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Biochemistry

☑ Sheet ○ Slides

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Title: TCA Cycle's Steps

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- Krebs cycle is the combination of **Acetyl CoA** with **oxaloacetate** (two carbon atoms with four carbon atoms)→6 carbon atoms molecule which is called **citrate** and this through citrate synthase enzyme.
- The enzyme which converts citrate to isocitrate is called Aconitase and it plays a role in regulation.
- Citrate has three carboxylic groups, same story with isocitrate
- Aconitase does process of isomerisation; the purpose of isomerisation is to make oxidation available because oxidation occurs for secondary alcohol not tertiary.
- Then, isocitrate will be converted into **α-**ketoglutarate by the enzyme isocitrate dehydrogenase, which is the most important enzyme in citric acid cycle(converts 6 carbon molecule to 5 carbon molecule); which means one carbon atom gets out of the cycle in the form of carbon dioxide. it is a dehydrogenase, isocitrate dehydrogenase removes hydrogen out so the electrons are loaded on NAD+ molecule and this is where the first NADH molecule is produced.
- When **α-**ketoglutarate (oxidation-reduction reaction)
  1) there will be an oxidative decarboxylation breaks COO- so it breaks bond (oxidizing)
  2) through this reaction you are converting ketone to acidic group (succinate),its acidic because when binding with CoA and CoA it remains ketone because it is bind CoA and when CoA leaves again it is back to an acidic
- it is dehydrogenase , electrons will be loaded on NAD+ molecule resulting in the second NADH molecule .
- succinyl –CoA is a four carbon molecule ,this structure has to be converted to the form when we started (oxaloacetate)
- How to convert succinyl-CoA to oxaloacetate?
  First CoA is removed, when it leaves, it gives energy, this energy is used to make GTP molecule which will be converted instantly to ATP
- CoA is out ,GTP is produced and the same 4 carbon molecule oxidized to an acid called succinate.
- To convert back succinate to oxaloacetate ,an enzyme called succinate dehydrogenase ,it is dehydrogenases the succinate ,removes two hydrogen and forming double bond
- Double bond formation converts succinate into fumarate
- Electrons from hydrogen will be loaded in FAD so will have $FADH_2$
- There is n enzyme called fumarase it is a hydratase enzyme it adds water to the molecule ,when we add water to double bond a hydrogen will be attached and hydroxyl group will be attached, resulting in what we call malate (is an alcohol).
- Alcohol is the difference between malate and oxaloacetate
- There is an oxidation step between malate and oxaloacetate ,the carbon will be oxidized ,two hydrogen removes and there will be ketone group in place
- Enzymes that catalyses this step from malate to oxaloacetate is called malate dehydrogenase

- NOTE: you have to identify the structures of all eight molecules and enzymes that catalyses these steps. from the name of these enzymes you can identify these reaction, the only exception that cannot be identified from type of the reaction is Aconitase because the name gets from the intermediate in between isomerisation process citrate converts to isocitrate
- The enzyme that converts succinyl-CoA to succinate is succinate thiokinase (thio because it is deal with thio groups present in carbon present in CoA ,, kinase because it phosphorylates ADP,GDP to ATP)
- Formation of citrate to isocitrate is an isomerisation process on tertiary alcohol to secondary alcohol as it can be oxidized
- From isocitrate there is an oxidative decarboxylation so we have carboxylic group out of the reaction so the number of carbon will be reduced by one resulting in what we call **α-** ketoglutarate
- Equilibrium found between citrate an isocitrate makes more citrate

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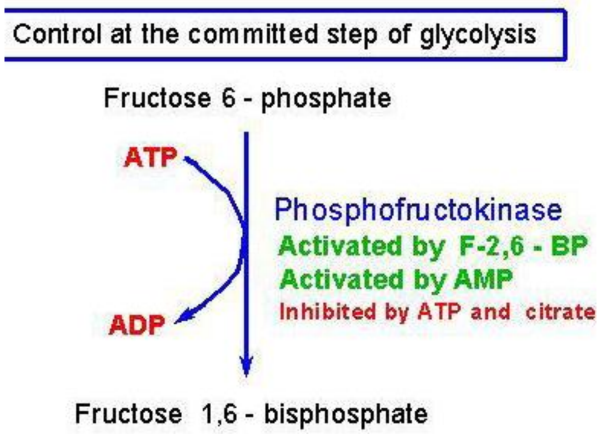

- Citrate works as an inhibitor in the rate limiting step of glycolytic pathways catalysed by the enzyme phosphofructo kinase which catalyse the step of conversion fructo-6-phosphate to fructose 1,6- bisphosphate

- Why citrate inhibits phosphofructo kinase ??

  Because citric acid cycle has high amount of citrate!

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α-ketoglutarate to succinyl CoA

- We reach a point that oxidative decarboxylation from isocitrare to α-ketoglutarate also when yhis step because it is a five carbon molecule there should be another carbon molecule to get out of α-ketoglutarate resulting in a four carbon molecule ,how it is removed?
  Another oxidative decarboxylation process occurs and another carboxylic group gets out of the molecule
- This reaction occurs by α-ketoglutarate dehydrogenase which has 3 coenzymes (TPP,pyrocic and FAD)
- At the end, ketone group on α-ketoglutarate is converted to an acidic group in the sufficient form and energy is loaded on NAD+ molecule ,so second NADH molecule gets out of the cycle.
  - In subsequence step the conversion of succinyl –CoA to succinate provides you with energy, in the form of GDP which is converted into ATP.
  - How do we usually make ATP or what is the process responsible for producing ATP ?
    Oxidative phosphorylation
- Oxidative phosphorylation reaction cannot occur without the presence of oxygen.
- If you make ATP through the use of oxygen it is named oxidative phosphorylation
- If you make ATP without the need of oxygen then it is called substrate level phosphorylation
- Few reactions inside the body result in the substrate level phosphorylation; one of them is getting ATP from GTP.
- **The enzyme which catalyses substrate level phosphorylation is succinate thiokinase**

  - How many dehydrogenase we have in the cycle ??

  Four dehydrogenase -→ 3 dehydrogenases result in NADH molecules (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase), the only one which is the exception (succinate dehydrogenase results in the production of $FADH_2$

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- CoA is used in two reactions :
  1-with acetyl CoA →it provides energy for anabolic reaction of combining acetyl CoA with oxaloacetate
  2-with succinyl CoA → excess energy providing from breaking down thioester bond used for the substrate level phosphorylation attaching the inorganic phosphate with GTP molecule

- All reactions of citric acid can go in both direction (reversible reaction).however essentially it goes only in one direction.
- α-ketoglutarate dehydrogenase → one big complex enzyme that has 3 enzymes involved in E1,E2,E3(three big enzymes in one complex)
- one of the ways to regulate enzymes is compartmentalization and complex of enzymes .

- we have 3 enzymes (complexes) have the same structure , we have pyruvate dehydrogenase (after glycolsis you will end up with pyruvate which is converted to acetyl CoA so it can be used in citric acid cycle .
- What is the enzyme that converts pyruvate to acetyl CoA ?
  Pyruvate dehydrogenase

- So pyruvate dehydrogenase, α-ketoglutarate dehydrogenase in citric acid cycle and branched chain α-ketoglutarate in amino acid metabolism
- Those three enzymes adopts the same structure ,the same free big molecule and adopts free coenzymes in each one
- Coenzyme involved in α-ketoglutarate dehydrogenase :
  Thiamine in the form of the TPP
- Vitamin B1 in the form of TPP
- Thiamine works at acyl carrier groups.
- Acyl group : compound has 3 carbon or more
- Acetyl group: compound has 2 carbon
- E1 works as decarboxylase (co2 get out) ,then E1 directly binds to thiamine. (enzymes at the end are converted back to original form without change), so E1 has to donate R groups back to its original structure,enzyme number 2 is Transacylase, it transfers acyl group through this big complex

- Transacylase enzyme has coenzyme (lipoic acid) →has 2 sulfurs to the disulfide bridge

- First enzyme do decarboxylation then the carbon reactive ,reactive is attached to thymine ,thymine donated back to trans Ac through its coenzyme (lipoic acid) which has disulfide bridge

- How acyl group binds disulfide group?

The disulfide bridge will be reduced through attachment this acyl group with sulfur ,other sulfur will abstract H from the solution and this is where CoA comes, when free acyl group binds the attached acyl group ,leaving the second sulfur also free ,the second sulfur will abstract also leaving disulfide bridge .

(R groups : might be succinate)

- Transacylase abstracts these two hydrogen getting back E2 to its original form on disulfide bridge ,these 2 hydrogen atoms are loaded on E3 (FAD molecule) resulting in $FADH_2$, At the end electrons are loaded on NAD+
- Electrons are transferred from $FADH_2$ to NAD+ resulting in NADH molecules and thus E3 is back to its original form FAD.
- The end result of to α-ketoglutarate dehydrogenase is NADH.
- Started with to α-ketoglutarate ending up with succinyl CoA and the electrons are loaded on NAD+ molecule to have NADH

- Co enzymes used in to α-ketoglutarate in the conversion process from to α-ketoglutarate ,succinyl-CoA
  1-thaymine pyrophosphate
  2- lipoic acid
  3-CoA
  4-NAD
  5-FAD
- How many FAD are involved in the one citric acid cycle?
  Two → one through the α-ketoglutarate dehydrogenase complex, the second in the succinyl dehydrogenase.

- Thiamine deficiency: α-ketoglutarate, pyruvate, & branched chain α-keto acids accumulate in the blood and enzymes do not work properly.
- Co enzymes are essential for enzyme to work.

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- How can we calculate the efficiency of citric acid cycle ?
- The results of TCA cycle are : 3NADH, 1FADH$_2$, 1GTP
- You compare it to acetate molecule, you bring acetate and burn it in a chamber and you will see how much energy comes out and (the amount of calories)=228 kc/mol
- Every NADH molecule = -53kc/mol , FADH2 = -41 , GTP near to ATP= -7
  (3*53+91+7)=207
  - EFFICINCY = (207/228)*100%
    = 90% → this number hardly find in life

  - Why is the acetate? because this is what gets to the cycle → other materials still inside the cycle nothing has been consumed
  - Entry→acetate molecule through acetyl CoA and the products are 3NADH,FAH2,GTP

  - All reaction can go both ways (reversible) however the cycle is essentially irreversible why ?because you have 3 reactions which has a very large negative ΔG:

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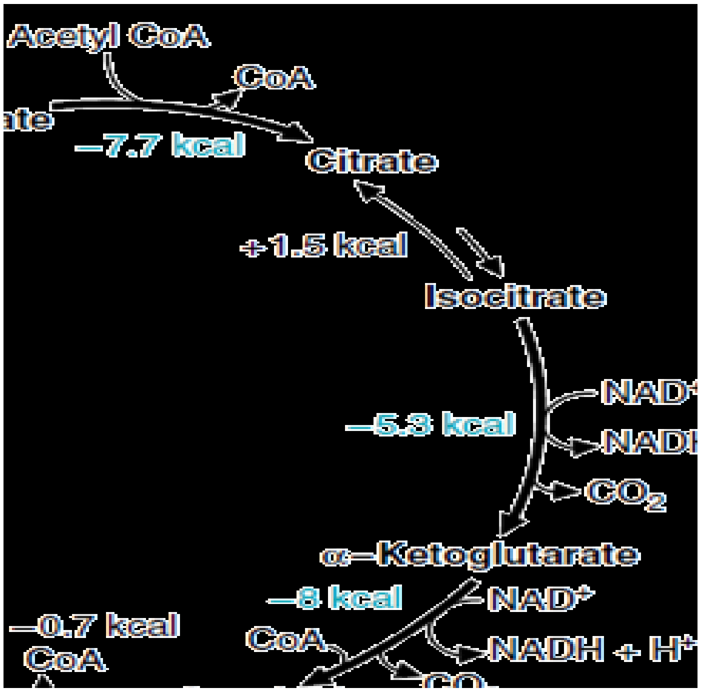

- Those reactions drive the reaction forward all the time
- Aconitase → citrate to isocitrate, keeps citrate in high concentration because it has allostric effect to other enzymes one of them is phosphofructo kinase and directly CoA is used in other process -→fatty acid synthesis and cholesterol synthesis
- Malate dehydrogenase also has + ΔG so it favors malate over oxaloacetate so you need more malate to overcome this reaction
- Malate is one of the main molecules which is being used in gluconeogenesis (in carbohydrate metabolism)→now you can provide glucose to the body from other sources
- It has + ΔG which means that the reversible reaction has – ΔG and that's why it favors malate over oxaloacetate .

GOOD LUCK ALL ☺