Faculty Science
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M.Sc. III Sem Paper- H3002 (Phytochemistry and Metabolism),

Topic- Tricarboxylic Acid Cycle and its regulation

The citric acid cycle (also called the tricarboxylic acid cycle or the Krebs cycle) is used to oxidize the pyruvate formed during glycolytic breakdown of glucose into carbon dioxide and water. It also oxidizes acetyl CoA arising from fatty acid degradation and amino acid degradation products. The cycle also provides precursors for many biosynthetic pathways. The citric acid cycle occurs in mitochondria of eukaryotes and in the cytoplasm of prokaryotes. Succinate dehydrogenase, the only membrane bound enzyme in the citric acid cycle, is embedded in the inner mitochondrial membrane in eukaryotes and in the plasma membrane in prokaryotes. The cycle occurs in close proximity to the reactions of electron transport, which oxidize the reduced coenzymes produced by the cycle. The TCA cycle is thus an aerobic pathway because oxygen is required as the final electron acceptor. The cycle is the most important central pathway connecting almost all the metabolic pathways.

Oxidative decarboxylation of pyruvate- Pyruvate is transported by specific pyruvate transporter into mitochondrion. Once in the matrix, pyruvate is converted to acetyl CoA by the enzyme pyruvate dehydrogenase complex. The reaction involves both the oxidation and the loss of CO_2, the process is known as oxidative decarboxylation.

Difference between synthase and synthetase

Synthase catalyzes condensation reactions in which nucleoside triphosphate (ATP,GTP etc) is not required as an energy source (e.g. citrate synthase). Synthetase catalyzes condensations in which ATP or other nucleoside triphosphate is used as a source of energy for the synthetic reactions (e.g. succinyl CoA synthetase).

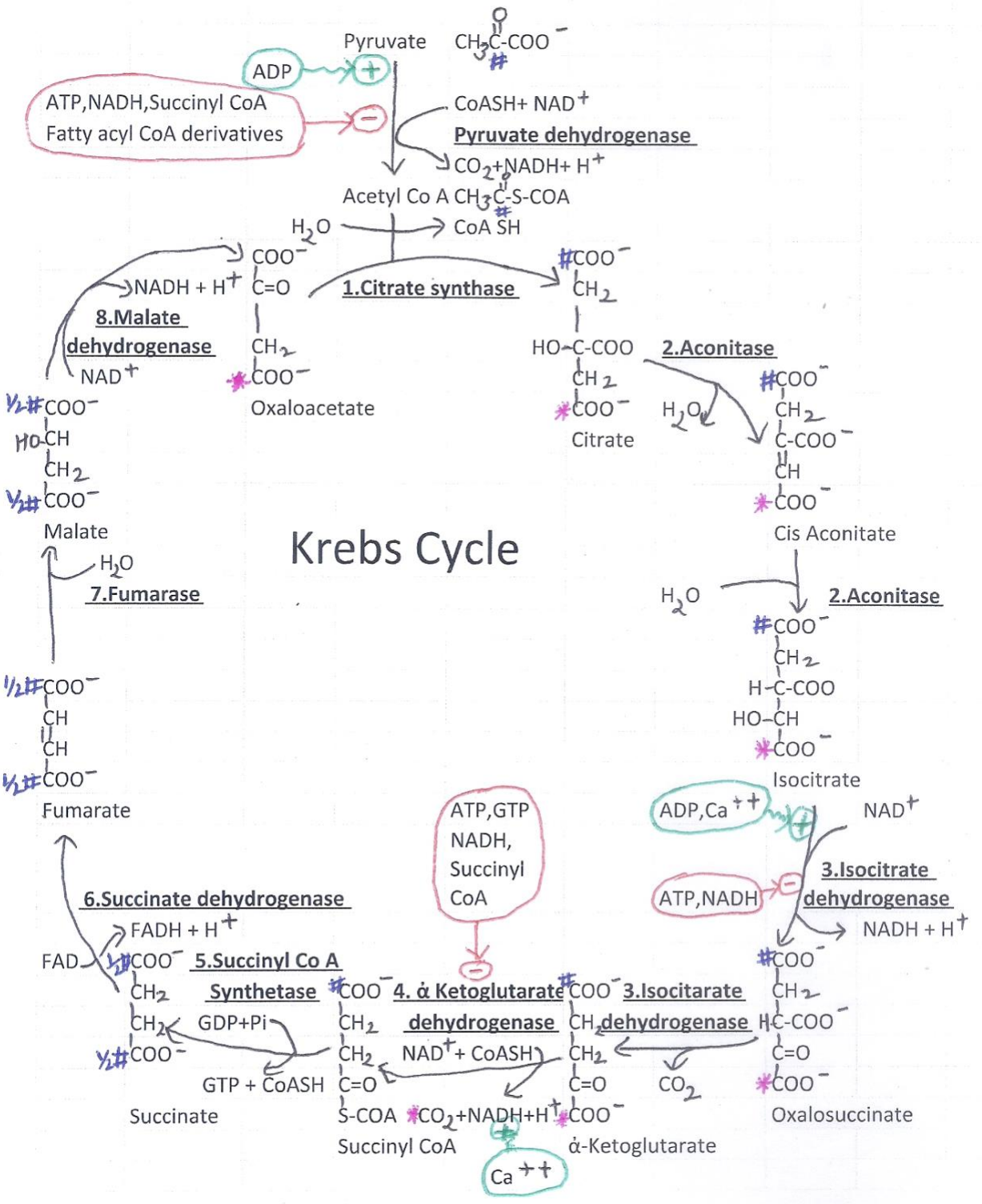
Krebs Cycle
Pyruvate
ADP
ATP,NADH,Succinyl CoA
Fatty acyl CoA derivatives
CoASH+ NAD+
Pyruvate dehydrogenase
CO2+NADH+ H+
Acetyl Co A
H2O
CoA SH
1.Citrate synthase
Citrate
2.Aconitase
Cis Aconitate
H2O
2.Aconitase
Isocitrate
ADP,Ca++
NAD+
ATP,NADH
3.Isocitrate dehydrogenase
NADH + H+
Oxalosuccinate
CO2
4. ά Ketoglutarate dehydrogenase
NAD+ + CoASH
ά-Ketoglutarate
CO2+NADH+H+
Ca++
ATP,GTP
NADH,
Succinyl CoA
Succinyl CoA
5.Succinyl Co A Synthetase
GDP+Pi
GTP + CoASH
Succinate
6.Succinate dehydrogenase
FADH + H+
FAD
Fumarate
7.Fumarase
H2O
Malate
8.Malate dehydrogenase
NADH + H+
NAD+
Oxaloacetate

Figure-1

Note: An isotopic label at C4 of oxaloacetate (*) becomes C1 of α-ketoglutarate and is released as CO_2in reaction 4. An isotopic label at C1 of acetyl CoA (#) becomes C5 of α-ketoglutarate and is scrambled in reaction 5 between C1 and C4 of succinate (1/2#**)**. Inhibitors (-) and activators **(+)** of the cycle**.**

Citric acid cycle carries out the oxidation of acetyl groups from acetyl CoA to the carbon dioxide with the production of four pairs of electrons, stored initially in the reduced electron carriers NADH and $FADH_2$.

The cycle has 8 steps.

1. **Synthesis of citrate-** The first reaction of cycle is the condensation of acetyl CoA with oxaloacetate to form citrate. This reaction is catalyzed by citrate synthase. One molecule of water is involved in the reaction and free CoA is released with citrate.
2. **Formation of isocitrate via cis-aconitate-** The enzyme aconitase catalyze the reversible transformation of citrate to isocitrate through the intermediary formation of cis-aconitate. Aconitase can promote the reversible addition of water.
3. **Oxidative decarboxylation-** Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate to form α-ketoglutarate and the first NADH molecule and the first molecule of carbon dioxide is released.
4. **Oxidative decarboxylation of α-ketoglutarate-** α-ketoglutarate is converted into succinyl CoA and carbon dioxide by the action of α-ketoglutarate dehydrogenase complex. NAD^+ serves as electron acceptor and CoA as the carrier of succinyl group.
5. **Cleavage of succinyl CoA-** Succinyl CoA is converted into succinate by the enzyme succinyl CoA synthetase. The reaction uses the energy released by cleavage of the succinyl CoA bond to synthesize either GTP (mainly in animals) or ATP (in plants) from Pi and, respectively GDP or ADP.
6. **Oxidation of succinate-** Succinate is oxidized to fumarate by the enzyme succinate dehydrogenase, producing reduced coenzyme FADH2.

7. **Hydration of fumarate-** Fumarate is hydrated to malate by the enzyme fumarase. This is a hydration reaction requiring the addition of a water molecule.
8. **Oxidation of Malate-** Malate is oxidized to oxaloacetate by the enzyme malate dehydrogenase. This reaction produces the third and final NADH of the cycle.

   The carbon atoms of the two molecules of CO_2 produced in one round of the cycle are not the two carbons of acetyl group that began the round. These acetyl carbon atoms are lost in subsequent rounds of the cycle (As shown in figure-1). However, the net effect of each round of the cycle is the oxidation of one acetyl group to 2 CO_2.

Energy produced by the TCA cycle

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

Regulation of citric acid cycle- The rate of cycle is determined by the substrate availability, inhibition by accumulating products and allosteric feedback inhibition by subsequent intermediates in the cycle. The most likely sites for regulation are the reactions catalyzed by citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. The cycle is also regulated by the enzyme pyruvate dehydrogenase which converts pyruvate to acetyl CoA to enter the cycle.

1. **Citrate synthase** is inhibited by citrate and also by ATP.
2. **Isocitrate dehydrogenase** is inhibited by an NADH and ATP but activated by ADP and Ca++.
3. **α-ketoglutarate dehydrogenase** is inhibited by NADH, ATP, GTP, acetyl CoA and succinyl CoA. It is stimulated by Ca++.
4. **Pyruvate dehydrogenase** is inhibited by NADH, acetyl CoA, succinyl CoA and fatty acyl CoA. In eukaryotes the enzyme is also controlled by

phosphorylation/ dephosphorylation via pyruvate dehydrogenase kinase/ phosphatase. The kinase catalyzes the phosphorylation of pyruvate dehydrogenase and this inactivates the enzyme. Dephosphorylation by phosphatase reactivates the enzyme. Increasing the NADH/ NAD+, acetyl CoA/ CoA or ATP/ ADP ratio stimulates phosphorylation and hence inactivates pyruvate dehydrogenase. As pyruvate builds up, it inhibits the kinase and hence reactivates the pyruvate dehydrogenase, thus stimulating pyruvate conversion to acetyl CoA.

Overall, the cycle speeds up when the cellular energy levels are low and slows down as ATP accumulates.

References:

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2. Richard A. Harvey, Denise R Ferrier (2014) in "Lippincott's Illustrated Reviews: Biochemistry". 6th edition, Lippincott Williams &Wilkins, USA.
3. Bios Instant Notes Biochemistry, 3rd edition (2007). Garland Science, USA.