate level phosphorylation
- Hydrolysis of thioester bond of succinyl CoA $-\Delta G^\circ$
- GTP used by G proteins or converted to ATP by NDPK
- two forms exist - in birds ATP is produced

Succinate Dehydrogenase (SDH)

- Succinate + FAD \rightarrow fumarate + FADH₂
- only non-matrix enzyme
- found bound to inner mitochondrial membrane
- facilitates transfer of FADH₂ electrons to electron transport system
- iron sulfur center
- FAD generally acts to oxidize C-C to C=C
- While NAD⁺ \rightarrow C-OH to C=O (aldehydes or ketones)
- FAD is covalently bound to protein - consequence?

Fumarase

- fumarate \rightarrow malate
- specific addition of H₂O

Malate Dehydrogenase (MDH)

- malate + NAD⁺ \rightarrow malate + NADH
- oxidation to reform oxaloacetate

Energetics

- NADH \rightarrow 3 ATP, FADH \rightarrow 2 ATP, GTP or ATP
 - one turn produces 12 ATP
 - 1 molecule of glucose \rightarrow 2 CO₂ + ATP (w/O₂)
 - glycolysis - 8 ATP
 - PDH - 6 ATP \rightarrow total of 38 ATP
 - TCA - 24
 - energy liberated = -688 kcal/mol
 - energy conserved = +266 kcal/mol
 - 39% efficiency based on ΔG NOT - ΔG°
 - lost energy used in thermogenesis

Regulation of PDH and the cycle

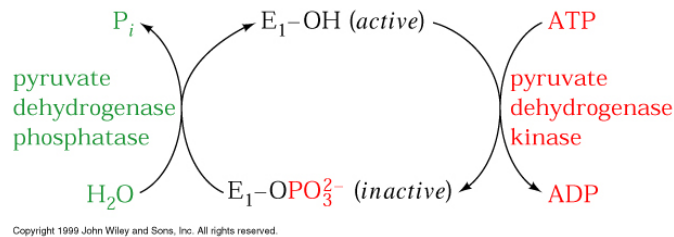
Regulated to meet needs of cell - don't waste energy

1. Feed back inhibition at PDH

- ACoA and NADH are allosteric inhibitors
 - Act by inhibiting E2 - shifting the equilibrium towards the acylated form. Leads to TTP build up and will decrease decarboxylation

2. PDH - phosphorylated/dephosphorylated

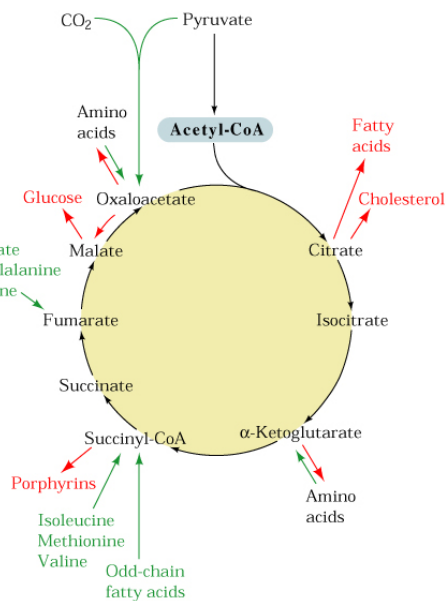
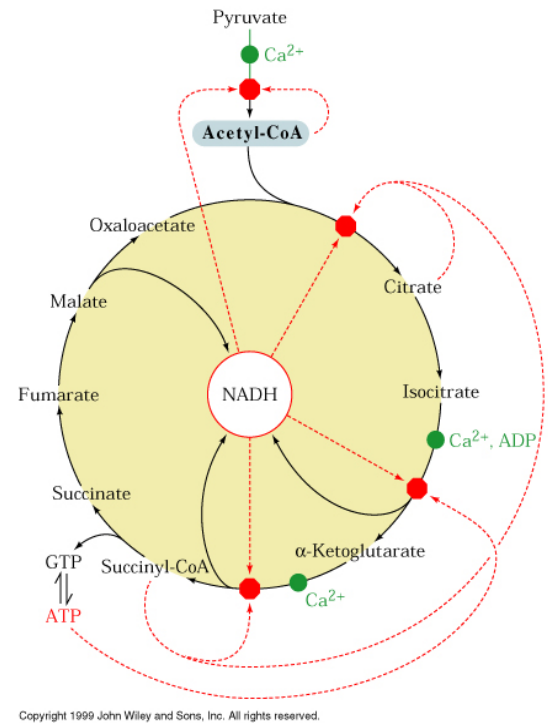
- phosphorylated PDH inactive
- ACoA, NADH - product activate kinase
- Pyruvate, CoASH, NAD⁺ - inhibit kinase
- Low [ATP] activate phosphatase
- Insulin activates the pyruvate DH phosphatase



Regulation of the cycle

Cycle enzymes

- CS - inhibited by S-CoA, citrate, NADH, and ATP
- IDH - inhibited by - NADH & ATP
- stimulated by NAD & ADP
- α KDH - Inhibited by S-CoA, NADH and high ATP/AMP



Feeding the cycle

- Addition of amino acids via transamination and oxidative deamination
- Odd chain fatty acids add
- Citrate can be transported into the cytosol to be converted to FFA and cholesterol

Transamination

Oxidative Decarboxylation

Metabolon theory

- The protein concentration is so high proteins aggregate