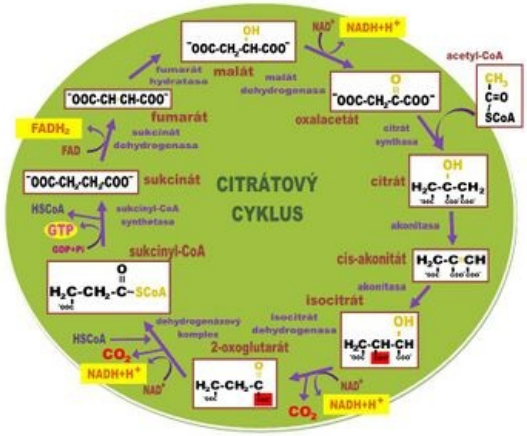
Citrate cycle

Citrate cycle, **tricarboxylic acid cycle**, **TCC**, **citric acid cycle**, **Krebs (large) cycle** [1][2] is a metabolic pathway of aerobic metabolism (vzniká $NADH + H^+$, is produced , which is consumed in the respiratory chain), although most anaerobic organisms use it to synthesize other metabolites through **cata-** and **anaplerotic reactions** (amphibolic nature of TCC, see below, that's why TCC is so complex). In aerobic organisms such as humans, in addition to this, it primarily serves to obtain $NADH + H^+$ and then ATP from pyruvate (product of glycolysis) or acetyl-S·CoA (product of β-oxidation). Enzymes involved in CC reactions are located in the mitochondria except for the succinate dehydrogenase enzyme located in the inner mitochondrial membrane.

An overview of enzymes

1. Pyruvate dehydrogenase

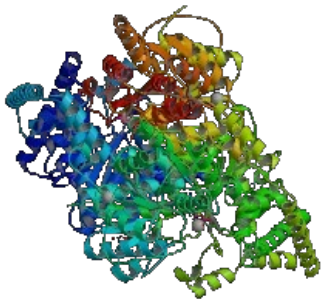


The **pyruvate dehydrogenase complex** is a complex of three enzymes inside the mitochondria: pyruvate decarboxylase, dihydrolipoyltransacetylase, and dihydrolipoyldehydrogenase. The complex works as a whole in the presence of coenzymes TPP, NAD^+, lipoate in the form of lipoamide, FAD and coenzyme A. Pyruvate dehydrogenase catalyzes the oxidative decarboxylation of pyruvate with the binding of acetyl to TPP, dihydrolipoyltransacetylase catalyzes the transfer of acetyl from TPP via lipoamide to coenzyme A, and dihydrolipoyldehydrogenase regenerates lipoamide using FAD, from which $FADH_2$ is formed, which regenerates in turn by NAD^+, from which $NADH + H^+$ is formed. The enzyme is inhibited by arsenic in the oxidation state of As(III) (arsenates,...), which blocks lipoamide.

2. Citrate synthase

It catalyzes the transfer of acetyl from acetyl-S·CoA to oxaloacetate with the entry of citrate.

3. Aconitase



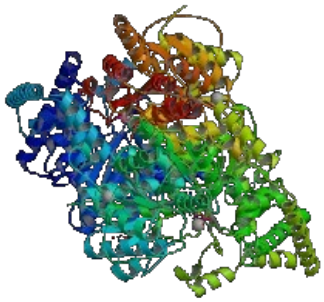
Pyruvate dehydrogenase

It catalyzes the conversion of citrate to isocitrate via cis-aconitate. Its active center consists of a cuboidal cluster of four sulfur atoms and four iron atoms, bound via sulfur from cysteine side chains. This enzyme is stereospecific with respect to the **prochiral** properties of citrate. (This can be demonstrated by labeling pyruvate with carbon ^{14}C). It is inhibited by (2R,3R)2-fluorocitrate.

4. Isocitrate dehydrogenase

It carries out oxidative decarboxylation of the carboxyl group on the tertiary carbon of isocitrate while simultaneously dehydrogenating the hydroxy group, changing NAD^+ to $NADH + H^+$. This enzyme uses **manganese** or magnesium **ions** as a **coenzyme**.

5. α-ketoglutarate dehydrogenase



Pyruvate dehydrogenase

It catalyzes oxidative decarboxylation with the simultaneous binding of an α-keto carbon to coenzyme A. Thus, sukcinyl-S·CoA and $NADH + H^+$ from NAD^+ are formed.

6. Succinyl coenzyme A synthetase

It performs the exact opposite reaction of what it is named after. It catalyzes the hydrolysis of succinyl coenzyme A with simultaneous **phosphorylation at the substrate level**, i.e. GTP is produced directly from GDP (and not in the respiratory chain).

7. Succinate dehydrogenase

Performs succinate dehydrogenation. $FADH_2$ is formed from FAD. Dehydrogenation is highly stereospecific and only fumarate and not maleate is formed. The enzyme is competitively inhibited by malonate, which is one carbon shorter than succinate, and therefore dehydrogenation cannot occur.

8. Fumarase

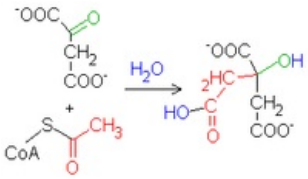
It catalyzes the alkaline hydration of fumarate to form malate. OH^- carries out a nucleophilic attack on carbon, which has acquired a partial positive charge due to the resonance of π-electrons, thereby introducing a carbanion to which H^+ is bound.

9. Malate dehydrogenase

It catalyzes the dehydrogenation of malate to oxaloacetate, which regenerates the substrate of the entire cycle. This enzyme may also be involved in the malate shuttle.

Overview of reactions

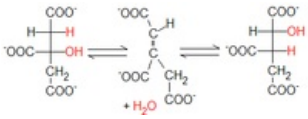
Acetyl-CoA + oxaloacetate



1. Acetyl-CoA + oxalacetate + H_2O → citrte + HS-CoA

- catalyzes the regulatory enzyme **citrate synthase**

Citrate – isocitrate



2. Citrate → cis-aconitate + H_2O → isocitrate

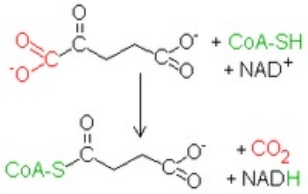
- catalyzes **aconitase**

Isocitrate – 2-oxoglutarate

3. isocitrate – 2-oxoglutarate 3. Isocitrate + NAD^+($NADP^+$) → 2-oxoglutarate + **NADH+H$^+$**(NADPH+H$^+$) + CO_2

- is catalyzed by regulatory **isocitrate dehydrogenase**

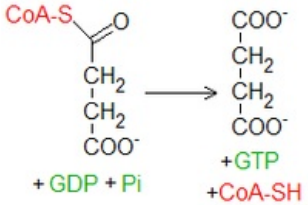
2-oxoglutarate – succinyl-CoA



4. 2-oxoglutarate + NAD^+ + HS-CoA → succinyl-CoA + **NADH+H$^+$** + CO_2

- catalyzes the regulatory **2-oxoglutarate dehydrogenase complex**

Succinyl-CoA – succinate



5. Succinyl-CoA + GDP → succinate + **GTP** + CoA

- catalyzed by **succinate thiokinase**

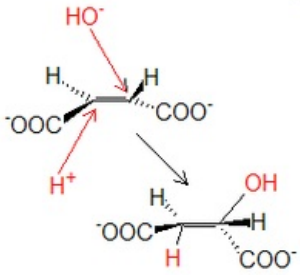
The macroergic succinyl coenzyme A is hydrolyzed to form succinate and the energy is used for substrate-level phosphorylation. This is an evolutionarily old reaction, which is why guanosine is used instead of adenosine in the conversion of GDP to GTP[3].

Succinate – fumarate

6. succinate – fumarate 6. Succinate + FAD → fumarate + $FADH_2$

- catalyzed by **succinate dehydrogenase**

Fumarate – L-malate



7. Fumarate + H_2O → L-malát

L-malate

- catalyzes **fumarate hydratase**

L-malate – oxaloacetate

8. L-malate – oxaloacetate 8. L-malate + NAD^+ → oxaloacetate + **NADH+H**$^+$

oxalacetát + **NADH+H**$^+$

- catalyzed by **malate dehydragenase**

The whole cycle is driven by these reactions [3]:

- Decarboxylation of isocitrate — the balance is shifted in favor of α-ketoglutarate as the resulting carbon dioxide is removed from the cells and exhaled.
- Decarboxylation of α-ketoglutarate — the balance is shifted in favor of succinyl coenzyme A — the same as in the previous case.
- Oxidation of malate to oxaloacetate — the balance is shifted in favor of oxaloacetate by the resulting NADH + H^+ being consumed in the respiratory chain.

Overview of cataplerotic reactions

Cataplerotic reactions are reactions that deplete TCC intermediates.

1. **Gluconeogenesis**;
2. **Synthesis of fatty acids** and **cholesterol** – starts from acetyl-S·CoA;
3. **Amino acid synthesis** – α-ketoglutarate jis the source for glutamate;
4. **Synthesis of porphyrins** – starts from acetyl-coenzyme A;
5. **oxidation of amino acids** – amino acids can be metabolized into individual TCC intermediates, which are transformed into oxaloacetate, which can be degraded using phosphoenolpyruvate carboxykinase to phosphoenolpyruvate, then to pyruvate, then to acetyl-S·CoA, which is oxidized in TCC to CO_2 and carbons from amino acid chains are exhaled.

Overview of anaplerotic reactions

Anaplerotic reactions (Greek ana = up, pleroein = to replenish) are reactions that replenish TCC intermediates.

1. **Oxidation of odd-numbered fatty acids** replenishes succinyl-S·CoA.
2. **Degradation of isoleucine, methionine and valine** eplenishes succinyl-S·CoA.
3. **Transamination and deamination** of amino acids leads to the formation of α-ketoglutarate and oxaloacetate.
4. **Carboxylation** of pyruvate - oxaloacetate.
5. **Oxidative decarboxylation** of pyruvate- Acetyl Co A.

> In the course of the citrate cycle, intermediate products are necessarily pumped out. Coupled reactions – the formation of other intermediates outside the cycle and from the synthesis cycle – reduce the concentration of intermediates. In order not to slow down the cycle, there are reactions by which the amount of intermediate products is replenished. The sum of these pathways is called anaplerosis. Of the several anaplerotic reactions, the best known is the one that replenishes the content of*oxaloacetate*. The metabolic product of glucose − *pyruvate*, which is carboxylated, is decisive for anaplerosis. Anaplerotic reactions take place in **mitochondria**, mainly in **muscles and kidneys**. *Pyravate* carboxylation is endergonic in nature and requires ATP. The catalyzing enzyme is *pyruvate carboxylase*, which includes the biotin cofactor, its own carboxyl carrier. CO_2 binds to the nitrogen of biotin to form *carboxybiotin*; this factor also carboxylates other substances. The allosteric enzyme *pyruvate carboxylase* is activated by acetyl coenzyme A. The need for a higher content of *oxaloacetate* is signaled by a high level of *acetyl conzyme A*. Further anaplerotic reactions lead mainly to the formation of *2-oxoglutarate*, mainly through transamination or even oxidative deamination of *glutamate*.

Regulation of the citrate cycle

Regulation of the Krebs cycle is carried out in several ways:

- **enough substances** for the course of the cycle, especially Ac-CoA and oxaloacetate.
- elimination of catabolites (see above)
- the cycle is inhibited by an increased **ATP:AMP ratio**
- **respiratory control** – the respiratory chain works (the NAD+:NADH+H^+ ratio increases)
- **enzymatic regulation**:
    - citrate synthase
    - isocitrate dehydrogenase
    - α-ketoglutarate dehydrogenase

+++++extras :

Citrate synthase, isocitrate dehydrogenase, and the α-ketoglutarate dehydrogenase complex are enzymes that are allosterically **inhibited by high concentrations of ATP.** This prevents excessive consumption of acetyl-CoA.

Some enzymes**are inhibited** if there is a **large amount of reduced coenzymes** in the mitochondria. The rate of reverse oxidation of coenzymes depends on the respiratory chain, i.e. **also on the availability of oxygen**. **Absence or partial lack of O_2** causes complete or partial**inhibition of the cycle.** On the contrary, the increased rate of respiration, which occurs with an increased need for ATP, also increases the activity of the citrate cycle.

Links

Related articles

- Glycolysis
- Coenzymes
- Acetyl-CoA
- ATP

External links

- Citric Acid Cycle (https://www.wiley.com/college/pratt/0471393878/student/animations/citric_acid_cycle/index.html)

Reference

1. VOET, Donald – VOET, Judith. *Biochemistry.* 3rd edition. John Wiley & Sons Inc, 2004. vol. 1616. pp. 765–795. ISBN 0-471-19350-X.
2. NELSON, David L – COX, Michael M. *Lehninger Principles of Biochemistry.* 4th ed edition. 2004. vol. 1130. pp. 601–623. ISBN 0-71674339-6.
3. LIBERDA, Jiří. *Citrátový cyklus* [lecture for subject Cvičení z biochemie (https://is.cuni.cz/studium/predmety/index.php?do=predmet&kod=MC250C23), specialization Chemie v přírodních vědách, Přírodovědecká fakulta UK]. Praha. 7.4.2011.

Used literature

- LEDVINA, Miroslav. *Biochemie pro studující medicíny. I. díl.* 2. edition. Karolinum, 0000. vol. 269. pp. 103-106. ISBN 978-80-246-1416-8.
- MATOUŠ, Bohuslav. *Základy lékařské chemie a biochemie.* 1. edition. Galén, 2010. vol. 540. ISBN 978-80-7262-702-8.

Recommended literature

See references.