**Krebs', citric acid, or TCA cycle**

* Why is it called Krebs' cycle?
* Because the scientist who discovered it is called Hans Krebs
* Why is it called citric acid cycle?
* Because its first product (intermediate) is citric acid or citrate
* Why is it called tricarboxylic acid cycle?
* Because citric acid contains 3 carboxylic groups
* **Note**: it contains 6 carbons, 3 is the number of carboxylic groups
* One of the reasons we need citric acid cycle is to extract electrons and carry them (upload them) on molecules that can carry electrons (electron carrying molecules),
* What are the main electron carrying molecules?
* **NAD+**
* **FAD**
* Location and where is it in the picture?
* After the degradation of fats, proteins, and carbohydrates, they go back to their monomers, then they enter the mitochondria
* Their pathways of degradation give us what is called acetyl coA,
* Acetyl coA enters the Krebs' cycle,
* Krebs' cycle gives us NADH and FADH2 which enter the electron transport chain which is located on the inner mitochondrial membrane
* **Important notes:**
* All steps of energy production procedures happen in the matrix, except for **glycolysis** which happen in the **cytosol**
* All steps of Krebs' cycle happen in the matrix, except for the catalyzation by succinate dehydrogenase (we will see where that happens later)
* What is the difference between NAD+ and FAD?
* They both can carry electrons
* NAD+ can take 2 electrons at a time
* FAD can take 1 electron at a time
* They both can carry 2 electrons eventually
* Because FAD can take 1 electron at a time 🡪 two different sources, so it can go to the free radical state, therefore it is scary, and must be contained all the time within proteins
* NAD+ can take 2 electrons at a time, it doesn’t go through the free radical state, therefore the latisse monument solutions do not essentially need to be within proteins
* **Note:** in Krebs' cycle, you must memorize 8 compounds, 8 enzymes, and the substrate and product for each enzyme
* **Steps of the Citric Acid Cycle:** (refer to the image in slide #7)

1. Acetyl coA, which is formed of 2 carbon units, enter the cycle. The coA then gets out, and the 2 carbon units remain. We introduced 2 carbon units to the cycle, the first intermediate must be formed of 6 carbon units (citrate), so those 2 carbon units must interact with a 4-carbon unit substance.

* **Note:** in the citric acid cycle there will be production of CO2 without the introduction of O2

Acetyl coA unites with a 4-carbon unit substance called Oxaloacetate,

* **Note:** when we need energy, we need to oxidize material, we need to break them down, reduction is the opposite of oxidation, reduction is concerned with building up material

Citrate is now formed.

* The enzyme responsible for synthesizing citrate is **Citrate Synthase**

1. We cannot oxidize citrate, because it is a tertiary alcohol, therefore, we must reformulate citrate to a form that can be oxidized, that can be done by transporting the (OH) from carbon number 3, to carbon number 2, by that we will have a secondary alcohol that can be transformed into a ketone.

* All enzymes in the citric acid cycle refer to their function, except for the **aconitase** which transforms citrate into **isocitrate**
* It is called aconitase because while the citrate is being transformed into isocitrate, it enters and intermediate phase called **Aconitate**
* **Notes**:
* As we can see, we started the process with 2 carbon units, they united with 4 carbon units.
* through the process CO2 is produced (at two steps)
* In the same cycle, the carbon units which are introduced to acetyl coA, are not the same ones which are exiting as CO2

1. As mentioned earlier, isocitrate can be oxidized,

* Inside the enzyme, isocitrate will undergo many process in order to be converted into a ketone
* The first process is decarboxylation (the removal of a carboxylic group), this is how the first CO2 is produced
* When the CO2 is removed, the 6-carbon molecule is transformed into a 5-carbon molecule
* The ketone produced after the oxidation of the Isocitrate is called **alpha- ketoglutarate**
* The isocitrate is transformed into a ketone (alpha- ketoglutarate) by the enzyme **isocitrate dehydrogenase**
* The process of transforming isocitrate into alpha ketoglutarate is an oxidation process, the alcohol is turned into a ketone, 2 H+ are removed, the enzyme must be a dehydrogenase, so because the substrate is isocitrate, the enzyme is called isocitrate dehydrogenase

1. Let's go to the next enzyme, it removes one carbon unit and catalyze the oxidation of the acidic state, so it is called **alpha ketoglutarate dehydrogenase**

* Because the previous two process produce electrons (H+) the must be carried by electron carrying molecules,
* Isocitrate dehydrogenase produce 2 electrons, they are carried by **NAD+**, this is the first NADH produced
* Alpha ketoglutarate dehydrogenase does the same thing, another NADH molecule is produced (the sum is 2 NADH molecules produced until now)
* In the previous 2 steps of citric acid cycle, 2 CO2 molecules, and 2 NADH molecules are produced

1. We were in a 5- carbon unit state, alpha ketoglutarate dehydrogenase turned the 5-carbon molecule into a 4-carbon molecule (by the removal of CO2), there is now an activated carbon that will abstract co-enzyme A

* A 4-carbon unit is called succinate, since it is attached to coenzyme A it will be called succinyl coA
* We are in the 4-carbon unit state, here we are at the half of the cycle, since it is a cycle, the aim here is to go back to the first substance that entered the cycle which is (oxaloacetate)
* In the first half of the cycle, 2 carbon units were added to 4 carbon units, so we had a 6 carbon unit substance, then we consumed 2 carbon molecules to go back to a 4-carbon unit state
* In the second half of the cycle, this 4 carbon unit (succinyl coA) will undergo changes in order to go back to the oxaloacetate form
* coA will detach from the 4 carbon molecules, producing energy, this energy is used for building up
* the excess energy produced by the separation of coA from the 4 carbon unit is consumed in building GTP by adding a phosphate group to the GDP
* **note**: in order to build energy molecules within the human body, which are ATP and its equivalents GTP, UTP, and CTP, you have 2 sources:

1. **by the use of oxygen**: **oxidative phosphorylation**
2. **without the use of oxygen**: **substrate level phosphorylation**, you are directly phosphorylating the substrate

* There are three main reactions which include substrate level phosphorelation: 2 in the carbohydrate metabolism, 1 in the krebs cycle
* The enzyme that transforms succinyl coA into succinate is called **succinate thiokinase**

1. **Succinate dehydrogenase** removes 2 hydrogens from succinate to transform it into fumarate, this is an oxidation process, the substrate is succinate, the product is fumarate

* **Succinate dehydrogenase is present in the inner mitochondrial membrane**
* The oxidation of succinate into fumarate is **the only step in the citric acid cycle that doesn’t occur in the matrix, it occurs in the inner mitochondrial membrane**, this step is step number 6
* Succinate is part of the citric acid cycle that takes place in the matrix, and it is also part of complexes present in the inner mitochondrial membrane, therefore it is the only direct link between Krebs' cycle and oxidative phosphorylation

1. We now have fumarate, the enzyme **fumarase** adds water to the double bond of fumarate to transform it into **malate**, and H will be added to one of the carbons of the double bond, OH will be added on the other, giving us a secondary alcohol
2. Secondary alcohols can be oxidized into ketones, so malate will be oxidized back to the first molecule that entered the cycle, the oxaloacetate, by the enzyme **malate dehydrogenase** (malate is a substrate in the this reaction)

* The 2 produced H+s will be carried on NAD+
* **Question on Krebs' cycle**:
* How many molecules of NADH are produced in citric acid cycle?
* 3
* How many molecules of FADH2 are produced in citric acid cycle?
* 1
* How many GTPs are produced in the cycle?
* 1, Some books say that ATP is a product of the krebs' cycle, this is because GTP can be easily transformed into ATP
* Is the CO2 produced in the krebs' cycle the same as the CO2 that we exhale?
* Yes, eventually
* **Questions on Glycolysis:**
* What is the end result of glycolysis?
* Pyruvate
* Why do we need pyruvate?
* To break it down into acetyl coA
* How many pyruvate does 1 glucose molecule give?
* 2
* How many acetyl coA does 1 pyruvate molecule give?
* 1
* Glycolysis 🡪 pyruvate 🡪 acetyl coA 🡪 enter krebs cycle
* When do we need glycolysis?
* When we don’t have energy
* The rate limiting step and the committed step in the glycolytic pathway is the transformation of fructose 6- phosphate into fructose 1,6- phosphate, this is catalyzed by the enzyme **phosphofructokinase**
* If you have excess ATP, excess citrate, do you need the glycolytic process to proceed?
* No, excess ATP and citrate inhibit the glycolytic process
* **The 3 reactions that represent substrate level phosphorylation are**:

1. phosphoglycerate kinase,
2. pyruvate kinase (catalyze the last step of glycolysis)
3. and succinyl coA synthase

* We have 3 enzymes all with almost similar structures, which are **pyruvate dehydrogenase** (which transforms pyruvate into acetyl coA), **alpha ketoglutarate dehydrogenase**, and **branched chain alpha keto acid dehydrogenase** (in protein metabolism), they also behave similarly
* **Mechanism of alpha ketoglutarate dehydrogenase:**
* catalyzes the transformation of alpha ketoglutarate into succinyl coA, 5-carbon molecule into 4-carbon molecule,
* One of the ways to regulate enzymes is enzyme complexing
* Pyruvate dehydrogenase, alpha ketoglutarate dehydrogenase, and branched chain alpha keto acid dehydrogenase are complex enzymes, each one is formed of 3 smaller enzymes attached together ( E1, E2, E3 )
* We said that the previous enzymes convert a 5-carbon unit molecule, into a 4-carbon unit molecule, so what is the function of ( E1, E2, E3 ) ?
* For example, in alpha ketoglutarate, **E1 is a decarboxylase**, it removes the last carbon unit represented by a carboxylic group so the carbon unit is separated from the molecule and becomes CO2, transforming the 5-carbon unit into a 4-carbon unit. When that happens, there is now an active carbon in the molecule, it must be captured by something
* E1 carries the new 4-carbon molecule on a co-enzyme that is present in it, which is the **thiamine** (vitamin B1), that is why thiamine must be present in sufficient quantities in our bodies, it is a co-enzyme for alpha ketoglutarate dehydrogenase in the sub-complex E1
* Thiamine is united with the 4-carbon unit molecule, E1 function is now over, but it has to go back to its original form, it must get rid of those 4 carbon units, so it gives them to the **sub-complex E2**
* **E2 is a transacylase**, it contains lipoic acid, the structure of lipoic acid includes **disulfide bridge**, E2 attaches the acyl group to one of the sulfurs, by this E1 goes back to its original structure,
* When the acyl group is attached to one of the sulfurs, the disulfide bridge breaks, the next sulfur is active, it will abstract hydrogen from the solution, becoming an SH
* E2 must go back to its original form as any other enzyme, a coA from the solution takes the acyl group, this gives us an acyl coA, which is the succinyl coA
* When the coA took the acyl group from the sulfur, an H+ attaches with the sulfur in that place, we now have 2 SH groups which are the reduced form of the sulfur
* E2 must go back to its original form, the H+s must be removed, we need a dehydrogenase, **E3 is a dehydrogenase**
* E3 removes 2 hydrogens, carries them on **FAD**, getting E2 to its original form, the disulfide bridge is formed again,
* We now have **FADH2**, it must go back to FAD, it gives its electrons to **NAD+,** which then becomes **NADH** that exits the solutions
* The enzyme now returned fully to its original form
* This mechanism of action applies to all 3 similar enzymes mentioned earlier
* How many co-enzymes did we use in the previous process? And what are they?
* 5 co-enzymes: Thiamine, lipoic acid, FADH2, NADH, and the co-enzyme A
* Sometimes, there may be a genetic deficiency in E1, so in this case it will not function, in this case high amounts of pyruvate or alpha ketoglutarate will be found in blood,
* Thiamine deficiency will also cause high amounts of pyruvtae or alpha ketoglutarate in blood because E1 will not function
* There is another way to regulate pyruvate dehydrogenase enzyme, we talked about enzyme complexing, another way is **phosphorylation:**
* When we phophorylate pyruvate dehydrogenase it becomes inactive
* We need the pyruvate dehydrogenase **active** when we need energy, we need **inactive** when we don’t
* So we **dephosphorylate** it when we **need energy**, **phosphorylate** it when we **don’t need energy**
* The enzyme that performs phosphorylation is **kinase**, the enzyme that removes phosphate is **phosphatase**
* We use **kinase** when we **don’t need energy**, that is when we have excess pyruvate or excess acetyl coA or excess NADH, because NADH eventually gives us ATP
* **Arsenic** is a very poisonous substance, it attaches to the lipoic acid when it is in the SH form, and prevents it from going back to its original form
* Citric acid cycle is one of the best machines in the world, its efficiency is 90%,
* **Regulation of the citric acid cycle:**
* It is logical, always control the **first step of the pathway**, control the **rate limiting step** in any pathway, control the **steps which give products** because products may cause feedback inhibition,
* **The regulation of the citric acid cycle is played by 2 main players:**

1. **The ratio of ADP/ATP:** higher ADP = higher regulation, higher ATP = higher inhibition
2. **The NAD+/NADH ratio:** higher NAD+ = activation, higher NADH = inhibition

* FADH2 doesn’t regulate the citric acid cycle, it regulates specific enzymes (regulates the enzyme itself)
* **NADH** regulates the enzymes that produce it, which are **isocitrate dehydrogenase**, **alpha ketoglutarate dehydrogenase**, and **malate dehydrogenase**
* The only enzyme which regulates allosterically the citric acid cycle by ADP is **isocitrate dehydrogenase**, it is **the rate limiting step** in the citric acid cycle, activated by ADP, inhibited by NADH
* The regulation of **citrate synthase**, which is a simple enzyme, is by the **feedback inhibition**

**Best wishes, :)**

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