Overview and Integration of Cellular Metabolism

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Lecture 05: Biochemistry of TCA Cycle (I)

Hello everyone. So, in today's session of Overview and Integration of Cellular Metabolism, we will discuss TCA cycle, the biochemistry of TCA cycle. The concept here will be covered are conversion of pyruvate to acetyl coenzyme A and the different biochemical steps of TCA cycle. Now, before discussing TCA cycle, I would like to highlight that in the previous session we have seen that glucose is the major source of fuel and that can undergo aerobic glycolysis as well as anaerobic glycolysis. In mammalian cell which is which is where the cells are basically aerobic mostly, there this pyruvate actually enters TCA cycle by forming acetyl coenzyme A. And then at the end of TCA cycle, there is formation of fuel reducing equivalent which is utilized to form energy in the form of ATP in oxidative phosphorylation.

Now, this is the basic concept of cellular respiration which occurs in 3 different phases. In cellular respiration, there are different metabolic fuels mainly carbohydrate, lipid and proteins. Now carbohydrate, lipid in the forms of fatty acid and few amino acids as well, they are entering in TCA cycle in the form of acetyl coenzyme A. Then that acetyl coenzyme A is forming different intermediate in TCA cycle tricarboxylic acid cycle forming different reducing equivalent.

And at the end stage, these reducing equivalent they undergo oxidative phosphorylation which forms ATP and regenerate those oxidized form of the equivalence. So, this is the concept of cellular respiration. Now acetyl coenzyme A is the form by which actually pyruvate which comes from glucose or acetyl coenzyme A formed from different fatty acid or some of the amino acid, they enter TCA cycle. So, formation of acetyl coenzyme A is required. Now pyruvate, the pyruvate we get from glycolysis, it forms acetyl coenzyme A with the help of the enzyme.

Now formation of pyruvate was in cytosol because glycolysis occurs in cytosol, but this enzyme pyruvate dehydrogenous complex is present in mitochondria in a mitochondrial membrane. So, basically conversion of pyruvate to acetyl coenzyme A occurs in

mitochondria. So, definitely acetyl coenzyme A must enter mitochondria. How it enters mitochondrial matrix with pyruvate hydrogen simport. This is a transporter which actually carries acetyl coenzyme A inside mitochondrial matrix.

Now in mitochondrial matrix, the enzyme pyruvate dehydrogenous complex is present in the inner mitochondrial membrane. Now this is this enzyme complex is a very interesting is a very complex enzyme complex where there are 3 different enzyme activity basically there are 3 different enzymes present. One is pyruvate dehydrogenous enzyme 1, then enzyme 2 is dihydrolypyl transacetylase and then dihydrolypyl dehydrogenase. So, there are 3 different enzymes present in this enzyme complex not only that there are 5 coenzymes or prosthetic groups. What are those? Thiamine pyrophosphate, flabine adenine dinucleotide, coenzyme A, nicotinamide adenine dinucleotide as well as lipo.

These are the 5 different prosthetic groups or coenzyme. Now nutritionally this enzyme complex is also important because there are 4 vitamins which are required. Now what are those vitamins? Thiamine which is present in thiamine pyrophosphate, riboflavine present in FAD flabine adenine dinucleotide, niacin present in NAD and pantothanate present in coenzyme A. So, you can see in these enzyme complex there are 3 different enzymes present 5 prosthetic groups of or coenzyme present as well as 4 vitamins required for these enzymes activity. So, how this conversion of pyruvate is happening with the help of pyruvate dehydrogenase complex? So, we can see there are sequential enzyme reaction by which pyruvate is converted to acetyl coenzyme A.

This is the whole sequence of the reactions which is occurring inside the enzyme complex. Now in the first step you can see pyruvate is converted to pyruvate is actually releasing carbon dioxide. So, there is decarboxylation as well as the acetyl group is attached to TPP molecule. Now this TPP after taking of the hydroxyl after taking of the acetyl group it is converted to hydroxy ethyl TPP. Then in the second reaction with the help of pyruvate dehydrogenase this acetyl group is transferred to the core enzyme.

What is the core enzyme? Dihydro lipoene transacetylase this is the core enzyme and in this core enzyme there are 2 sulfhydryl group. This acetyl group is taken up by the oxidized lipolysin group of core enzyme. So, this is the oxidized lipolysin group of core enzyme and it after taking the acetyl group from TPP it forms the acyl lipolysin this is the acyl lipolysin ok. Now, in the third reaction there is coenzyme A which is actually taking up this acetyl group from the core enzyme and it forms ultimately the acetyl coenzyme A which regenerates reduced lipolysin. So, this is the reduced lipolysin you can see the sulfhydryl groups are here reduced.

So, all these are occurring inside the core enzyme that is dihydro lipolyne transacetylase. Now this reduced lipolysin actually it gives of this hydrogen atoms to FAD. FAD present in the third enzyme that is dihydro lipoene dehydrogenase. FAD is reduced to form FADH 2 and in the core enzyme once again the oxidized lipolysin is form once again you can see that from here the oxidized form is there. Now this reduced FADH 2 they it gives of the hydride to generate NADH from NAD.

So, once again the pyruvate dehydrogenase complex with its prosthetic groups with the help of the vitamins they are ready for the next cycle of conversion of acetyl coenzyme A from pyruvate. So, at the end of the reaction what we are getting that pyruvate releases carbon dioxide decarboxylation as well as it produces acetyl coenzyme A and also produces NADH or reducing equivalent. So, now this acetyl coenzyme A enters the TCA cycle proper. Now what happens in TCA cycle? Now, TCA cycle is tricarboxylic acid cycle also known as citric acid cycle also known as Krebs cycle because definitely with the name of the discoverer Hans Krebs it is named Krebs cycle. So, definitely it is a cyclical reaction or cyclical metabolic pathway where there are series of cyclical reaction inside mitochondria which oxidizes the acetyl residue of acetyl coenzyme A and the reduced coenzyme form is form reduced coenzyme they undergo reoxidation in electron transport chain which forms ATP.

So, this is the crux of TCA cycle. Now, TCA cycle is also the final common pathway for Arabic oxidation of carbohydrate lipid as well as protein because as we have already discussed acetyl coenzyme A comes from comes not only from glucose through pyruvate, but also from fatty acid by fatty acid oxidation and also from different different amino acids. Then TCA cycle plays a central role in neoglucogenesis, lipogenesis and also inter conversion of amino acids we will discuss definitely in the later sessions. And with respect to TCA cycle liver is the most important organism because this is the only tissue where all these metabolic pathways are happening. So, this is the this is liver, liver is the only tissue where all of these metabolic pathways are happening in a significant extent.

Now, this is the TCA cycle let us see what is happening. So, you can see here acetyl coenzyme A which is formed from pyruvate is entering TCA cycle it come it is combined with oxaloacetate a 4 carbon molecule to generate citrate the name is citric acid cycle because now citric citric acid will undergo cyclical conversion to different molecules. So, there is formation of citrate with the help of the enzyme citrate synthase with the help of the enzyme citrate synthase. Now, citrate synthase why it is important it is basically helping to form direct bond between carbon carbon the acetyl group of acetyl coenzyme A and the carbonyl group of carbonyl group of oxaloacetate to form citrate. Now, citrate undergoes isomerization to form isocitrate.

Now, this isomerization process is a 2 step reaction and the enzyme is econitase in the 2 step reaction the enzyme is econitase. First citrate is citrate undergoes dehydration to form cis econitate. Cis econitate once again undergo rehydration to form isocitrate. So, this is the isomer of citrate. Now, this enzyme econitase is inhibited by fluoroacetate is inhibited by fluoroacetate.

Now, what happens fluoroacetate it forms fluoroacetyl coenzyme A in the form of fluoroacetyl coenzyme A it enters the TCA cycle combines with oxaloacetate to form fluorocitrate. Now, this fluoroacetate inhibits econitase. So, basically fluoroacetate undergoes modification to fluorocitrate which inhibits econitase. Next isocitrate undergoes oxidative decarboxylation to form alpha ketoglutarate. Again this is a 2 step reaction.

So, in this process the first product is oxalo succinate isocite from isocitrate oxalo succinate is formed by dehydrogenation with the help of the enzyme isocitrate dehydrogenous. As there is dehydrogenation there is formation of NADH from NAD, then there is decarboxylation again the enzyme is isocitrate dehydrogenous decarboxylation. So, there is release of carbon dioxide finally, forming a 5 carbon compound alpha ketoglutarate. Next this alpha ketoglutarate once again undergo oxidative decarboxylation another step of oxidative decarboxylation with the help of the enzyme alpha ketoglutarate dehydrogenase. Now alpha ketoglutarate dehydrogenase is a complex which is similar to pyruvate dehydrogenase complex.

The whole enzyme reaction is just similar with pyruvate dehydrogenase complex. So, basically there are 3 different enzymes, there are 5 different prosthetic groups as well as 4 vitamins which are required for the enzyme activity. So, because that is decarboxylation reaction there is release of carbon dioxide and also formation of NADH. Now succinyl coenzyme A is converted to succinate. Now this is one substrate level phosphorylation.

What happens in succinyl coenzyme A there is a high energy thioester bond which finally, helps in formation of ATP with the help of the enzyme succinate thiokinase. Remember in all the kinases there is magnesium ion required as cofactor. So, there is formation of succinate which is a 4 carbon compound. Now succinate undergoes different conversion to finally, form oxaloacetate. What are those intermediates? From succinate there is formation of fumarate with the help of the enzyme succinate dehydrogenase.

Now, succinate dehydrogenase is the enzyme which can be competitively inhibited by

malonate. And in this dehydrogenation there is formation of FADH 2. Now, fumarate is converted to malate with addition of one molecule of water. You can see that here water is coming out basically this is a reversible reaction and from malate to fumarate there is from release of water, but for formation of malate from fumarate there is addition of water and the enzyme here is fumarate. Now once again malate forms oxaloacetate by another dehydrogenation reaction with the help of malate dehydrogenase and there is formation of NADH.

So, as we have discussed that at the end of TCA cycle we are getting reducing equivalence and those reducing equivalence are generating ATP's. So, let us see what are the net ATP production at the end of TCA cycle. So, in TCA cycle there are reducing equivalence like NADH in these steps we are getting 3 NADH molecule. So, these are the 3 NADH molecule from where we are getting energy which is equivalent to from each NADH molecule we are getting energy equivalent to 2.

5 molecules of ATP fine. 7.5 molecules of ATP then there is another reducing equivalent FADH 2 1 molecules of FADH 2. Now FADH 2 on oxidation it gives rise to energy which is equivalent to 1.5 molecules of ATP remember previously the concept was 2 molecules of ATP are generated from FADH 2, but if the recent concept is that there is 1.5 molecules of ATP generated from FADH 2.

So, 1.5 ATP from FADH 2 then there is substrate level phosphorylation. So, in substrate level phosphorylation we are getting 1 ATP substrate level phosphorylation we are getting 1 ATP. So, at the end of this 1 TCA cycle we are getting 10 molecules of ATP. Now remember that after glycolysis from 1 molecule of glucose we were getting 2 pyruvates which was which is actually generating 2 molecules of acetyl coenzyme A. So, basically 2 molecules of acetyl coenzyme A undergo 2 cycles of TCA cycle.

So, there is 20 ATP formation plus if you do remember once again from pyruvate to acetyl coenzyme A there were formation of 1 molecule of NADH. So, from 2 molecules of 2 molecules of acetyl coenzyme A formation we are getting 2 molecules of NADH actually. So, these 2 molecules of NADH are giving rise to 5 molecules of ATP. So, at the end of glycolysis we were getting 7 molecules of ATP glycolysis we were getting 7 molecules of ATP and at the end of TCA cycle we were getting 25 molecules of ATP. So, the calculation which we can see that at the end of 1 molecule of glucose oxidized through aerobic glycolysis following TCA cycle to up to the end of electron transport chain you can see there is 25 plus 7 that is 32 molecules of ATP's are generated.

So, these are the key points discussed today that is TCA cycle is occurring inside mitochondria pyruvate is converted to acetyl coenzyme A that is also inside

mitochondria with the help of pyruvate dehydrogenase complex. The resultant acetyl coenzyme A enters TCA cycle in TCA cycle NADH, FADH 2 and by following substrate level phosphorylation ATP are formed and these electron carriers they finally, enters electron transport chain and undergoes oxidative phosphorylation to form ATP's. So, this is the energy production through glycolysis and TCA cycle. Thank you.