Medical Committee
The University of Jordan

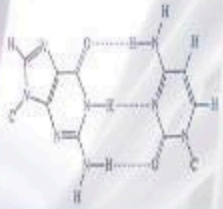
SLIDE SHEET

LECTURE#: 10

DR.NAME: Nafeth Abu Tarboush

DONE BY: Layan Attili

Biochemistry



Majida Al-Foqaraa'

The citric acid cycle (2)

We have already talked about the citric acid cycle (Krebs cycle or tricarboxylic acid cycle) and here is an overview :

It's an eight-step cycle that takes place in the mitochondrial matrix, each step is catalyzed by a different enzyme.

Pyruvate is first converted into acetyl CoA, this being the first step which is the junction between the process of glycolysis and the citric acid cycle, after that the resulting molecule will enter the cycle for further oxidation through the following reactions catalyzed by certain enzymes :

1 – Formation of citrate catalyzed by Citrate Synthase

2- Isomeration catalyzed by Aconitase

3- **Isocitrate** loses a CO_2 molecule (decarboxylation) and the resulting compound is oxidized reducing NAD+ to NADH (oxidation) this is catalyzed by Isocitrate Dehydorgenase

4- One more CO_2 molecule is lost and another NADH is formed (oxidative decarboxylation), this step is catalyzed by α-Ketoglutarate Dehydrogenase

5- Substrate level phsophorylation to form one GTP molecule (or ATP) catalyzed by **Succinate thiokinase (know also as Succinyl-CoA synthetase)**

6- Oxidation of succinate and transfer of two hydrogens to FAD to form $FADH_2$ catalyzed by Succinate Dehydrogenase

7-Hydration of Fumarate, catalyzed by Fumarase

8- Oxidation of Malate to regenerate oxaloacetate and form NADH, catalyzed by Malate Dehydrogenase

Written by Layan Attili

»3 NADH molecules, 1 $FADH_2$, 1 ATP and 2 CO_2 molecules are produced for each turn

Note : One glucose molecule will give two Pyruvate molecules, each goes into krebs cycle so if we want to know the number of NADH, $FADH_2$, ATP and CO_2 molecules formed we multiply the numbers we got by 2 .

Inhibition of Glycolysis by Citrate

We are all familiar with the process of glycolysis which occurs in the cytosol .

The rate -limiting step of the gylcolytic process is the conversion of Fructose -6-phosphate to Fructose1,6-bisphosphate and this reaction is catalyzed by **Phosphofructokinase** by adding a phosphate group to Fructose -6- phosphate.

Citrate (the first product of the citric acid cycle, a process that takes place in the mitochondria), inhibits the process of Glycolysis by inhibiting the rate-limiting enzyme of this process (Phosphofructokinase) , since Citrate (found in the mitochondria) is the inhibitor of glycolysis which takes place in the cytosol there must be a way for citrate to be translocated from the mitochondria to the cytosol so that it can exert its effect on the enzyme.

This means that the use of Citrate isn't limited to the citric acid cycle as it:

1- Controls the Glycolytic process.
2- Can be broken down to acetyl CoA (in the cytosol) to participate in the synthesis of fatty acids and cholesterol.

How does Citrate inhibit the process of glycolysis ?

As we already know, the final product of glycolysis is Pyruvate, which is converted to Acetyl CoA in a reaction catalyzed by the enzyme Pyruvate Dehydrogenase. Acetyl CoA which in turn gets into the citric acid cycle to form citrate.

When the concentration of Citrate increases, citrate will leave the mitochondria to the cytosol and inhibit the process of glycolysis and thus the formation of Pyruvate the material which gives us Acetyl CoA.

---

*Succinate Thiokinase : is the enzyme which results in the formation of GTP molecule in the citric acid cycle. There are several names for this enzyme, but we're only required to know this name because it's the most informative of the enzyme's function.

**Coenzyme A (you have to be familiar with its structure) :

1-Adenosine

2-Pantothenic Acid (vitamin B5)

3-Cysteine

The thiol group in Cysteine forms a bond with carbon, this bond releases energy when it's broken down. The amount of energy released upon the breaking of S-C bond is comparable to the ATP being hydrolyzed. (has a close value to that resulting from the hydrolysis of ATP.)

α- Ketoacid Dehydrogenase complexes :

One of the ways to control the enzymatic function is Multi-enzyme complexing.

What exactly is multi-enzyme complexing?

If the product of an enzyme is only used as a substrate for another enzyme, and that's why it is only logical to have these two enzymes (or more) together, this way there won't be much energy spent to move

the material between different enzymes and that's exactly what happens in multi-enzyme complexes.

The α-Ketoacid dehydrogenase complexes are composed of three enzyme complexes which adopt the same mechanism and similar structure (not identical), these complexes are :

1- **α-ketoglutarate dehydrogenase** complex –converts α-ketoglutarate to succinyl CoA
2- **Pyruvate dehydrogenase** complex – converts pyruvate to acetyl CoA
3- **Branched chain α- keto acid dehydrogenase** complex –this enzyme is involved in the metabolism of branched-chain containing amino acids, also it gives intermediates that are connected to CoA

The products of these three enzymes are always attached to Coenzyme A , like acetyl CoA , succinyl CoA.

α-ketoglutarate dehydorgenase complex is composed of three enzymes E1, E2 and E3. This complex catalyzes an oxidative decarboxylation reaction as it decarboxylates the substrate and oxidizes it through a dehydrogenation reaction, that's why the complex should have three enzymes one to decarboxylate the substrate, another to dehydrogenate it and the third enzyme to attach the carbons to Coenzyme A.

The first enzyme (E1) is a decarboxylase, it takes out a carbon unit in the form of CO_2 , the rest of the carbons, which are activated carbons, will be loaded on the enzyme.

Each of the three enzymes is attached to a coenzyme, the first enzyme (E1) is attached to Thiamine (vitamin B1) ,whose active form is Thiamine pyrophosphate (thiamine attached to two phosphate groups). after the decarboxylation process (taking out of CO_2) the carbon becomes reactive, this reactive carbon will be loaded on the coenzyme (thiamine), at this point the enzyme will still be in a form different from

the original one it took before the reaction, and because any enzyme must go back to its original state after the reaction is finished, it has to transfer an acyl group (3 or more carbon units).

The acyl group will be transferred from the first enzyme to the second and with that the first enzyme will go back to its original form. The second enzyme is close to the first one and is called Transacylase (transfers the acyl group), Transacylase is attached to a coenzyme called Lipoic acid (Lipoate).

**The structure of Lipoate is characterized by two sulfur atoms connected through a disulfide bond.

When the reactive carbon is transferred from the first enzyme it will attach to one of the Sulfur atoms of the Lipoic acid, while the other sulfur (which becomes reactive after breaking the disulfide bond) will extract a proton from the solution forming a thiol group.

The acyl group will detach, leaving the sulfur reactive, this reactive sulfur will extract a second proton from the solution and that will result in two thiol groups.

The carbon units are also reactive after the detachment that's why these carbon units will attach to CoA and the product of the reaction will be produced at this stage (the product of the enzyme α-ketoglutarate dehydrogenase is succinyl CoA and it is produced at enzyme number 2)

** if it is Pyruvate dehydrogenase complex, the product will be acetyl CoA and it will be produced at the second enzyme.

The product (acyl – CoA) has now already left the complex , but the lipoic acid still has two thiol groups attached, and this isn't the original form of the enzyme (which has two sulfur atoms connected through a disulfide bond) , so there should be a third enzyme that will take away the two hydrogens of the two thiol groups so that the disulfide bond will be reformed and the second enzyme will go back to its original form.

The enzyme which takes out hydrogens is named a Dehydrogenase, this dehydrogenase enzyme will take out two hydrogens, load them on the Coenzyme (attached to enzyme number three) FAD to give $FADH_2$ and by this the second enzyme has already gone back to its original form.

Now $FADH_2$ isn't the original form of the third enzyme, to restore the original form of the enzyme $FADH_2$ must donate its electrons to NAD+ which gets converted to NADH at the end of the reaction.

** If you look at the cycle, you can see that the first CO_2 molecule and NADH are produced when Isocitrate is converted to α-ketoglutarate .

The second CO_2 and NADH are a result of the conversion of α-ketoglutarate to succinyl CoA , so the product of this conversion is NADH (this point was only brought up to show that the final product of the action of α-ketoglutarate dehydrogenase enzyme is NADH not $FADH_2$)

There are five coenzymes :

1-Thiamine

2-Lipoic acid

3-Coenzyme A

4-FAD

5-NAD+ (which extracts the electrons from enzyme number three)

Thiamine at <u>enzyme number 1</u> , Lipoic acid and CoA at <u>enzyme number 2</u>

FAD and NAD+ at <u>enzyme number 3</u> .

Written by Layan Attili

α- ketoglutarate dehydrogenase complex – a summary :

- Catalyzes an oxidative decarboxylation reaction to convert α-ketoglutarate to succinyl CoA
- Is composed of three enzymes E1, E2 and E3
  - E1 is a decarboxylase
  - E2 is a tranacylase
  - E3 is a dehydrogenase

- Each of the three enzymes is attached to one or more coenzymes
  - E1's coenzyme is Thiamine
  - E2's coenzymes are Lipoic acid and CoA
  - E3's coenzymes are FAD and NAD+
- Each of the three enzymes will give one product
  - E1 will give CO_2
  - E2 will give succinyl CoA
  - E3 will give NADH

  Together, these three products constitute the products of α-ketoglutarate dehydrogenase enzyme.

Thiamine Pyrophosphate

Is a coenzyme (vitamin B1) found in both α-ketoglutarate dehydrogenase complex and pyruvate dehydrogenase complex (as we have already mentioned , all three complexes adopt the same mechanism).

In cases of thiamine (vitamin B1) deficiency, the coenzyme won't be there and the complex won't be functional and this would result in the accumulation of the substrates in tissues and they will eventually exist to the blood.

A person who suffers from thiamine deficiency will have high concentrations of pyruvate and α-ketoglutarate in the blood.

Bioenergetics of the citric acid cycle :

(note : there is a mistake in the slide it's 1 ATP instead of 10)

we've already mentioned that the citric acid cycle has an efficiency of 90% .

what keeps the cycle running in one direction?

Most of the reactions of the citric acid cycle are reversible, so what makes these reactions go in one direction and not the other? The cycle has three large negative ΔG reactions, the excess energy makes these reactions go in the forward direction and essentially, physiologically irreversible, however these reactions are reversible (in nature).

Physiologically these reactions only occur in one direction which is the forward direction to generate oxaloacetate at the end of the pathway

Are there any reactions with a positive ΔG in the cycle ?

Yes , in fact there are two of them

1-the conversion of citrate to Isocitrate by the enzyme Aconitase

2-the conversion of Malate to Oxaloacetate by Malate dehydrogenase

Both of which have physiological implications as citrate isn't only used in the citric acid cycle but it (citrate) also:
1) Inhibits the glycolytic process (after it exits the mitochondria to the cytosol) and 2- to be broken down again to acetyl CoA to participate in the processes of fatty acid and cholesterol synthesis .

If the concentration of citrate increases it will exit the mitochondria to participate in other processes in the cytosol.

How does the concentration of citrate increase ?

At equilibrium, the enzyme Aconitase (the enzyme which converts citrate to isocitrate) favors citrate.

And because the concentrations of products and reactants aren't necessarily equal at equilibrium, the ratio of citrate to isocitrate is equal to (20:1)

And this is important to maintain the needed levels of citrate concentration for the physiological processes.

**This same story happens with Malate and oxaloacetate, as Malate is needed for a very important process (in the liver and kidney) which is gluconeogenesis

Gluconeogenesis : the process of making glucose from non-carbohydrate sources, this is important in cases of starvation or when we're fasting .

Malate is a crucial compound in this process, that's why when a person is fasting or is starved malate is needed in high concentrations on the expense of oxaloacetate.

Regulation of the citric acid cycle :

The citric acid cycle just like any other pathway is regulated by enzymes that are affected by a feed-back mechanism / by the products of the reactions.

The products of the citric acid cycle are : NADH , $FADH_2$, CO_2 and GTP, GTP however isn't a main product as only one molecule is produced per turn and because there aren't GTP binding sites on other enzymes.

Can $FADH_2$ be considered as a regulator of the cycle ?

$FADH_2$ plays a role of regulation, but it's not a regulator of the citric acid cycle and that's mainly because $FADH_2$ is attached to the enzyme that converts succinate to fumarate (succinate dehydrogenase enzyme) and it cannot detach and leave to the solution and affect the other enzymes . An enzyme responsible for the regulation of a pathway should have a freedom of movement and several sites for the attachment of different

enzymes to exert an effect on more than one enzyme , however, these characteristics can be found in NADH and that's what makes NADH a good regulator of the cycle .

The main regulators of the citric acid cycle are :

1-NADH , the concentration of NADH, which is expressed by the ratio of NADH to NAD+, regulates the cycle.

High concentrations of NADH will inhibit the cycle and high concentrations of NAD+ will activate the cycle .

2- Adenine's ratio , ADP/ATP :

both $FADH_2$ and NADH are destined to make ATP through the electron transport chain (in an oxidative phosphorylation process), and when the ATP levels increase the electron transport chain and the citric acid cycle will be shut down

generally speaking, how are metabolic pathways controlled by enzymes regulated within the body ?

- Control of the first step of the pathway, to prevent energy loss when unneeded intermediates are made.
- Control of the rate-limiting step (slowest step in a reaction or pathway), because it's the step that determines how fast the pathway will go .
- Control of the committed step (irreversible enzymatic reaction that occurs at a branch point during the biosynthesis of some molecules)of any pathway .
- Multi-enzyme complexing.
- Compartmentalization .

We have already mentioned that the main regulators of the citric acid cycle are the ADP/ATP ratio and NAD+ /NADH ratio, enzymes affected by these ratios are dehyadrogenases (the enzymes that give NADH). For NADH to inhibit a pathway through feedback inhibition mechanism, it

should inhibit an enzyme that produces NADH and in the citric acid cycle, these enzymes are :

- Isocitrate dehydrogenase
- α-ketoglutarate dehydrogenase
- malate dehydrogenase

Citrate synthase (a simple enzyme) :

The enzyme that converts oxaloacetate to citrate, this enzyme is regulated by two main compounds : the product and the substrate, citrate and oxaloacetate respectively.

Citrate regulates this enzyme through a feed-back mechanism, as citrate inhibits the enzyme (citrate synthase) when it is found in high concentrations, but we have already explained that citrate is usually found in high concentrations (because aconitase enzyme favors citrate over isocitrate) and that means the enzyme is usually inhibited and that's what the body needs, to prevent excess production of energy when it isn't needed.

In **Malate Dehydrogenase** ,We have already mentioned that malate dehydrogenase favors malate on the expense of oxaloacetate and that's why oxaloacetate will be found in low concentrations most of the time and the ratio of oxaloacetate (the substrate) will be lower than the K_m of the enzyme citrate synthase and this will increase the inactivity of the enzyme citrate synthase

---

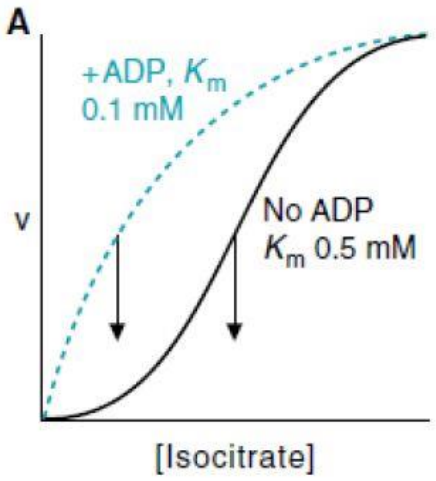
Isocitrate dehydrogenase (an allosteric enzyme) :

*It is the only allosteric enzyme within the citric acid cycle.

*It can be activated and inhibited allosterically , ADP activates the enzyme while NADH inhibits it (because it is the product)

Written by Layan Attili

*As we already know, allosteric enzymes have a sigmoidal plot, if an activator is added to the enzyme, the plot will shift to the left resulting in a rather hyperbolic shape than sigmoidal , so when ADP is added to the enzyme (isocitrate dehydrogenase) the plot will shift to the left.

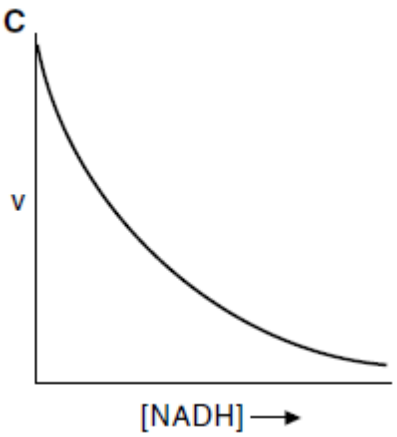


ADP is an allosteric activator of the enzyme and it doesn't affect enzymes other than isocitrate dehydrogenase (in an allosteric manner).

** the step catalyzed by isocitrate dehydrogenase is the slowest/rate-limiting step in the cycle.

**if NADH is added to the enzyme it will inhibit it allosterically (not in a simple manner) causing a shift of the plot to the right

and the plot will look like this :



**As we increase the concentration of NADH ,the velocity (V) will be decreased.

α-ketoglutarate dehydrogenase (a simple enzyme) :

*is inhibited by NADH in a simple manner and so is malate dehydrogenase and is activated by Ca^{+2} (and so is isocitrate dehydorgenase) .

Most enzymes that are involved in energy processes are activated by Ca^{+2} . why?
Because of the muscle contraction process, where Ca^{+2} is secreted from the sarcoplasmic reticulum to affect all enzymes involved in energy production because the body needs energy during muscle contraction.

The citric acid cycle intermediates :

These are all compounds in the citric acid cycle other than acetyl CoA , the use of these compounds/intermediates isn't limited to the citric acid cycle, they can be used in different pathways in the body, such as : citrate (as we have already mentioned previously) and α-ketoglutarate.

**Pyridoxal / pyridoxamine/ pyridoxine (vitamin B6) :is a coenzyme, its active form within the body is pyridoxal phosphate, it is important in transamination reactions (the transfer of an amino group) , if we add an amino group to a ketoacid it becomes an amino acid and if we remove an amino group from an amino acid it becomes a ketoacid .

**α-ketoglutarate is a ketoacid if an amino group is added to it, it will produce glutamate which can either participate in the synthesis of amino acids or get converted to GABA .

GABA (gamma-aminobutyric acid) : is an inhibitory neurotransmitter in the central nervous system

**pyruvate is a ketoacid if an amino group is added to it , it will produce alanine

**oxaloacetate is a keotacid if an amino group is added to it ,

it will produce aspartate.

**succinyl CoA can be converted to propionyl CoA which participates

In Heme biosynthesis.

**Malate is very important for the process of Gluneogenesis.

**oxaloacetate takes part in amino acid synthesis after being converted to aspartate.

Written by Layan Attili

Anaplerotic Reactions :

Reactions that can add their products to cover a deficiency/ compensate for a drop in the level of an intermediate

If there's a deficient intermediate / a decrease in the concentration of an intermediate what are the reactions that can compensate for this decrease ?

Logically they should be the opposite of reactions that give the previously mentioned intermediates :

Glutamate can produce α-ketoglutarate

Aspartate can give you oxaloacetate

Propionyl CoA (branched chain amino acids) can give us succinyl CoA

Other amino acids can give us fumarate

** one of the most important anaplerotic reactions which supplies one of the citric acid cycle intermediates is the one catalyzed by pyruvate carboxylase, an enzyme that carboxylates pyruvate (a 3 -carbon unit) to produce oxaloacetate (a 4-carbon unit).

If the concentration of oxaloacetate is reduced for any reason, pyruvate carboxylase will supply the cycle with the needed oxaloacetate through its action.

Best of luck, everyone!

Written by Layan Attili