Citric Acid Cycle

Cycle Overview

Metabolic Sources of Acetyl-Coenzyme A

Enzymes of the Citric Acid Cycle

Regulation of the Citric Acid Cycle

The Amphibolic Nature of the Citric Acid Cycle

Cycle Overview

(citric acid or Krebs or tricarboxylic acid cycle)

Amphibolic - operates catabolically and anabolically

$$\text{acetyl group} \rightarrow 2CO_2$$

Reactions of the cycle:

1. Citrate synthase
2. Aconitase
3. Isocitrate dehydrogenase
4. α-Ketoglutarate dehydrogenase
5. Succinyl-CoA synthase
6. Succinate dehydrogenase
7. Fumarase
8. Malate dehydrogenase

$$3NAD^+ + FAD + GDP + P_i + \text{acetyl-CoA} \rightarrow$$

$$3NADH + FADH_2 + GTP + CoA + 2CO_2$$

Cycle operates catalytically as a result of regeneration of oxaloacetate

Acetyl-Coenzyme A (acetyl-SCoA or acetyl-CoA) - common product of carbohydrate, fatty acid and amino acid breakdown ($\Delta G^{\circ\prime} = -31.5\ kJ{\cdot}mol^{-1}$)

Metabolic Sources of Acetyl-Coenzyme A

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH}$$

Pyruvate dehydrogenase multienzyme complex

pyruvate dehydrogenase (E_1)
dihydrolipoyl transacetylase (E_2)
dihydrolipoyl dehydrogenase (E_3)

Eukaryotic complex - 30 E_1 dimers + 6 E_3 dimers around a core of 60 E_2 monomers

Advantages of multienzyme complexes:

1. Rate enhancement due to shorter distances for diffusion of substrates
2. Channeling of intermediates, minimized side reactions
3. Coordinate control of reactions

Five cofactors required:

thiamine pyrophosphate (TPP)
lipoic acid
coenzyme A (CoA)
flavin adenine dinucleotide (FAD)
nicotinamide adenine dinucleotide (NAD^+)

Metabolic Sources of Acetyl-Coenzyme A

Pyruvate dehydrogenase multienzyme complex

Five reactions:

1. Pyruvate dehydrogenase (E_1) decarboxylates pyruvate (identical to pyruvate decarboxylase)
2. Hydroxylethyl group transferred to E_2
3. E_2 catalyzes transfer (transesterification) of acetyl group to CoA
4. Dihydrolipoyl dehydrogenase (E_3, lipoamide dehydrogenase) reoxidizes dihydrolipoamide (similar to glutathione reductase reaction in reverse)
5. Reduced E_3 reoxidized by NAD^+

Metabolic Sources of Acetyl-Coenzyme A

Pyruvate dehydrogenase multienzyme complex

Dihydrolipoyl transacetylase (E_2):

lipoyllysyl tether allows one E_1 subunit to acetylate many E_2 subunits and one E_3 subunit can reoxidize several dihydrolipoamide groups

Arsenic compounds covalently bind sulfhydryl groups, inactivates lipoamide-containing enzymes (pyruvate dehydrogenase and α-ketoglutarate dehydrogenase)

Protein X - facilitates binding of dihydrolipoyl dehydrogenase (E_3)

Control of Pyruvate Dehydrogenase

Pyruvate dehydrogenase (E_1)

Product inhibition by NADH and acetyl-CoA

NADH and acetyl-CoA compete with NAD^+ and CoA
Drive reversible E_2 and E_3 reactions backwards

Covalent modification by
phosphorylation/dephosphorylation

pyruvate dehydrogenase kinase - inactivates E_1 subunit by phosphorylating Ser residue
pyruvate dehydrogenase phosphatase -reactivates E_1 subunit by dephosphorylating Ser residue

Enzymes of the Citric Acid Cycle

Citrate synthase

$$\text{acetyl-CoA} + \text{oxaloacetate} \rightarrow \text{CoA} + \text{citrate}$$

Ordered sequential mechanism - oxaloacetate adds first

His274, Asp375, and His320 general acid-base catalysis

Rate determining step - formation of enol form of acetyl-CoA

Formation of enzyme-bound citryl-CoA

Hydrolysis of citryl-CoA to citrate and CoA

$$\Delta G^{\circ\prime} = -31.5\ kJ \cdot mol^{-1}$$

Stereospecific Aldol-Claisen condensation at the *si* face

Enzymes of the Citric Acid Cycle

Aconitase

citrate ⇔ aconitate ⇔ isocitrate

Prochiral center

First stage - dehydration reaction (trans elimination)

Second stage - rehydration reaction (stereospecific trans addition)

Asp, His, and Ser catalytic residues

[4Fe-4S] iron sulfur cluster

180˚ flip of aconitate intermediate

Enzymes of the Citric Acid Cycle

NAD^+-dependent isocitrate dehydrogenase

isocitrate + $NAD^+ \rightarrow$ α-ketoglutarate + CO_2 + NADH

First production of CO_2 and NADH

Requires Mn^{2+} or Mg^{2+} cofactor

Enzymes of the Citric Acid Cycle

α-Ketoglutarate dehydrogenase multienzyme complex

α-ketoglutarate + acetyl-CoA + NAD^+
$\rightarrow$ succinyl-CoA + CO_2 + NADH + H^+

α-ketoglutarate dehydrogenase (E_1)
dihydrolipoyl transsuccinylase (E_2)
dihydrolipoyl dehydrogenase (E_3)

Similar to pyruvate dehydrogenase complex (2-keto-acid dehydrogenase family)

No covalent modification system

Formation of "high-energy" thioester

Enzymes of the Citric Acid Cycle

Succinyl-CoA synthetase (succinate thiokinase)

$$\text{succinyl-CoA} + \text{GDP} + P_i \rightarrow \text{succinate} + \text{CoA} + \text{GTP}$$

Phosphoryl-enzyme intermediate (OPO_3-His)

Successive synthesis of "high-energy" compounds:

succinyl phosphate
3-phosphohistidine residue
GTP

Up to this point:

$$\text{one acetyl} \rightarrow 2CO_2 + 2\text{NADH} + \text{GTP(ATP)}$$

Enzymes of the Citric Acid Cycle

Succinate dehydrogenase

$$\text{succinate} + \text{FAD} \rightarrow \text{fumarate} + \text{FADH}_2$$

Stereospecific

Bound to inner-mitochondrial membrane (only citric acid cycle enzyme membrane bound)

FAD covalently linked to enzyme, reoxidized by electron transport chain

Enzymes of the Citric Acid Cycle

Fumarase

$$\text{fumarate} + H_2O \rightarrow \textit{S}\text{-malate}$$

Hydration reaction

Two possible mechanisms:

carbocation intermediate

carbanion intermediate - established by ^{18}O exchange experiments, product release is rate determining step

Enzymes of the Citric Acid Cycle

Malate dehydrogenase

$$S\text{-malate} + NAD^+ \rightarrow \text{oxaloacetate} + NADH + H^+$$

$$\Delta G^{\circ\prime} = +29.7\ kJ \cdot mol^{-1}$$

[oxaloacetate] kept low (high $\Delta G^{\circ\prime}$ of citrate synthase drives cycle 1st reaction)

Oxidation-reduction reaction

Similar to lactate dehydrogenase and alcohol dehydrogenase

Enzymes of the Citric Acid Cycle

Integration of the citric acid cycle

One cycle:

1. One acetyl oxidized to two CO_2 (8 e^- process)
2. Three NAD^+ reduced to NADH (6 e^-)
3. One FAD reduced to $FADH_2$ (2 e^-)
4. One GTP (ATP) produced

Electrons pass to the electron transport chain

$$O_2 \rightarrow H_2O \text{ (4 } e^- \text{ process)}$$

$$\text{NADH} \cong 3 \text{ ATP}$$

$$FADH_2 \cong 2 \text{ ATP}$$

$$\text{one cycle} \cong 12 \text{ ATP}$$

These are approximate (maximum) number of ATP as we shall soon see

Regulation of the Citric Acid Cycle

Rate-controlling enzymes:

citrate synthase
isocitrate dehydrogenase
α-ketoglutarate dehydrogenase

Dioxygen consumption, NADH reoxidation, and ATP production are tightly coupled

Regulatory control:

1. Substrate availability - oxaloacetate stimulates citrate synthase
2. Product inhibition - citrate competes with oxaloacetate for citrate synthase, NADH inhibits isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, succinyl-CoA inhibits α-ketoglutarate dehydrogenase
3. Competitive feedback inhibition - NADH inhibits citrate synthase, succinyl-CoA competes with acetyl-CoA in citrate synthase reaction

Most crucial regulators:

substrates -acetyl-CoA and oxaloacetate
product - NADH

Regulation of the Citric Acid Cycle

Allosteric control of cycle enzymes:

isocitrate dehydrogenase
α-ketoglutarate dehydrogenase
pyruvate dehydrogenase phosphatase

ADP - allosteric activator of isocitrate dehydrogenase

ATP - inhibits isocitrate dehydrogenase

Ca^{2+} - activates pyruvate dehydrogenase phosphatase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase

The Amphibolic Nature of the Citric Acid Cycle

Amphibolic - both anabolic and catabolic

intermediates must be replaced

Pathways that utilize citric acid cycle intermediates:

1. Glucose biosynthesis (gluconeogenesis) - oxaloacetate (transported as malate)
2. Lipid biosynthesis - acetyl-CoA from ATP-citrate lyase

   ATP + citrate + CoA $\Leftrightarrow$

   ADP + P_i + oxaloacetate + acetyl-CoA
3. Amino acid biosynthesis - α-ketoglutarate (glutamate dehydrogenase or transamination) and oxaloacetate (transamination)
4. Porphyrin biosynthesis - succinyl-CoA

The Amphibolic Nature of the Citric Acid Cycle

Reactions that replenish citric acid cycle intermediates:

anaplerotic "filling up" reactions

Pyruvate carboxylase

Pyruvate + CO_2 + ATP + $H_2O \Leftrightarrow$

oxaloacetate + ADP + P_i

Oxidation of fatty acids - succinyl-CoA

Breakdown of amino acids (Ile, Met, Val) - succinyl-CoA

Transamination and deamination of amino acids - α-ketoglutarate and oxaloacetate