The Citric Acid Cycle

Introduction

- The **citric acid cycle** is the **final common pathway** for the **oxidation** of **fuel molecules**.
- Most fuel molecules **enter** the **cycle** as **acetyl coenzyme A**.
- In **Eukaryotes**, the reactions of the citric acid cycle take place **inside mitochondria**. The only exception is **succinate dehydrogenase**, which is present in the **inner mitochondrial membrane**.
- The cycle is also an **important source** of **precursors** for the **building blocks** of many other molecules.
- **Fuel molecules** can be **oxidised** (**lose electrons**). The **role** of the **citric acid cycle** is to **oxidise** an **acetyl group** to **two molecules of CO_2**, thereby generating **high-energy electrons** in the form of 3 **NADH** and 1 **$FADH_2$** for each turn of the cycle. These **electrons** are then used to **power** the **synthesis** of **ATP**, but this is **not** the **role** of the **cycle**.

The Entry of Pyruvate into the cycle

Carbohydrates, most notably glucose, are processed by glycolysis into **pyruvate**. Under **aerobic conditions**, this is transported into the mitochondria by a **specific carrier protein** embedded in the **mitochondrial membrane** and is then **oxidatively decarboxylated** by the **pyruvate dehydrogenase complex** to form **Acetyl CoA**:

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetylCoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

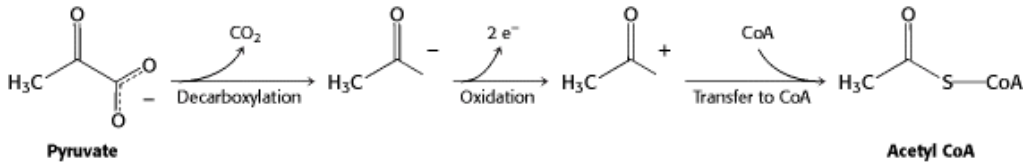
This reaction is **irreversible** and is the **link** between **glycolysis** and the **citric acid cycle**.

The **pyruvate dehydrogenase complex** contains **three enzymes**. It is a member of a family of homologous complexes that include the **α-ketoglutarate**

dehydrogenase complex. These are **giant complexes**, with molecule masses ranging from **4 million** to **10 million** Daltons. The three enzymes are:

- **Pyruvate dehydrogenase** – E_1
- **Dihydrolipoyl transacetylase** – E_2
- **Dihydrolipoyl dehydrogenase** – E_3

The actual reaction consists of three steps – **decarboxylation**, **oxidation** and **transfer** of the resultant acetyl group to CoA:



The actual steps are (these achieve the coupling of the release of CO_2 [lots of energy] to the subsequent reactions, which need the energy):

1. **Pyruvate** combines with a **coenzyme** – TPP [**thiamine pyrophosphate**] – and is then **decarboxylated** to give **hydroxyethyl-TPP**. **CO_2** is released.

   This is catalysed by **E_1**, and **TPP** is that enzyme's **prosthetic group**.
2. The **hydroxyethyl group** attached to TPP is **oxidised** to form an **acetyl group** and simultaneously **transferred** to **lipoamide** (a derivative of **lipoic acid** linked to the side chain of a **lysine residue <u>in the E_2 enzyme</u>** by an **amide linkage**). This results in an **energy rich thioester bonds** and gives **acetyllipoamide**.

   The **oxidant** is the **disulfide group** of **lipoamide** (–S–S–) which is **reduced** to its **disulfhydryl** form (ie: two –SH groups, one of which is used for the thioester bond).

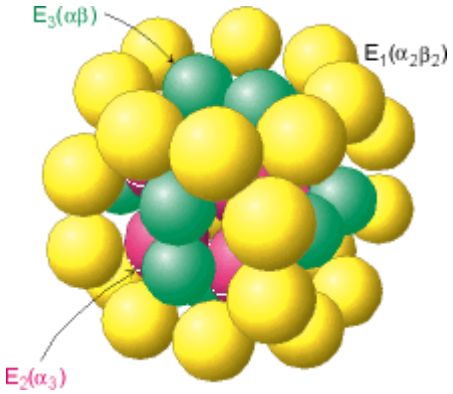
   This is also catalysed by **E_1**, and **lipoamide** is the **coenzyme**.
3. **Acetyl CoA** is formed. The **acetyl** group is transferred from **acetyllipoamide** to **CoA** to form **Acetyl CoA**.

   This reaction is catalysed by **E_2** – note that the energy-rich thioester bond has been preserved).

4. Finally, the **lipoamide** must be re-generated. This is achieved by **E_3**, in two steps:
    a. **Dihydrolipoamide** is **reduced** to **lipoamide** [two electrons are transferred to an FAD prosthetic group, to make $FADH_2$].
    b. The electrons are then transferred to **NAD^+**, to give **NADH** and one **proton**. This is rather unusual, since usually FAD *receives* electrons from NADH. In this case, however, the FAD's **electron transfer** potential is increased by its association with the enzyme.

    [Note that such enzymes tightly bound to **FAD** to **flavin mononucleotide** (**FMN**) are called **flavoproteins**].

The **structure** of the **pyruvate dehydrogenase** complex helps these reactions to take place.



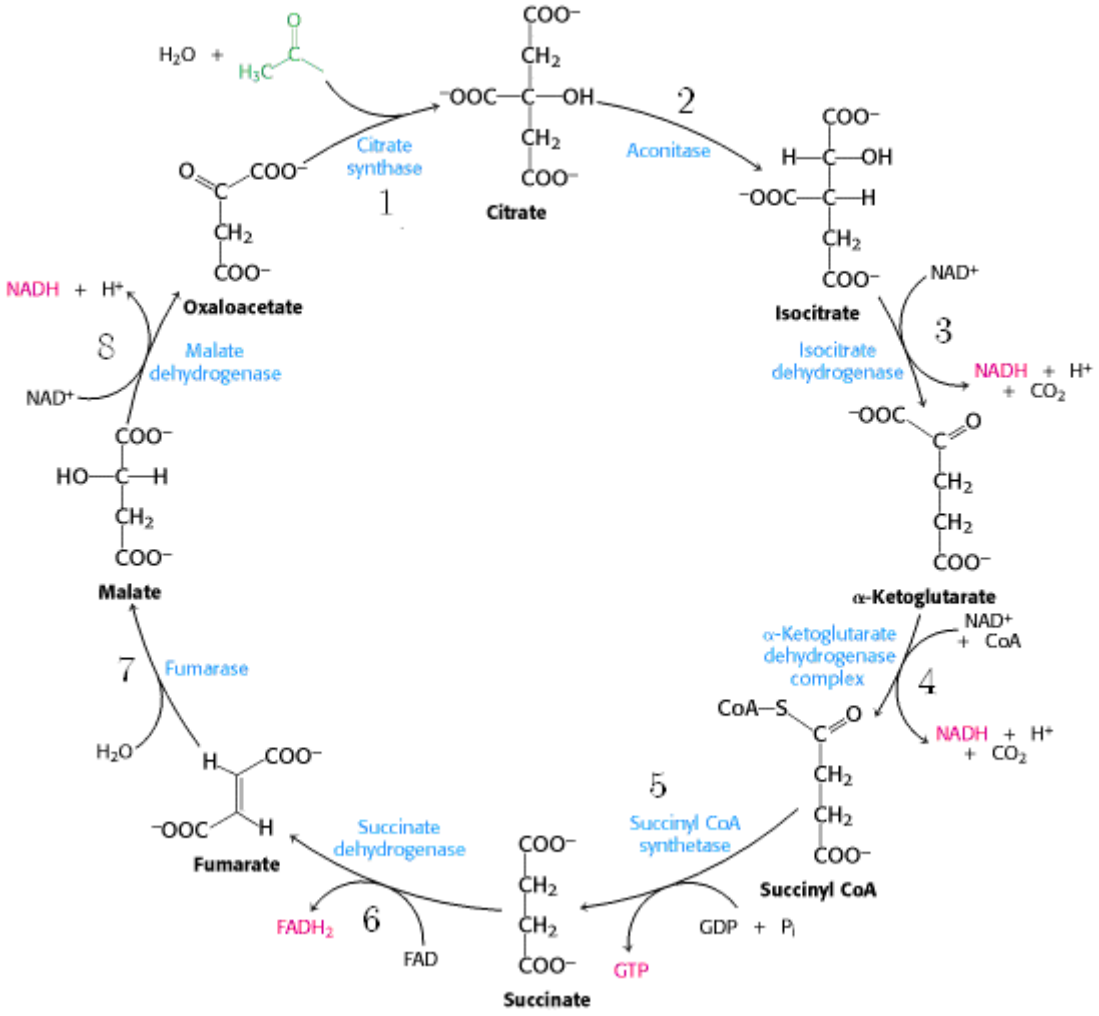
Notes:

- The **E_2** component consists of **eight trimers**, which form a **cube**. Each of the three subunits forming a trimer has three major domains:
    - At the **amino terminus**, a **flexible lipoamide cofactor** is bound.
    - Next, there is a small codomain that interacts with E_3.
    - Finally, there is a **larger transacetylase** domain, that completes **E_2**.
- What then happens is this:
    1) **Step (1)** occurs in the active site of **E_1**. This lies **deep in E_1**, and is connected to the **enzyme surface** by a 20Å long **hydrophobic channel**. **CO_2** leaves.
    2) **E_2** inserts its **lipamide arm** into the **deep channel**.
    3) **E_1 catalyses** the transfer of the **acetyl group** to the inserted **lipoamide**.
    4) **E_2** withdraws the **acetylated** arm, and enters it into the **E_2** cube.

5) The **Acetyl moiety** is transfer to **CoA**, and **Acetyl CoA** leaves the cube.
6) The arm then swings to the active site of the **E_3 flavoprotein**.
7) Step (4) happens – a new cycle can begin.

- This makes the **coordinated catalysis** of a **complex reaction** possible. The **proximity** of the enzymes **increase the overall rate** and **minimises side reactions**. All the **intermediates** remain **bound** to the complex at **all times**.

The Cycle Itself



The Citric Acid **oxidizes two-carbon units**. We look at each of the reactions in more detail:

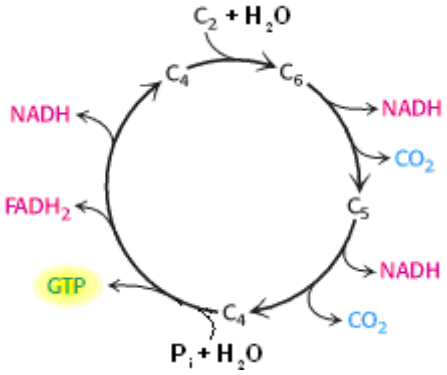
- Reaction 1
  - In essence, the **hydrolysis** of the **thioester bond** in **Acetyl CoA** powers the synthesis of a **new molecule** from **two precursors**.
  - **Citrate synthase** prevents side-reactions [especially the hydrolysis of the thioester bond by water] thanks to **induced fit** [like **hexokinase**] – when, and only when, the **oxaloacetate** binds to the enzyme, it undergoes a **conformational change** and **gains** the **ability** to bind to Acetyl CoA. Without the oxaloacetate, the enzyme has **no Acetyl CoA binding site**.
- Reaction 2
  - This is accomplished by a **dehydration** step followed by a **hydration** step, which basically interchanges an H and an OH group.
  - The intermediate is called ***cis*-aconitase**.
  - **Aconitase** is an *iron-sulfur protein*, or a *nonheme-iron protein*. Its four iron atoms are **complexed** to **four inorganic sulfides** and **three cysteine sulphur atoms**. This leaves **one iron available** to bind to **citrate** (though one of its COO^- groups) and an OH group.
  - **Aconitase** binds **asymmetrically** to **aconitase**, even though **citrate** is a **symmetrical molecule**, so the labelled precursors are not **randomized** at this stage.
- Reaction 4
  - This is carried out by the **α-ketoglutarate dehydrogenase complex**. The reactions of the two complexes are entirely analogous (**pyruvate** is also an **α-ketoacid**), and both include the **decarboxylation** of the acid and the subsequent **formation** of a **thioester linkage** with CoA.
- Reaction 5
  - The ΔG for the hydrolysis of succinyl CoA is about –33.5 kJ mol^{-1}, which is comparable to that of ATP. This is thanks to the **high energy thioester bond**.

- The hydrolysis of this bond is **coupled** to the **phosphorylation** of a **puerine nucleoside diphosphate** (either GDP or ADP – the *E. coli* enzyme can use one or the other).
- This mechanism is catalysed by **Succinyl Coenzyme A Synthetase** in several steps:
    1. An **orthophosphate** displaces the **Coenzyme A** to form another high energy compound – **succinyl phosphate**.
    2. A **histidine residue** then **detaches** this phosphate group [at the free nitrogen atom, in fact], swings over to the **nucleoside diphosphate** to **phosphorylate** it.

- Reaction 6, 7 & 8
    - These **regenerate oxaloacetate** by **oxidating sucinate**. A **methylene group** (CH_2) is converted to a **carbonyl group** (C=O) in **three** steps: an **oxidation**, a **hydration** and a second **oxidation**.
    - More energy is extracted during these steps.
- Reaction 6
    - In two of these reactions, the electron acceptor is NAD^+, but in the first reaction, it's FAD, since because the free energy change is **not enough** to reduce NAD^+. [FAD is nearly always the electron acceptor in reactions that remove two hydrogen *atoms* from the substrate].
    - **Succinate dehydrogenase** is **embedded into the mitochondrial membrane** – in fact, it is **directly associated with the electron transport chain**. $FADH_2$ produced does **not** dissociate from the enzyme. Rather, two electrons are directly transferred from $FADH_2$ to the iron-sulphur clusters in the enzyme, whence they pass to **Coenzyme Q (CoQ)**, an important component of the electron transport chain.
- Reaction 7
    - **Fumarase** is **sterospecific** – it will only add OH^- to one side of fumarate. Hence, only **L-Malate** is formed.
- Reaction 8
    - Unlike others in the cycle, this reaction has a **significantly positive** ΔG (+29.7 kJ mol^{-1}). The reaction thus has to be driven by the

consumption of the products (NADH by the electron transport chain and oxaloacetate by the cycle).

So, basically:



Notes:

- Remember, also, that an **NADH** molecule is also produced by the **conversion** of **pyruvate** to **Acetyl CoA**.
- Two carbons enter, two carbons leave. This has *huge* repercussions:
  - Any **removal** of material from the cycle to form other molecules **depletes** the cycle. The cycle can then no longer operate at optimal rates (because **Acetyl CoA** can only enter the cycle by **condensation** with **oxaloacetate**). There therefore needs to be an **anaplerotic** (building up) **pathway** to "re-fill" the cycle. Mamals lack enzymes that can convert **acetyl CoA** to any citric acid cycle intermediate. Rather, **oxaloacetate** is formed by the **carboxylation of pyruvate** catalysed by **pyruvate carboxylase**, which only operates in the presence of **Acetyl CoA** (a build-up of which signifies the need for more oxaloacetate!).[1]
  - **Mamals** cannot **synthesise** glucose from fat. This is because mammals can obtain a **two carbon unit** from fat, which they can use to synthesise energy, but not the replenish the cycle. In the long term, this is what leads to **death** by **starvation** [since the brain needs some **glucose** to work (see later)].

[1] Note that some organisms *can* convert Acetyl CoA to a four-carbon sugar, though the **glycoxylate pathway**. In plants, these reactions take place in organelles called **glycoxysomes**.

- **Studies** using **isotope-labelling** have revealed that the two carbon atoms that **enter** the cycle are not the ones that **leave**. They are **incorporated** into the **four-carbon** unit and will leave in **another cycle**. Note that **succinate** is a **symmetric molecule** – the **identity** of the original carbons is **lost**.
- Evidence is accumulating that the enzymes of the cycle are **physically associated with each other**. This enhances the efficiency of the cycle because products can pass direction from one active site to the next, though connecting channels (**substrate channelling**).
- Each molecule of NADH eventually leads to 2.5 molecules of ATP, and each molecule of $FADH_2$ leads to 1.5 molecule of ATP.
- Oxygen does not participate directly in the cycle. However, it only occurs under **aerobic conditions**, because FAD and NAD^+ can only be **regenerated** in the mitochondrion by the transfer electrons to oxygen.

Measuring the rate of the citric acid cycle

There are several ways we can **monitor** the **rate** of the **citric acid cycle**:

- Make use of the **coupling** between the **citric acid cycle** and **oxygen consumption** by measuring the concentration of oxygen in one of two ways:
    - using an **oxygen electrode**.
    - in vivo, using **fMRI** scans (**functional magnetic resonance imaging**). **Paramagnetic substances** modify the signal, and **deoxyheamoglobin** is **paramagnetic**, where **haemoglobin** is **diamagnetic**.
- Use ^{13}C and ^{14}C labelling to "chase the label" round the cycle.