Overview and Integration of Cellular Metabolism

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Lecture 06: TCA Cycle (II) – Regulation and special characteristics.

Hello everyone. Welcome back to the course of Overview and Integration of Cellular Metabolism. So, we will be continuing TCA cycle, Tricarboxylic Acid cycle, the part 2 of this session where regulation and also the special characteristics will be discussed. So, let us see what are the concept here will be covered in this class. See in the previous class we already have discussed the conversion of pyruvate to acetylcoenzyme as well as the detailed biochemical steps of TCA cycle.

Now, in this class we will discuss different salient features of the enzymes there are different important enzymes present in TCA cycle, they are salient characteristics we will discuss as well as 2 important features of TCA cycle that is TCA cycle acts as amphibolic nature it acts in amphibolic nature and it has multiple anaplerotic reactions within it. So, these 2 things we will discuss and also the regulation of TCA cycle how TCA cycle is tightly regulated to its target we will discuss in this session ok. So, there are few points I will like to highlight about TCA cycle like vitamins play a key role in citric acid cycle. Now, if you remember from the previous class that there is pyruvate dehydrogenous complex and in that complex there are vitamins 4 vitamins play important role in driving the complexes activity.

So, these vitamins deficiency will manifest as deficiency of citric acid cycle. Next it play a the TCA cycle plays a pivotal role in metabolism. Metabolism of what? Metabolism of neo glucogenesis which we will discuss in next session also transamination and deamination these are very important you will see these 2 mechanisms are very important for metabolism of amino acids and proteins. So, TCA cycle has role in this metabolism as well. Next we already have highlighted that it acts as amphibolic pathway means it functions as anabolic pathway where oxidative degradation of products are happening also as anabolic pathway where synthetic different biosynthetic it acts as precursors for different biosynthetic molecules.

So, these are the important things which should be remembered about TCA cycle. Now,

you can see this is the TCA cycle from the previous class. Now, there are few important enzymes and their features I would like to discuss. Now, number 1 is econitase. Econitase is the enzyme is basically what it is doing it is actually isomer this is an isomerization and citrate is forming isocitrate with the help of this econitase enzyme.

Now, econitase has one iron sulfur center one important iron sulfur center which acts as a prosthetic group within this econitase enzyme molecule. Now, what happens whenever there is iron deficiency in the circulation it hampers the econitase activity what happens this iron sulfur iron sulfur center it is disassembled within this enzyme and what remains is the apo econitase form of the enzyme where iron sulfur center is not present. Now, this apo econitase form acts as iron responsive elements it signals the iron responsive element. It is just act like iron responsive element binding protein 1 this is a sort of this is one transcription factor. What it does? This iron responsive element binding protein it enters in the nucleus and signals in such a way that ferritin synthesis is decreased ferritin synthesis is decreased and transfer in receptor synthesis is increased.

So, what will be the effect due to decreased ferritin synthesis there will be reduced storage of iron. So, iron will be more available in the circulation and because there is increased transfer in receptor synthesis iron uptake inside the cell will be increased. So, ultimately there will be an increased iron concentration within the cell which will help to reform the iron sulfur center of econitase. So, this cis econitase will be forming econitase which is the enzyme and that econitase will be active. So, basically it is actually regulating its own activity by modulating the concentration of iron in circulation.

So, this is the role of econitase for which econitase is also known as moon lighting enzyme. Econitase is also known as moon lighting enzyme because of its dual nature of activity. Next is the enzyme isocitrate dehydrogenase as well as alpha ketoglutarate dehydrogenase. I have already told that these two enzyme are similar like pyruvate dehydrogenase complex. So, basically they are having both of these enzyme along with pyruvate dehydrogenase they are having three types of enzymes and also three types of enzymes along with the there are five types of cofactors and four vitamins which play important role in the enzyme complex function.

Apart from that isocitrate as you can see isocitrate here in citric acid cycle it utilizes NAD as cofactor. Now, isocitrate dehydrogenase in our body are of two types it has two isoenzymes. One type uses NAD which is present in mitochondria and acting in TCA cycle another form utilizes NADP, NADP ok and that form present both in mitochondria as well as cytosol and those isoenzymes are basically important for synthesizing NADPH. Then there is succinate thiokinase now once again succinate thiokinase here

you can see it is forming ATP and here the function of succinate thiokinase is basically dependent on the presence of ADP. Again I sorry again succinate thiokinase it has two isoform one isoform forms ATP from ADP another isoform utilizes GDP to form GTP.

So, both are having high energy phosphate bonds and actually the these thio ester bond is hydrolyzed to form these energy currencies like ATP or GDP. Now the important thing is whatever that whatever the isoform is present in TCA cycle the final product is ATP. It can be produced directly with the with conversion of ADP to ATP or even if there is formation of GDP to GTP these GTP actually reacts with ADP to form ATP and the G GTP is converted to GDP. Now these enzyme is known as nucleoside diphosphate kinase the enzyme here I am writing ok nucleoside diphosphate kinase. So, basically whatever iso enzymes or iso enzyme of iso succinate thiokinase is used for the final product is ATP at the end of the cycle.

Then succinate dehydrogenase so, the important feature of succinate dehydrogenase is this is the one enzyme of TCA cycle which is attached to the inner mitochondrial membrane all the other enzymes are actually present in mitochondrial matrix. Now, succinate dehydrogenase I have discussed in the previous class that the inhibitor here is malonate. Now malonate is a competitive inhibitor of succinate dehydrogenase and basically it is a structural analogue of succinate. So, basically it competes with succinate to bind with the enzyme succinate dehydrogenase. So, it is a it is a type of competitive inhibitor of succinate dehydrogenase.

So, remember whenever it is asked name one inhibitor which is a competitive inhibitor or or give an example of competitive enzyme inhibition remember the example is malonate which is an inhibitor which inhibits the enzyme succinate dehydrogenase ok. So, this is the one important things TCA cycle acts as amphibolic pathway because it react as anabolic pathway it acts also as catabolic pathway. So, catabolism part you all know we have already discussed that finally, all the metabolites say it glucose, say it fatty acids, amino acids they all enter as acetyl coenzyme A inside the TCA cycle and inside the TCA cycle there is formation of NADH or FADH 2 those are the reducing equivalence electron carriers which finally, enters the electron transport chain and forms ATP. So, this is the example of catabolism. Now, interesting thing is TCA cycle also acts as anabolic pathway it forms different precursor small u which are important for other biosynthetic pathway like what here you can see two most important thing are one is alpha ketoglutarate another is oxaloacetate.

Now, I gave an example of I gave I discussed about transamination and deamination. So, basically alpha ketoglutarate and oxaloacetate these are the two important intermediates of TCA cycle which by transamination reaction form glutamate from alpha

ketoglutarate and from aspartate from oxaloacetate. And these two molecules finally, are utilized to form other amino acids also nucleotides purines and pyremidions. Then again oxaloacetate forms phosphanal pyruvate by in while it enters the neo glucogenesis phosphanal pyruvate can be used to form these amino acids. Another intermediate succinyl coenzyme A it plays an important role in forming the porphyrin ring the porphyrin ring which is present in heme heme which you all know that heme is important for synthesis of hemoglobin it is basically the oxygen carrier and also present in myoglobin also this heme molecule is present in cytochrome molecule.

So, succinyl coenzyme A also acts as a precursor for porphyrin ring. So, this is these are the important role as important role of TCA cycle in anabolism synthesizing different precursor molecules this is the reason why TCA cycle is known as amphibolic pathway. Now, in TCA cycle you can see that intermediates are used up for synthesizing different other materials. So, the intermediates are siphoned off from the cycle. So, definitely it needs some replenishment the intermediate should be replenished inside the cycle how by different anaplerotic reactions.

Now, there are these 4 important anaplerotic reactions which occur in TCA cycle the most important one which occurs in liver and kidney remember these 2 liver you you will see that in metabolism liver plays an important role wherever some important pathway is happening liver has some role. So, here also TCA cycle with anaplerotic reactions one of the important one is conversion of pyruvate to oxaloacetate. So, basically here oxaloacetate the concentration of oxaloacetate is replenished and it is found from pyruvate with the help of the enzyme pyruvate carboxylase. Now, carboxylases these group of enzymes always require in majority of the cases always require biotin as cofactor. So, this pyruvate carboxylase is using biotin as cofactor and also PEP is basically phosphanol pyruvate PEP phosphanol pyruvate carboxylase where phosphanol pyruvate is also forming oxaloacetate to maintain the concentration of oxaloacetate.

Now remember whenever there is TCA cycle whenever there is slowing of TCA cycle TCA cycle is happening in a slower rate there is accumulation of TCA cycle intermediate as well as the intermediate of glycolysis. Now different intermediate influences these enzyme basically they influences in such a way that the piled up intermediates are used up. Now one important molecule is acetyl coenzyme A. Acetyl coenzyme A it basically it gives a positive influence over pyruvate carboxylase enzyme. Similarly another product of glycolysis which is fructose 1, 6 bisphosphate fructose 1, 6 bisphosphate this influence positively phosphanol pyruvate carboxylase it is basically allosteric activator of phosphanol pyruvate carboxylase.

So, whenever there is accumulation of the intermediates of glycolysis or even the or even acetyl coenzyme A they influence this anaplerotic reaction in such a way that the used up intermediates of TCA cycle accumulates to utilize the piled up intermediates of glycolysis or TCA cycle. So, it basically balances the cycle. So, these are the important anaplerotic reactions. Then we will go to regulation of TCA cycle. Now I will start from formation of pyruvate to acetyl coenzyme A to entrance of acetyl coenzyme A inside TCA cycle.

Now remember TCA cycle are tightly regulated at these two point. One is the formation of the product the formation of the substrate of the TCA cycle which is acetyl coenzyme A. So, basically the step which is conducted by the enzyme pyruvate dehydrogenous complex and the other tightly regulated step is the entry of acetyl coenzyme A in the TCA cycle which is done by citrate synthase enzyme. Now apart from that I have already discussed that acetyl coenzyme A can be formed from different other metabolic pathway. So, basically how those metabolic pathways are happening which means how much acetyl coenzyme A is available from other metabolic pathways this is also one determining factor in TCA cycle regulation.

Then those two important enzymes like isocitrate dehydrogenous and alpha ketoglutarate dehydrogenous they are also important in regulation of TCA cycle. Now let us see what happens. Now in pyruvate dehydrogenous complex regulation there are two different mechanism one is allosteric regulation and the other one is covalent modification of the enzyme. Now what happens in allosteric regulation that basically TCA cycle occurs when there is requirement of energy in the body. So, body needs more ATP for that the signals the molecules which basically signals for energy demand of body like AMP, ADP which are the broken down product of ATP actually.

So, basically there is no ATP those are used up and are have formed AMP or ADP. So, presence of AMP or ADP presence of NAD means NADH is used up. So, there is more NAD in the system. So, basically these are the molecules which signals for the energy demand of body. So, they allosterically activate TCA cycles enzyme.

So, you can see AMP, NAD they basic they are the allosteric inhibitor of TCA cycle. Similarly molecules which actually signal for energy excess in body like ATP enough ATP present in body NADH means the reducing equivalents are present in body they are the allosteric inhibitors of pyruvate dehydrogenous complex. Similarly acetyl coenzyme there is huge amount of acetyl coenzyme which are formed from different other metabolic pathway or also formed from pyruvate. So, in that case we do not need pyruvate dehydrogenous complex to run in that speed we need to slow it down. So, they are the allosteric inhibitors of pyruvate dehydrogenous complex.

Also long chain fatty acids it means if there is more long chain fatty acids they are actually oxidized to provide more amount of acetyl coenzyme A. So, these are the allosteric inhibitors of pyruvate dehydrogenous complex. So, this is allosteric regulation not only that there is covalent modification. Now, if you remember from the previous class that enzymes are modified by reversible phosphorylation and dephosphorylation and either of the form dephosphorylated or phosphorylated form can be active or inactive based on that the metabolic pathways are regulated. Here the E 1 enzyme 1 which is pyruvate dehydrogenous enzyme itself present in pyruvate dehydrogenous complex is basically covalently modified with the help of phosphorylation dephosphorylation.

Now, ATP here is the phosphorylating trigger which activates the kinase enzyme and when phosphorylated E 1 pyruvate dehydrogenous is inactive. Similarly, in absence of ATP there is activation of phosphatase. So, there is dephosphorylation of the enzyme which is the active form. So, basically this is the covalent mechanism by which PDH is regulated. Now, this is one important point I have told that vitamin deficiency manifest as TCA cycle deficiency also thiamine deficiency it gives rise to high level of pyruvate in blood because in absence of thiamine pyruvate dehydrogenous complex is inhibited.

So, the substrate here accumulates. Next are the other enzymes like citrate synthase. Now, citrate synthase also is triggered by the molecules of enzyme energy excess. So, it is deactivated by NADH it is deactivated by ATP also citrate if more product is available it can also allosterically inhibit the citrate synthase enzyme. Similarly, another intermediate succinyl coenzyme A can inhibit it and you can see ADP here it is activating because it signals for energy demand. Same for isocitrate dehydrogenous and alpha ketoglutarate dehydrogenous where the molecules of energy excess like ATP or NADH they signal for energy excess inhibits allosterically whereas, ADP is actually activating the enzyme.

Now, what happens when there is vigorous muscle contraction it is signal by the it signal by calcium. Calcium not only signals for muscle contraction it also signals for now when the muscle is supposed to be contracted it needs energy. So, TCA cycle should be influenced positively. So, basically calcium here acts as a positive influencer or positive allosteric activator of isocitrate dehydrogenous and alpha ketoglutarate dehydrogenous. So, these are the key points which I have discussed in TCA cycle that TCA cycle acts as amphibolic nature and also it has anaplerosis.

So, basically the intermediates of TCA cycle it can form different other biosynthetic precursors can be utilized in different other pathway. So, if the intermediates are depleting those can be replenished by anaplerotic reaction. The production of acetyl

coenzyme A for TCA cycle is by PDH complex and PDH complex is regulated allosterically as well as by covalent modification. And the trigger for all the enzymes like pyruvate dehydrogenous complex or citrate synthase or isocitrate dehydrogenous or alpha ketoglutarate dehydrogenous are the molecules with signals for energy excess or energy demand. And these fluxes are also determined by different substrate and products.

So, if there is more substrate available the cycle will be enhanced the rate of the cycle will be enhanced whereas, if the products are more it gives feedback inhibition of TCA cycle. So, this is all about TCA cycle these are my references. Thank you, see you in the next class.