Universiti Malaysia PAHANG
Engineering • Technology • Creativity

BIOCHEMISTRY

Citric Acid Cycle

by
Dr Jaya Vejayan
Faculty of Industrial Sciences & Technology
email: jayavejayan@ump.edu.my

Chapter Description

- Overview

  This chapter is first of 3 pathways known to be cyclic. The others are Calvin and urea cycles. Also known as krebs cycle with amphibolic capacity. It is directly connected with the electron transport chain.

- Expected Outcomes

  You should be able to have a general understanding on the location, connection, substrate level and oxidative phosphorylation contributions, regulation etc of this pathway.

- Other related Information

  Some relevant questions been provided for improving your understanding of the topic. You are expected to search for external sources for information to adequately answer the questions. All pictures and figures within this chapter categorized as creative commons for the purpose of education only.

CITRIC ACID CYCLE

- Operate under **aerobic condition** only.
- Occur **within mitochondria.**
- **Oxidizes the two carbon acetyl** group in acetyl-CoA to **CO_2**.
- Produces **reduced coenzymes NADH and $FADH_2$ and one ATP** directly.
- Proceeds in **2 phases**:
  1. Addition of 2C Acetyl-CoA + 4C Oxaloacetate (OAA) → 6C citrate
  2. Regeneration of OAA

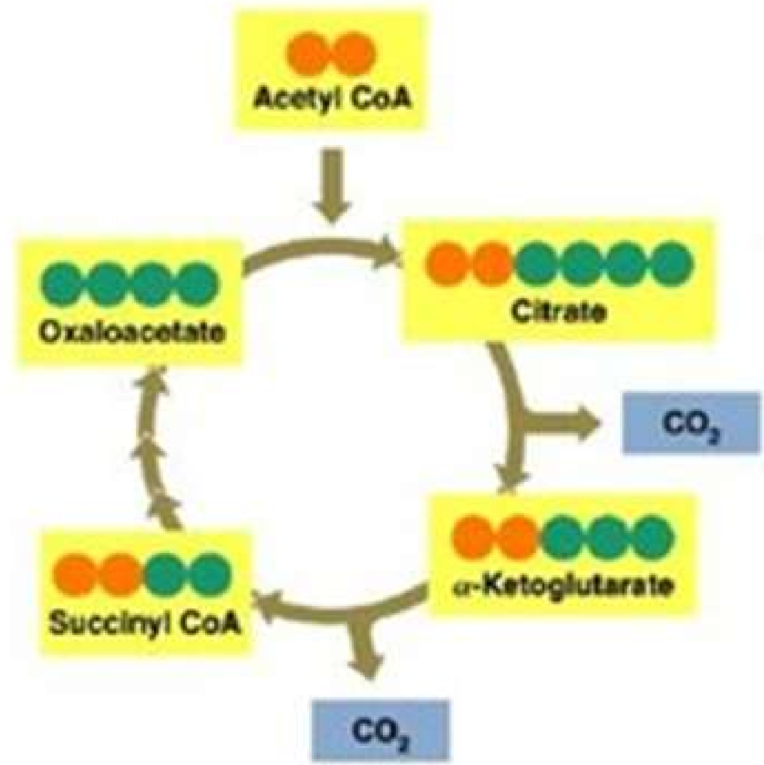
Acetyl-CoA

- Product of catabolic reactions in carbohydrate, lipid and amino acid metabolism.
- Synthesized from pyruvate.
- Product of β-oxidation of fatty acid.
- Certain reactions in amino acid metabolism.
- Because it cannot penetrate the inner mitochondrial, it is converted into citrate.
- Then, citrate is cleaved to form Acetyl-CoA & oxaloacetate (OAA) by citrate lyase.

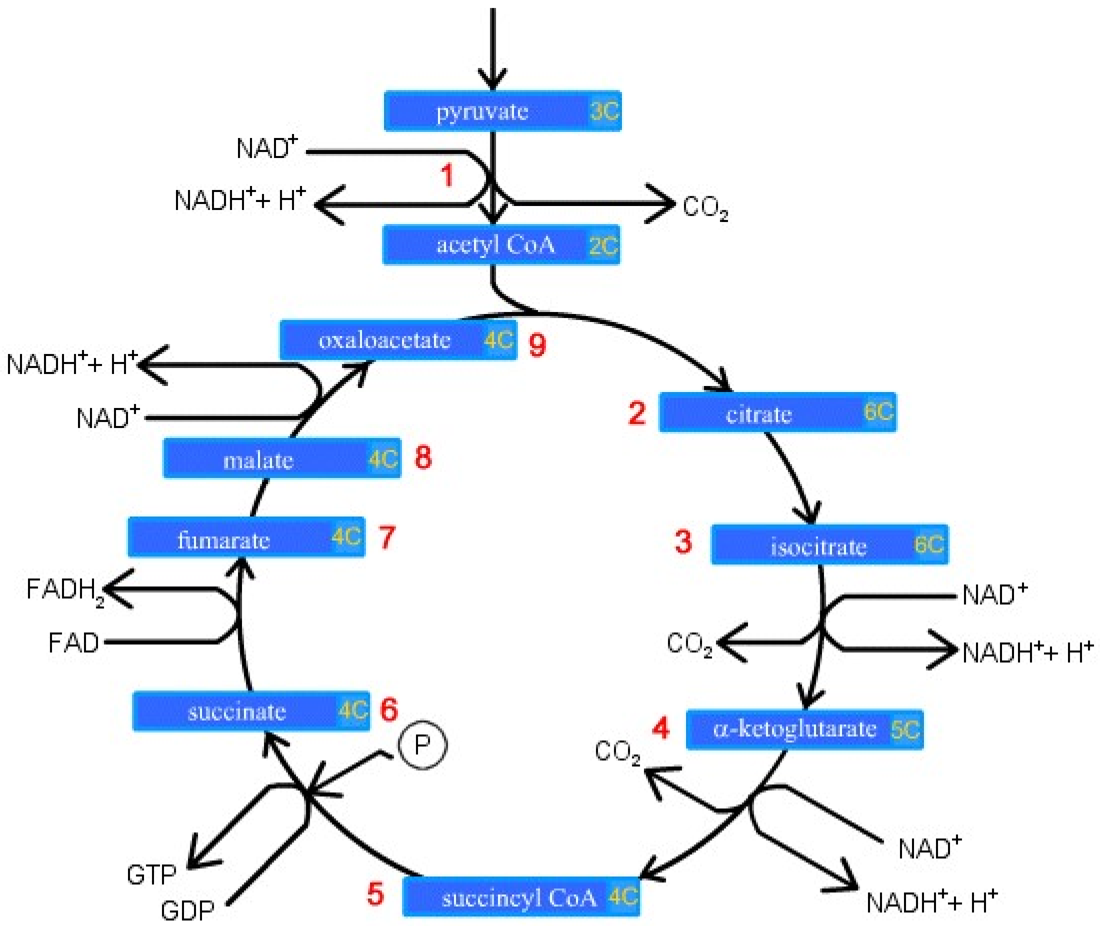
Citric Acid Cycle Overview

In the **citric acid cycle**:

- Acetyl (2C) bonds to oxaloacetate (4C) to form citrate (6C).
- Oxidation and decarboxylation convert citrate to oxaloacetate.
- Oxaloacetate bonds with another acetyl to repeat the cycle.



Citric acid cycle: Basic Outline

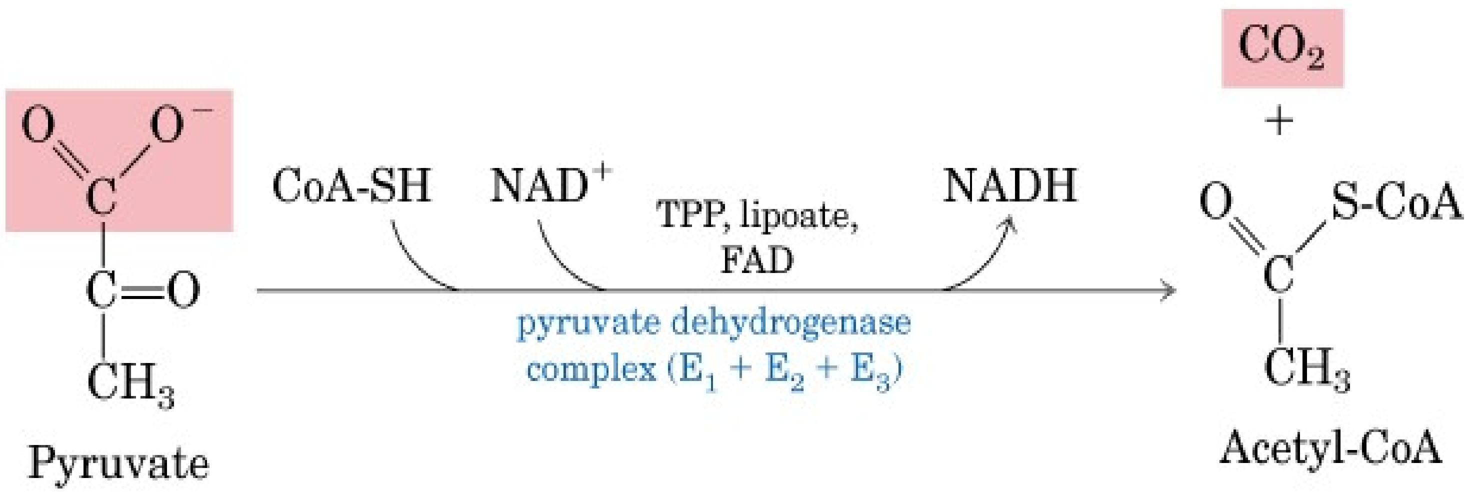


Conversion of pyruvate to Acetyl-CoA

- After its transport into the mitochondrial matrix, pyruvate is converted to Acetyl-CoA (catalyzed by the enzymes in the **pyruvate dehydrogenase complex**).

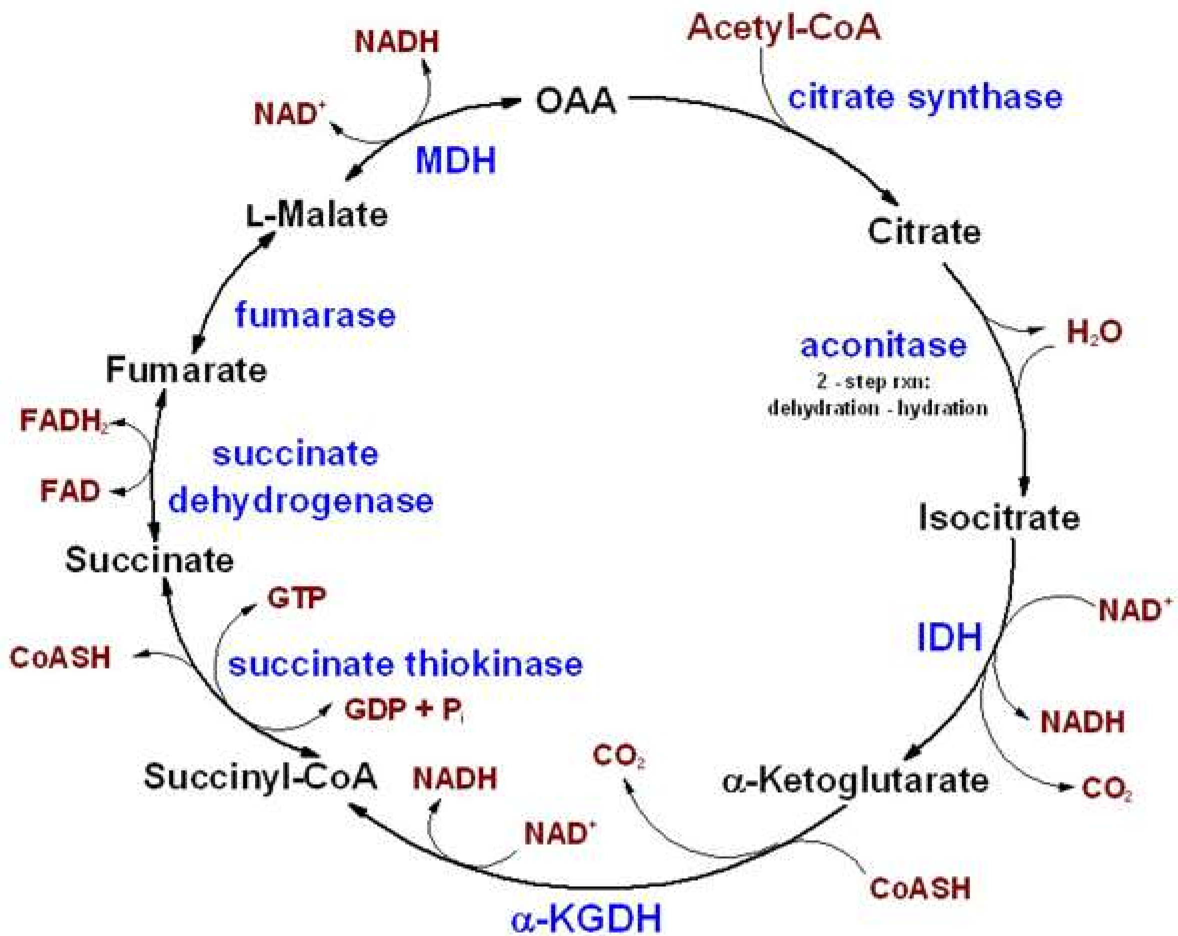
Pyruvate + NAD^+ + CoASH → Acetyl-CoA + NADH + H^+ + CO_2 + H_2O

Reaction of pyruvate dehydrogenase complex (PDC)



$\Delta G'^{\circ} = -33.4$ kJ/mol

Citric acid cycle: Intermediate Outline

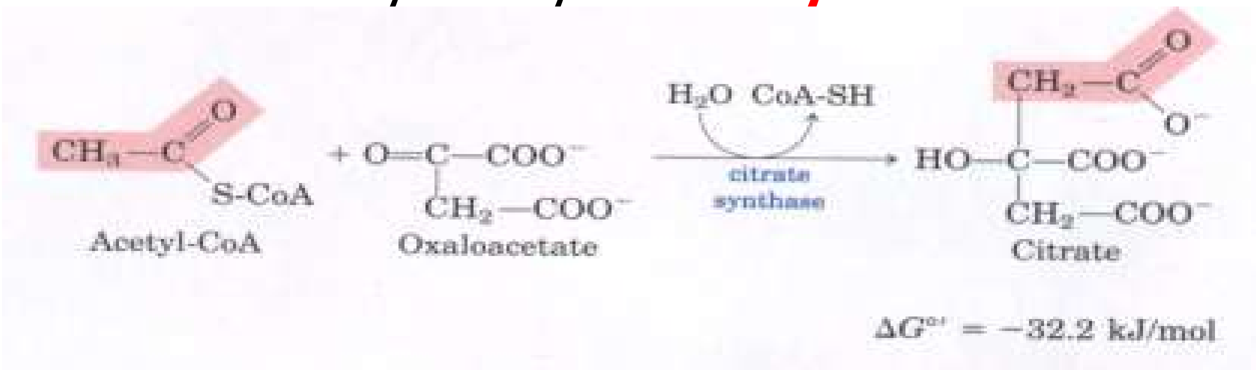


Reactions of the Citric Acid Cycle

Phase 1:

Step 1: Introduction of 2C atoms as Acetyl-CoA

- Initial reaction catalyzed by **citrate synthase**.

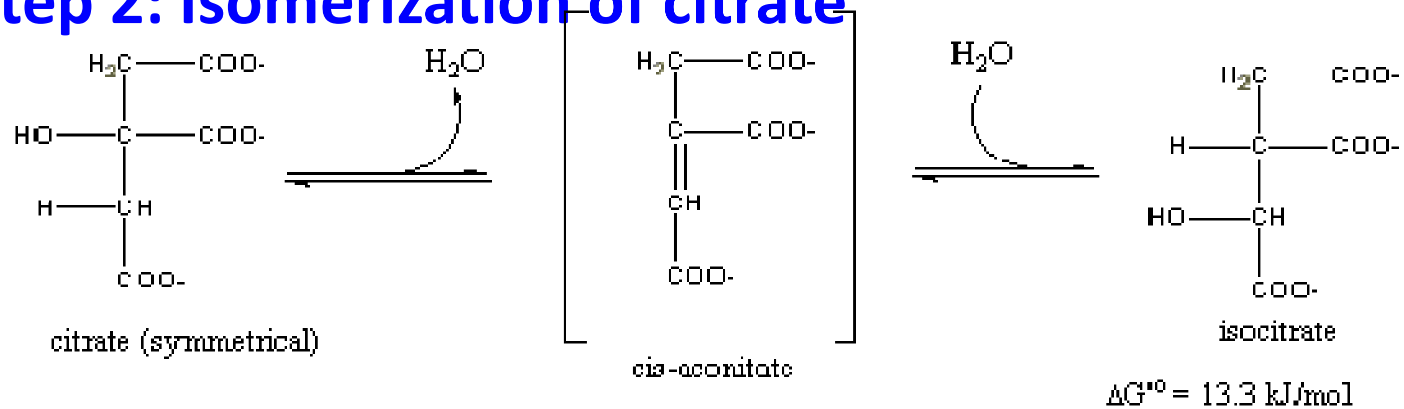


- Methyl carbon on Acetyl-CoA loses proton with nucleophilic attack on carbonyl carbon of OAA.
- This reaction generates the highly unstable Citroyl-CoA, which sponteneously hydrolyzes while enzyme bound, to yield the products.
- This reaction is highly exergonic

Reactions of the Citric Acid Cycle

<u>Phase 1</u>:

Step 2: Isomerization of citrate



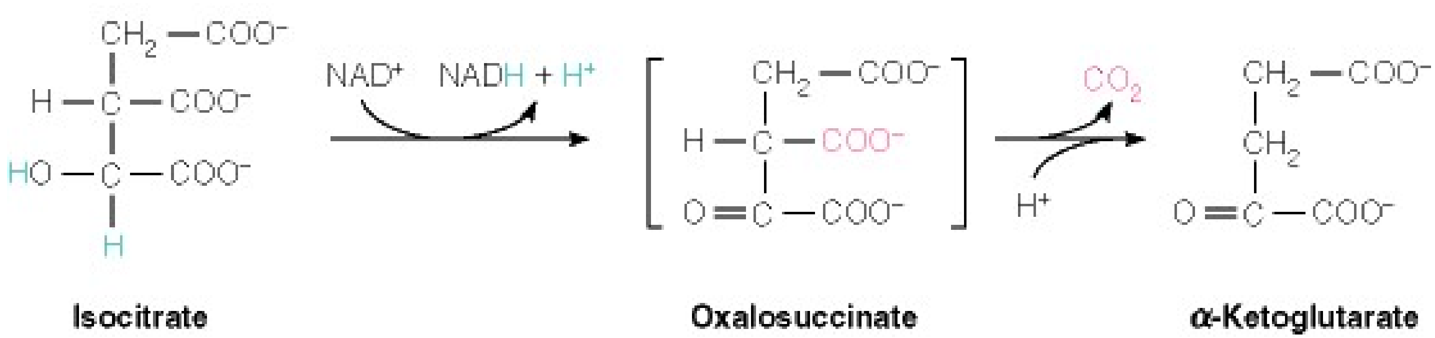
- Isomerization catalyzed by aconitase, generates the second alcoholic compound, isocitrate, which can be oxidized.
- The reaction involves successive dehydration & hydration, through cis-aconitate as dehydrated intermediates.

Reactions of the Citric Acid Cycle

<u>Phase 1</u>:

Step 3: Generation of CO2 by an NAD⁺ -Linked Dehydrogenase

- Isocitrate is oxidized to form oxalosuccinate.
- Intermediate decarboxylation of oxalosuccinate results in the formation of α-ketoglutarate.



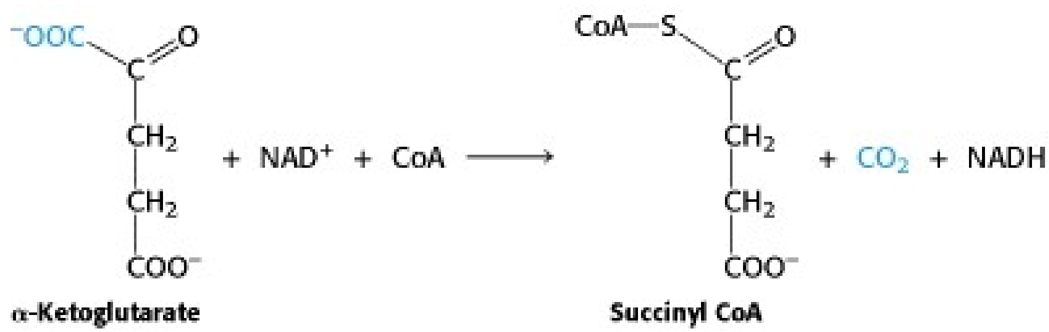
- Isocitrate dehydrogenase is specific enzyme for NAD+ (NAD+ present in both cytosol & mitochondria).

Reactions of the Citric Acid Cycle

Phase 1:

Step 4: Generation of second CO_2 by a multienzyme complex.

- An α-keto acid substrate undergoes oxidative decarboxylation, with concomitant formation of an acyl-CoA.
- This reaction catalyzed by the α-ketoglutarate dehydrogenase complex.

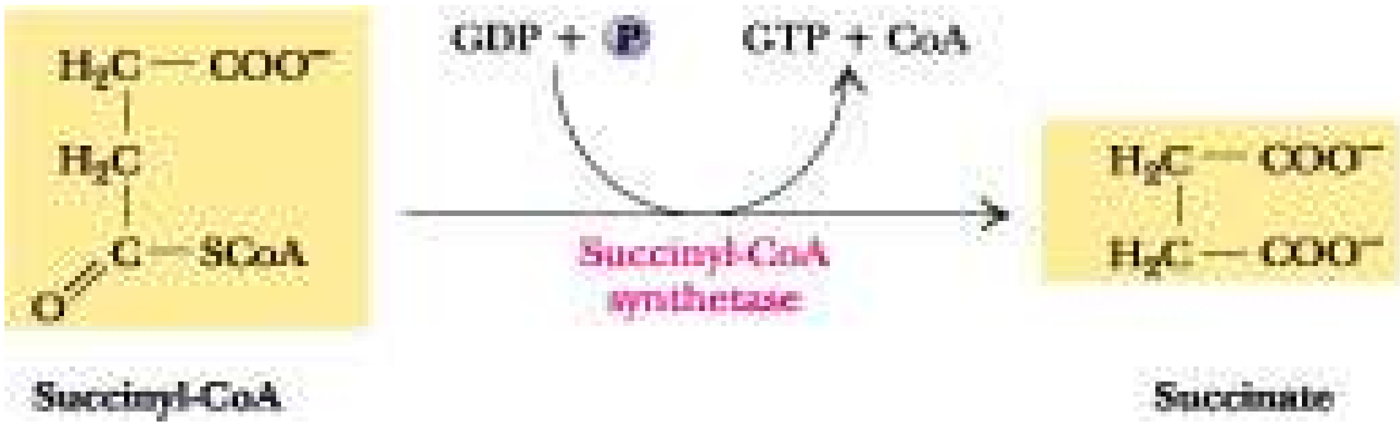


Reactions of the Citric Acid Cycle

Phase 2: Regeneration of Oxaloacetate (OAA)

Step 5: A substrate-Level Phosphorylation

- Succinyl-CoA is an energy-rich compound, & its potentially used to drive the formation of a nucleoside triphosphate from a diphosphate.
- Catalyzed by succinyl-CoA synthetase

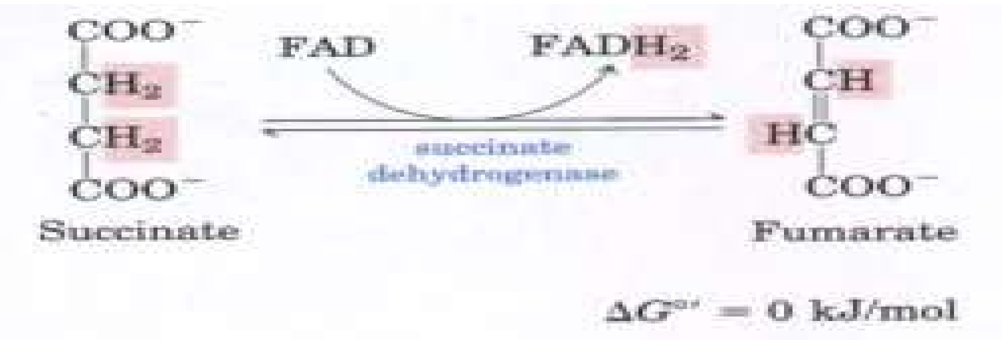


Reactions of the Citric Acid Cycle

Phase 2:

Step 6: A Flavin-Dependent Dehydrogenation

- **Succinate dehydrogenase** catalyzed the oxidation of succinate to form fumarate



- A C-C single bond is more difficult to oxidized than a C-O bond. Therefore, the redox coenzyme for succinate is not NAD^+ but the more powerful oxidant, FAD.

Reactions of the Citric Acid Cycle

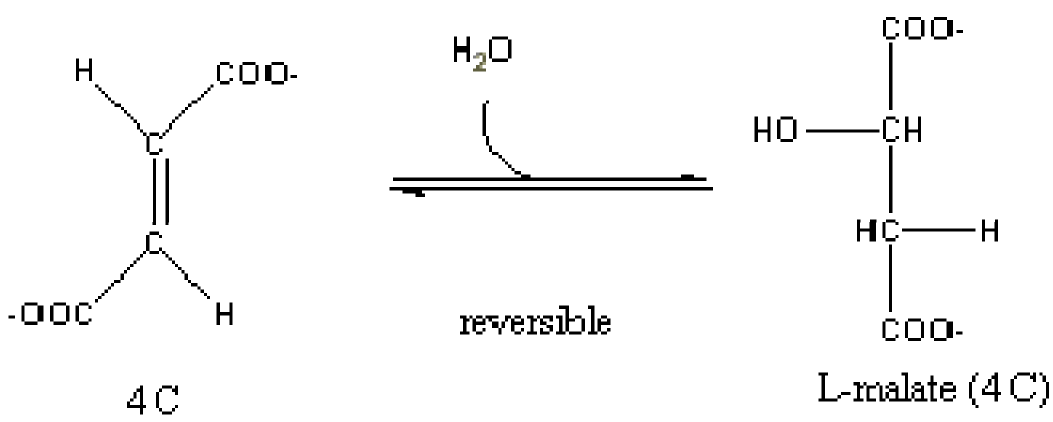
- FAD is bound covalently to the enzyme protein (E).
- The enzyme is tightly bound to the inner mitochondrial membrane.
- **Succinate dehydrogenase** is activated by high concentration of succinate and inhibited by oxaloacetate.

Reactions of the Citric Acid Cycle

Phase 2:

Step 7: Hydration of a C-C double bond

- Fumarate is converted to malate (L-malate)
- In reversible stereospesific hydration reaction catalyzed by **fumarase.**



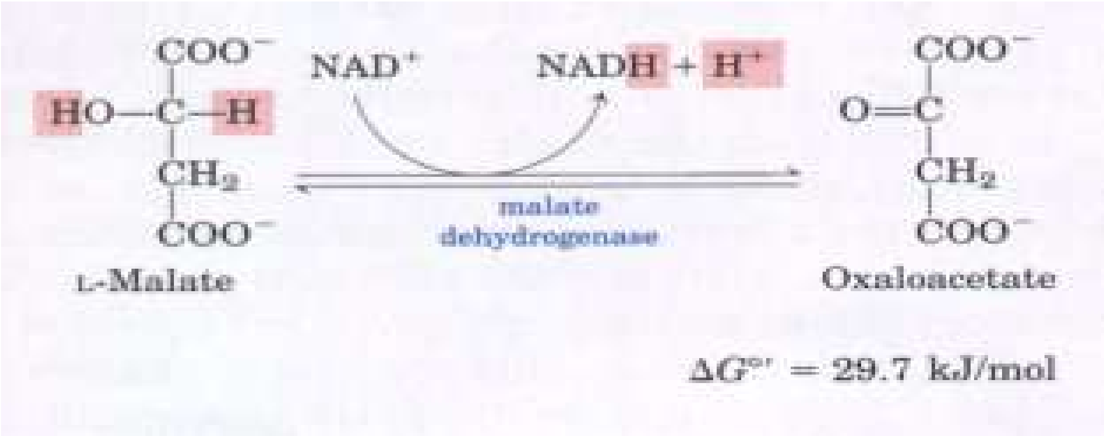
$\Delta G'^{0} = -3.8$ kJ/mol

Reactions of the Citric Acid Cycle

Phase 2:

Step 8: A dehydrogenation that regenerates OAA

- Oxaloacetate is regenerated with the oxidation of malate (L-malate).
- Malate dehydrogenase uses NAD+ as the oxidizing agent in a highly endergonic reaction.



The amphibolic Citric Acid Cycle

- **Amphibolic pathways** – can function in both anabolic and catabolic process.
- **CAC**:
  - obviously **catabolic**:

    : E.g.; acetyl groups are oxidized to form CO_2 & energy is conserved in reduced coenzyme molecule.

The amphibolic Citric Acid Cycle

- **CAC**:
  - also **anabolic**

    : Several CAC intermediates are precursors in biosynthetic pathways

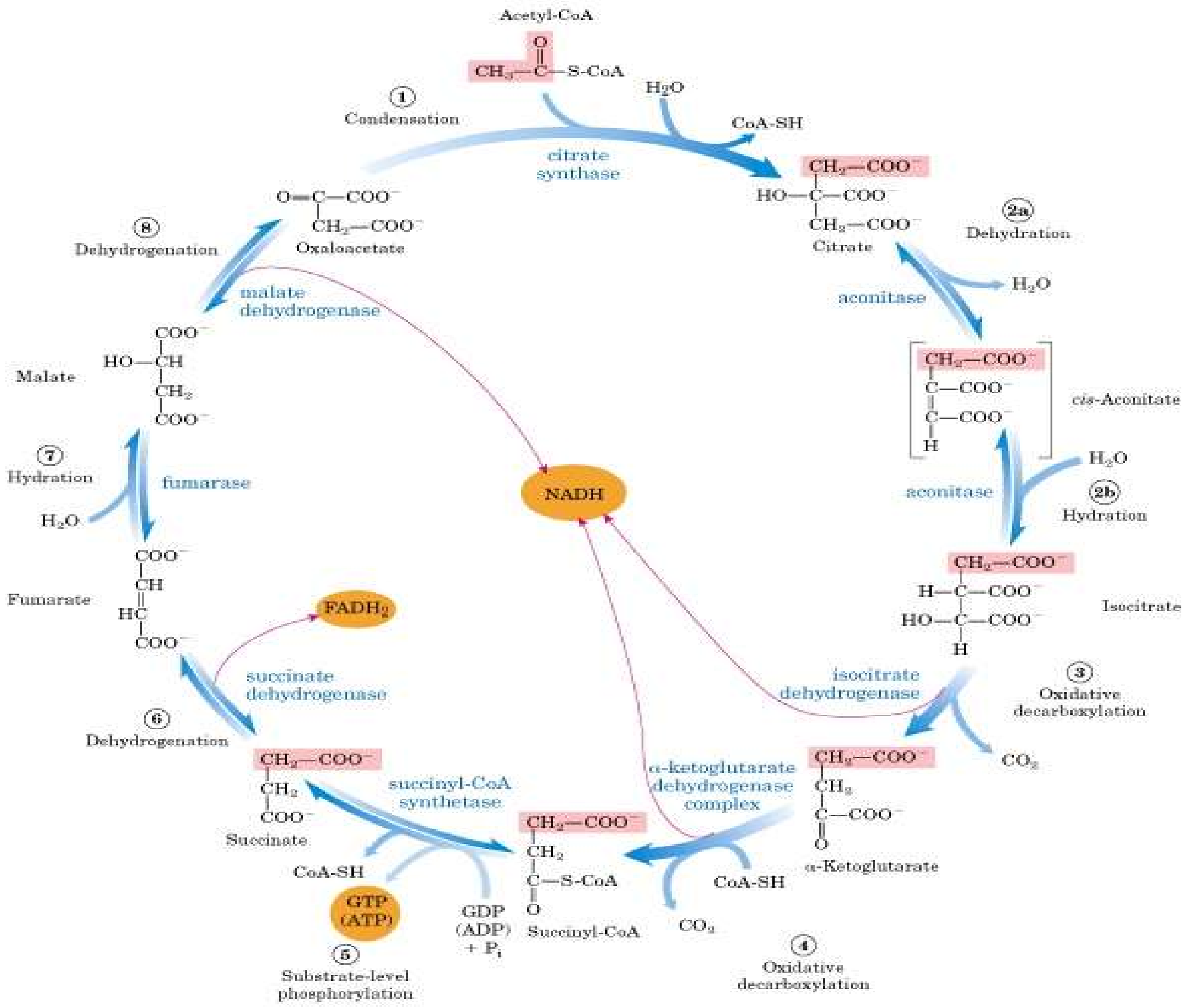
    : E.g.;

    i) **oxaloacetate** – used in both gluconeogenesis & amino acid synthesis.

    ii) **α-ketoglutarate** – plays important role in amino acid synthesis.

    iii) **Acetyl-CoA** – required in synthesis of fatty acid & cholesterol in cytoplasm.

Citric acid cycle: Advance Outline



The net reaction of CAC:

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + P_i + 2H_2O$$

$$\downarrow$$

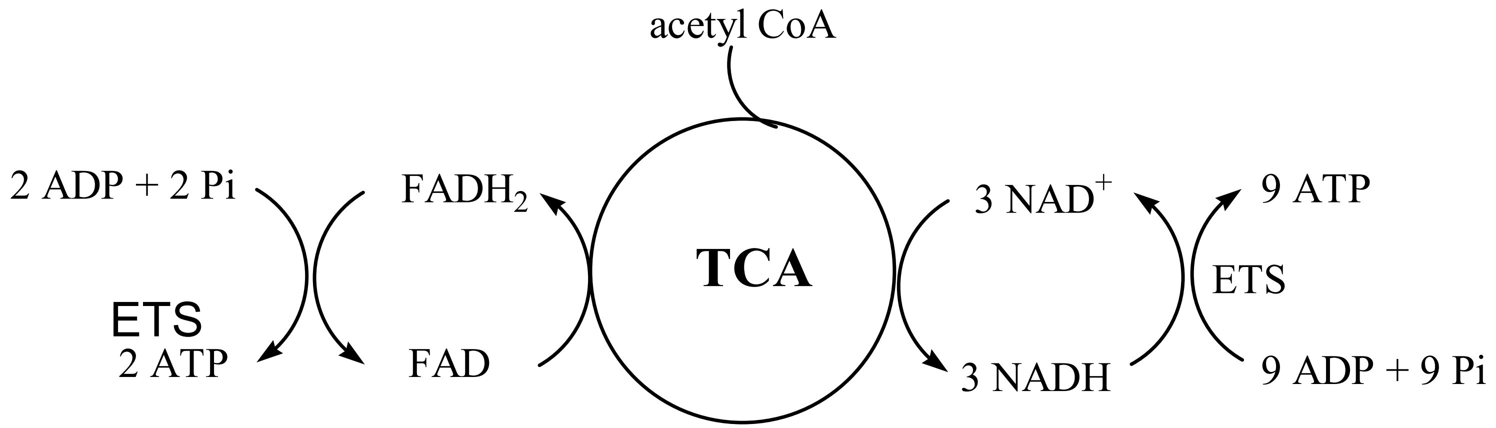
$$2CO_2 + 3NADH + FADH_2 + CoASH + GTP + 3H^+$$

Function:

- The common pathway leading to complete oxidation of **carbohydrates**, **fatty acids**, and **amino acids** to CO_2.
- Some ATP is produced, more NADH is made ,NADH goes on to **make more ATP** in electron transport and oxidative phosphorylation.
- A pathway providing many **precursors for biosynthesis**.

Energetics

- Energy is conserved in the reduced coenzymes NADH, $FADH_2$ and 1 GTP
- NADH, $FADH_2$ can be oxidized to produce ATP by oxidative phosphorylation



Regulation of the Citric Acid Cycle

- Regulation is achieved primarily by the modulation of key enzymes & availability of certain substrates.
- It is prominent role in energy production, & depends on a continuous supply of NAD^+, FAD & ADP.

Regulation of the Citric Acid Cycle

3 irreversible reactions are the key sites

- Citrate synthase
- Isocitrate dehydrogenase
- α -Ketoglutarate dehydrogenase

Student Activity

- Analyze glycolytic and krebs cycle. Explain these requisites i.e. starting material or precursor, reaction steps, location, connection with other pathways and end products.

References:

Title/URL	Author	Publisher	Year
Biochemistry (6th edition)	Campbell, M.K. and Farre	Thompson Brooks/C	
Biochemistry.2010	Garret, R.H., Grisham, C.	Thompson Brooks	2007
Biochemistry	Hames,D	USA: Taylor and Fran	-
Color Atlas of Biochemistry	Koolman, J., Roehm, K.H	Thieme Stuttgart	2005
Biochemistry demystified	Walker, S.	New York, USA; McGr	2008
Biochemistry, 7th Edition	Stryer	W.H Freeman and Co	2010
Biochemistry, 4th Edition	Donald Voet and Judith G	Wiley and Co	2011
Google with keyword of biochemistr	Various Online Biochemi	various	
Concepts in Biochemistry, 2nd ed	Boyer, R	Brooks/Cole/Thomsc	2002