

Citric acid cycle (FBLT)

Subchapter overview

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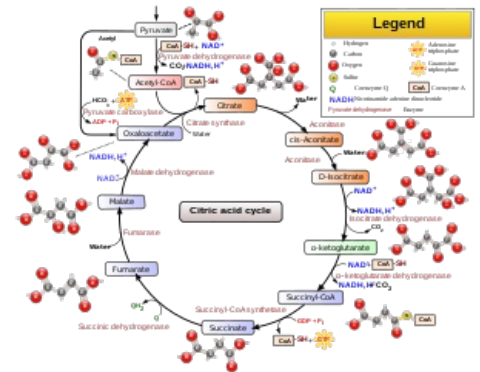
Introduction to the citric acid (TCA) cycle

The Krebs cycle (citrate cycle, citric acid cycle, TCA cycle) is a metabolic pathway located in the matrix of mitochondria. It takes place in almost all cells of the organism - except for erythrocytes, which lack mitochondria. Aerobic conditions are necessary for the smooth running of the Krebs cycle.

- More detailed information can be found on the [TCA cycle regulation](#) page.

Cells suffering from a lack of oxygen are speed limited. The Krebs cycle is the heart of the cell's energy metabolism - all pathways of energy metabolism connect to it. For example, the electron transport chain, gluconeogenesis, transamination and deamination of amino acids or lipogenesis. Therefore, it cannot be determined whether it is an anabolic or catabolic pathway. That's why we call it the amphibole pathway.

- For more detailed information, see the [Energy metabolism overview](#) page.



The citric acid cycle (diagram)

Significance of the citric acid (TCA) cycle

Oxidation of acetyl residues (supplied in the form of acetyl-CoA)

Oxidation of acetyl residues ($\text{CH}_3\text{-CO}^-$) to final CO_2 . The reaction is a source of reducing equivalents (H^+), which are transferred to cofactors NAD^+ or FAD to form reduced forms:

- $\text{NADH} + \text{H}^+$,
- FADH_2 .

Reduced cofactors saturate the electron transport chain, where they are regenerated - reoxidised, and therefore represent a mutual connection between the **Krebs cycle** and the **electron transport chain**. The Krebs cycle is the main supplier of reduced cofactors for the electron transport chain and therefore an important source of ATP for the cell. In the Krebs cycle itself, however, only one GTP is directly produced per one of its "turns".

The culmination of many catabolic pathways into the Krebs cycle

Many catabolic pathways produce Krebs cycle intermediates or metabolites such as pyruvate and acetyl-CoA. These can be oxidized to CO_2 , but also used as substrates for the synthesis of other substances.

Delivery of precursors to anabolic pathways

For example, gluconeogenesis, the biosynthesis of tetrapyrroles (heme), the formation of amino acids (e.g. glutamate, also the most abundant excitatory neurotransmitter in the brain) or the supply of acetyl-CoA for the synthesis of fatty acids.

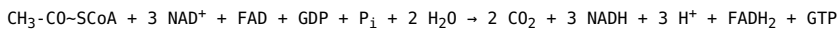
Participation in the excretion of amino nitrogen

The Krebs cycle is closely linked to the urea synthesis cycle and to the formation of glutamate, which are the two main reactions used to eliminate nitrogen derived from amino acids from the body.

Historical correlation: The Krebs cycle is named after Sir Hans Adolf Krebs (1900–1981), a German, later English physician and biochemist. He was awarded the Nobel Prize in Physiology and Medicine in 1953 "for his discovery of the citric acid cycle". He received the prize together with the German, later American biochemist Fritz Albert Lipmann, who received it "for his discovery of co-enzyme A and its importance for intermediary metabolism".

Reactions of the citric acid (TCA) cycle

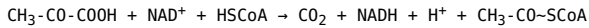
The summary equation describing the Krebs cycle:



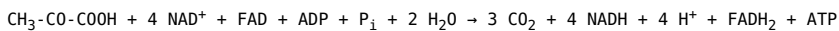
Most of the **acetyl-CoA**, which supplies acetyl residues to the Krebs cycle, comes from β -oxidation of fatty acids and from the pyruvate dehydrogenase reaction (processes taking place in the mitochondrial matrix).

Pyruvate dehydrogenase reaction

It is an irreversible oxidative decarboxylation of pyruvate.



If we connect the pyruvate dehydrogenase reaction and the summary reaction of the Krebs cycle, we get an equation describing the **complete oxidation of pyruvate**.



Individual reactions of the Krebs cycle

The oxidation of acetyl residues proceeds through several intermediate steps.

1. Acetyl residue (2C) transferred to oxaloacetate (4C)

- reaction catalysed by the enzyme citrate synthase - citrate (6C) is formed
- irreversible – regulatory reaction

2. Isomerization of citrate to isocitrate

- via aconitate, catalysed by the enzyme aconitate-hydratase (aconitase)
- freely reversible reaction

3. Oxidation of isocitrate to α -ketoglutarate

- catalysed by the enzyme isocitrate dehydrogenase
- **oxidative decarboxylation** – oxidation of the –OH group of isocitrate to a keto group (formation of NADH + H^+) with simultaneous cleavage of one carboxyl group in the form of CO_2
- irreversible – the most important regulatory reaction

4. Oxidation of α -ketoglutarate to succinyl-CoA

- catalysed by α -ketoglutarate dehydrogenase (multienzyme complex)
- it is oxidative decarboxylation – another CO_2 molecule is split off
- formation of NADH + H^+
- irreversible and regulatory reaction

5. Conversion of succinyl-CoA to succinate and coenzyme A

- catalysed by succinyl-CoA-ligase
- typical substrate phosphorylation
- reversible reaction
- formation of GTP, which can be converted into ATP.

In the previous reactions, the acetyl residue was completely oxidised to 2 CO_2 and oxaloacetate was reduced to succinate. The following three reactions regenerate oxaloacetate from succinate

6. Oxidation of succinate to fumarate

- using the succinate dehydrogenase enzyme (integral protein in the inner mitochondrial membrane, which is part of the electron transport chain – complex II)
- cofactor is FAD – FADH_2 is formed

7. Addition of water to the double bond in fumarate to form malate

- catalysed by the enzyme fumarate hydratase (fumarase)

8. Oxidation of malate to oxaloacetate

- using the malate dehydrogenase enzyme
- $\text{NADH} + \text{H}^+$ is formed
- closes the Krebs cycle

Krebs cycle products

In one turn of the Krebs cycle, **2 CO_2** , **3 $\text{NADH} + \text{H}^+$** , **1 FADH_2** and **1 GTP** (can be exchanged for ATP) are produced.

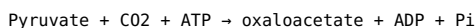
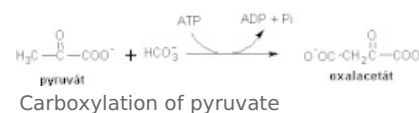
Carbon dioxide (CO_2) diffuses out of the mitochondria and is finally excreted in the lungs. **Reduced cofactors ($\text{NADH} + \text{H}^+$, FADH_2)** saturate the electron transport chain, which subsequently forms ATP. The energy balance of the Krebs cycle (direct formation of GTP and formation of ATP in the electron transport chain) varies between **10-12 ATP** per one molecule of acetyl-CoA. The situation is much more complicated and the exact number is problematic to determine.

Complementary (anaplerotic) reactions

Krebs cycle intermediates are found in very small amounts in mitochondria. On the one hand, anabolic pathways create the main outflow of intermediate products from the Krebs cycle - for example: **succinyl-CoA \rightarrow heme synthesis**, **oxaloacetate \rightarrow gluconeogenesis**. On the other hand, during the oxidation of acetyl residues, their constant regeneration occurs, and therefore their concentrations remain relatively stable over time. Reactions that replenish these losses of Krebs cycle intermediates are called **anaplerotic**.

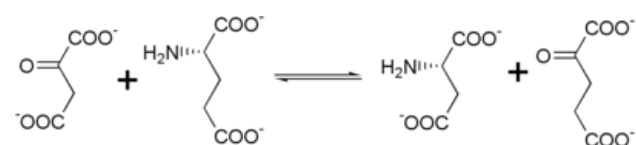
1. Carboxylation of pyruvate

- formation of oxaloacetate
- the reaction is catalysed by the enzyme *pyruvate carboxylase* (cofactor is biotin - vitamin B7)



2. Formation of oxaloacetate and α -ketoglutarate

- arising from the carbon skeletons of amino acids
- aspartate can be transaminated to oxaloacetate & glutamate can be transaminated to α -ketoglutarate



Aspartate transaminase (PLP-dependent) catalyses the transamination of oxaloacetate and glutamate to give aspartate and α -ketoglutarate. Transaminates most L-amino acids, e.g. phenylalanine, tyrosine and tryptophan.

3. Formation of succinyl-CoA from propionyl-CoA

- formed during the degradation of fatty acids with an odd number of C-atoms

Regulation of the citric acid (TCA) cycle

The regulatory points (enzymes) of the Krebs cycle are:

1. *Citrate synthase*
2. *Isocitrate dehydrogenase*
3. *α -ketoglutarate dehydrogenase*

The regulatory factors of the Krebs cycle are:

1. $\text{NADH} / \text{NAD}^+$ ratio - respiratory control;
2. $\text{ATP} / (\text{ADP} \text{ and } \text{AMP})$ ratio - energy control;
3. Availability of Krebs cycle substrates - substrate control.

$\text{NADH} / \text{NAD}^+$ ratio - respiratory control

The continuation of the Krebs cycle is the electron transport chain, where reduced cofactors are reoxidised. If NADH + H⁺ and FADH₂ accumulate (NADH / NAD⁺ ratio increases), *α-ketoglutarate dehydrogenase* and *isocitrate dehydrogenase* are inhibited.

ATP / (ADP and AMP) ratio - energy control

If there is enough energy, *α-ketoglutarate dehydrogenase* and *isocitrate dehydrogenase* are inhibited.

- ATP is their inhibitor;
- ADP and AMP, on the other hand, are activators.

Krebs cycle substrate availability - substrate control

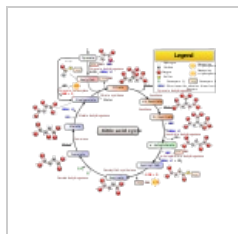
Substrate control is at the level of *citrate synthase*, which produces as much citrate as we supply it with oxaloacetate and acetyl-CoA.

Krebs cycle activity is also related to **O₂ availability**. Even though none of the reactions in the cycle require O₂, oxygen is needed for the electron transport chain because it serves as the final **electron acceptor**. In the electron transport chain, the following are **reoxidised**:

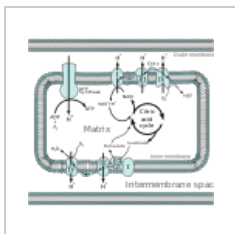
NADH → NAD⁺

FADH₂ → FAD

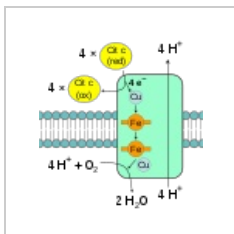
If the cell lacks O₂, the concentration of NAD⁺ and FAD decreases and consequently the activity of the Krebs cycle also decreases.



The citric acid cycle (diagram)



The electron transport chain (diagram)



Complex IV in the ETC (diagram)

References

Related articles

- Electron transport chain
- Electron transport chain (FBLT)

Bibliography

- FONTANA, J. – TRNKA, J. – MAĎA, P., et al. *Funkce buněk a lidského těla : Přeměna látek a energie v buňce* [online]. 3. lékařská fakulta UK, [cit. 2022-11-27]. <<http://fblt.cz/skripta/ii-premena-latek-a-energie-v-bunce/8-krebsuv-cyklus/>>.