Introduction to Biomolecules
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Lecture - 20
Citric Acid Cycle (Part 1/2)

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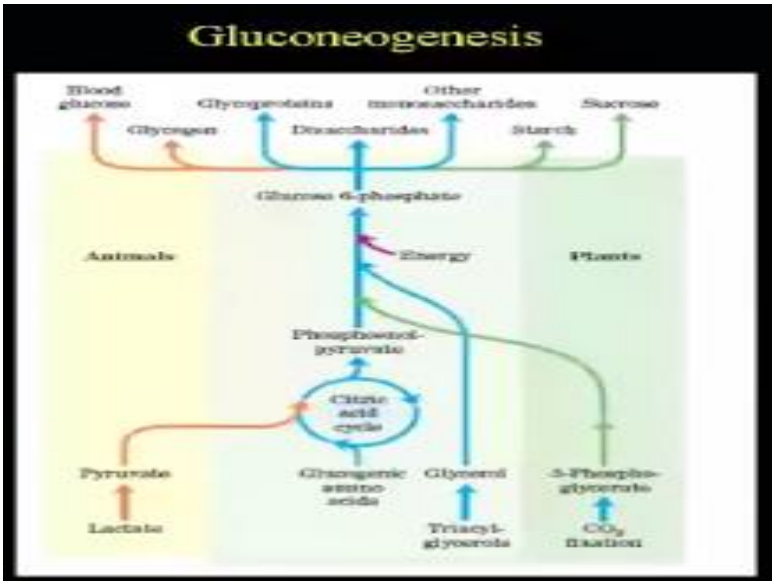

So say in the last class we completed glycolysis and then we looked briefly the gluconeogenesis. So today we will briefly spend couple of minutes on gluconeogenesis specific reverse directions in the glycolytic pathway.

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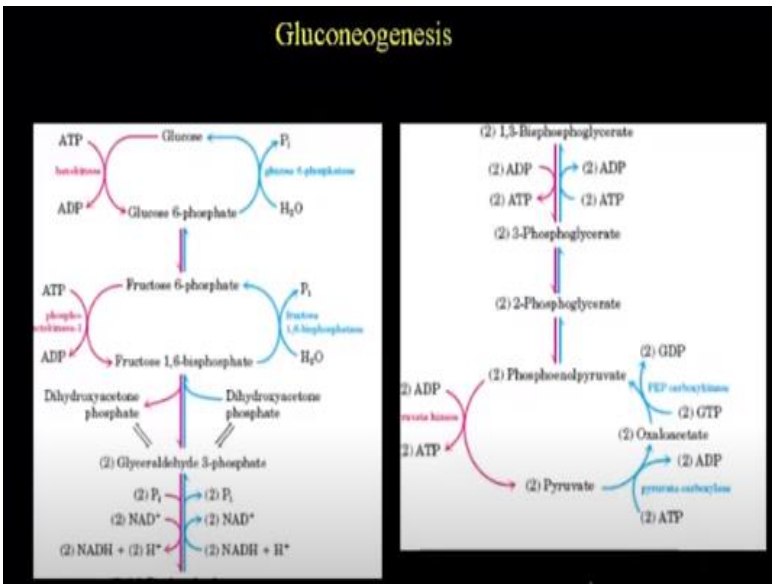

So that is shown in this slides. So looking backwards, so from pyruvate back to glucose, so the pyruvate to phosphoenolpyruvate so that is the glycolytic direction is not reversible by the same enzyme, pyruvate kinase will not go back in this direction. So that happens by two other enzymes pyruvate carboxylase to oxaloacetate, which is an intermediate in citric acid cycle that we will see soon.

And then a carboxykinase that finally forms the phosphoenolpyruvate. So this is one bypass okay, so bypassing the glycolytic direction of P P2 pyruvate a two enzyme based step and both require energy. And the second one is this fructose 6-phosphate to fructose 1,6-bisphosphate formation by PFK-1, phosphofructokinase. So that again is not reversible and therefore, you have a separate enzyme that does the job, opposite direction.

Then glucose 6-phosphate to glucose. So that is also by a separate enzyme. So these three steps, glucose to glucose 6-phosphate, fructose 6-phosphate to fructose 1,6-bisphosphate and 2-pyruvate. So these are irreversible reactions, which proceed with large negative free energy change. And the whole glycolysis itself, there is not any appreciable free energy change at all, it is nearly delta G is zero.

So due to this, the forward direction, glucose to pyruvate is essentially irreversible. And for the gluconeogenesis therefore a reciprocal pathway involving 1, 2, 3, 4 enzymes catalyzing these three steps are involved. So this is how these two pathways, these two directions are kept separate in the same cytoplasm where these enzymes are.

So the regulation is primarily by regulating the activity of these enzymes, these you know these specific steps, which are bubble like structures in this pathway. So this is the primary point we need to know about gluconeogenesis. So we are not going to since it is an introductory level biochemistry, we are not going into the details of these you know these enzyme mechanisms and in which tissue under what conditions they occur, etc.

The primary one brief point I want to make is glycolysis and gluconeogenesis, both in a multicellular organism like humans is regulated by hormones as well. Like for example, epinephrine, which is produced by adrenal gland, glucagon and insulin

produced by pancreas. So they play a major role in regulating these enzymes, okay. At an immediate term level the enzymes are regulated allosterically.

And in the long term, the genes involved in producing these proteins their expression is regulated, okay.

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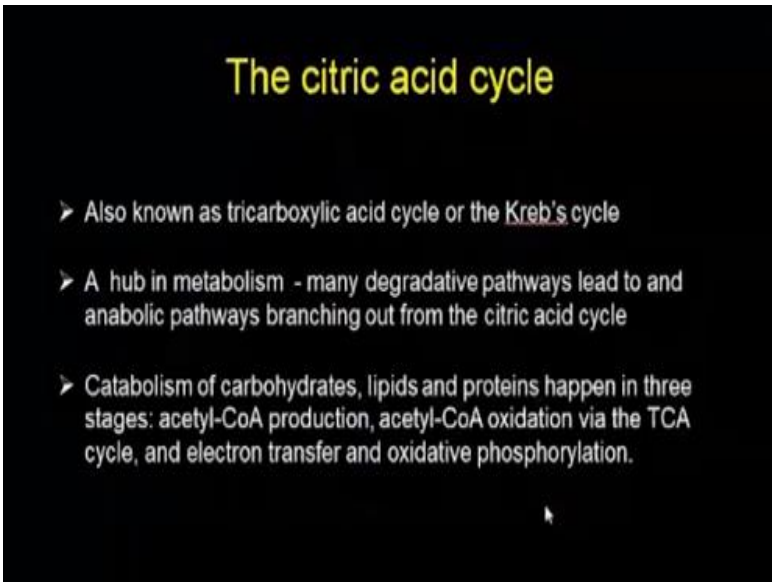

So now let us move on to the next step. So we saw the pyruvate formation via glycolysis. Then we also saw what are the fates of pyruvate. For example, in our muscles under anaerobic conditions, it will be reduced to lactate by lactate dehydrogenase, okay. And then we saw in yeast pyruvate decarboxylase and alcohol dehydrogenase will produce ethanol.

So that is how pyruvate gets reduced via acetaldehyde and involving a decarboxylation step. And other than these two, so in neither of these two it is not fully oxidized to carbon dioxide. So in the presence of oxygen or it gets oxidized to carbon dioxide. So how does that happen? That is where we are going to see citric acid cycle, okay.

So let us first go through the steps of this cycle and then I will briefly discuss about why such an elaborate way of oxidizing or the two carbons of pyruvate. So one of the pyruvate is a 3-carbon molecule, but one of them gets readily decarboxylated as we saw in yeast by pyruvate decarboxylase. So the remaining two, the acetyl group, why oxidizing? That should be an elaborate thing, we will discuss at the end.

So first let us go through the cycle. So this is known as citric acid cycle because citric acid is produced as an important molecule in this cycle. And second citric acid and other some of the other intermediates are 3 carboxyl groups and therefore this is called tricarboxylic acid cycle or TCA cycle, okay.

So T and then this C and then this A okay, TCA cycle. Or it is also known as Krebs cycle in honor of the scientist who elucidated this pathway first. So this is a hub in metabolism, okay. So this is a major cycle at the center of the metabolic pathways, various metabolic pathways. So many degradative pathways like glycolysis, they end up in one of the intermediates of citric acid cycle.

And many anabolic pathways take off the intermediates from the citric acid cycle for the biosynthesis of various molecules. So this is the reason why this is called a hub in metabolism, okay. So catabolism of meaning breakdown of carbohydrates, lipids, proteins, all of this produce intermediates of this pathway, okay. So the degradation of these compounds, these three macromolecules involve three stages.

The first is production of the acetyl-CoA, which is the one that is going to enter into TCA cycle. And the oxidation of this acetyl-CoA to carbon dioxide and this is what happens via TCA cycle. So when we are going to talk about TCA cycle what is accomplished is this okay, acetyl-CoA gets oxidized to carbon dioxide. And this is you know oxidation involving large free negative free energy release of a negative delta G.

And that is taken up, the electron flow. So remember the biological oxidation reduction means we are actually talking about an EMF. That is flow of electrons from molecules that have low affinity for electron to molecules that have high affinity for electrons. So here the ultimate molecule that is going to take up this is oxygen that is going to become CO2. And the electron flows from glucose, if we are talking about glycolytic pathway. So at least we can take acetyl-CoA which is common, okay.

So this is what is happening and that so therefore here oxidation means electron is being the electrons are being transferred from these molecules. And where does that

go? That goes into temporary electron carriers. We already know NAD and FAD and those two are the main ones here. So that is accomplished in the second step.

The third step is those electrons in the NADH and FADH2, so they are ultimately transferred oxygen via the oxidative phosphorylation in the mitochondria, which sets up a proton gradient. And that proton gradient is used to make ATP, okay. So again, the three steps are the first is, from the macromolecules you produce acetyl-CoA. Then the acetyl-CoA is oxidized via TCA cycle.

And in that process, electrons are obstructed and those obstructed electrons in the third step flow via oxidative phosphorylation to oxygen. So these are the three major steps of biological oxidation, okay. So now, so therefore in all of these things TCA cycle is an intermediate. So do not think TCA cycle only as an intermediate step in glucose oxidation, okay.

So many a times people think that way because they begin with glucose, go to pyruvate and then from pyruvate to acetyl-CoA and then go through TCA cycle. But the acetyl-CoA need not come only from pyruvate. It can come from multiple sources namely lipids as well as proteins, okay. So with this background let us get into the individual steps.

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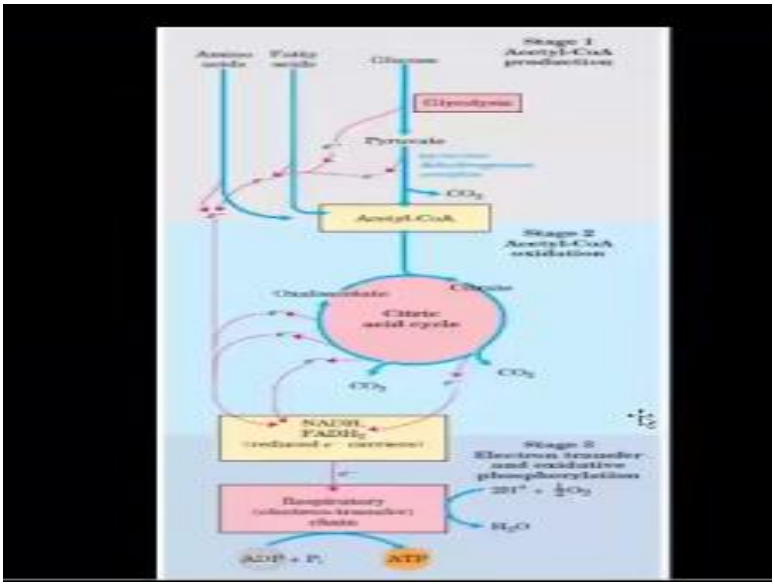

Okay, here is one more, this one I will try to copy paste and blow up a little bit so that it is little bigger.

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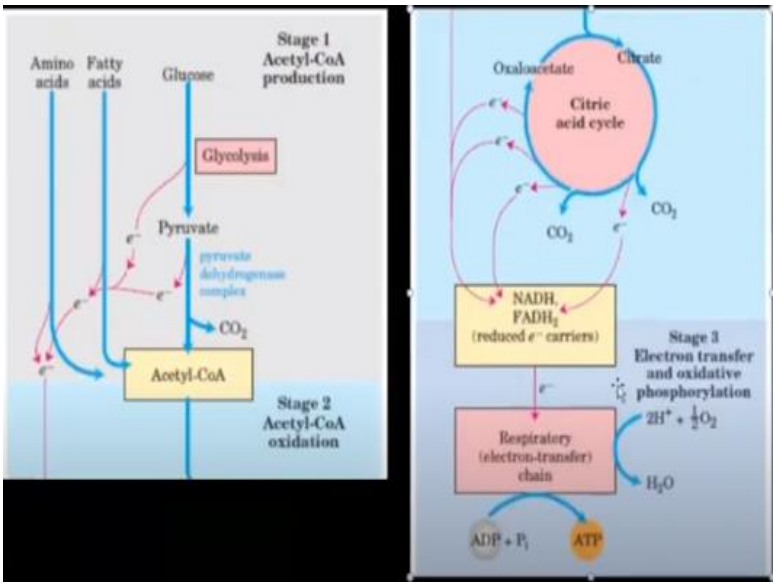

Yeah, I think it is little better, better than that full thing. So as you can see here, so this we already know the central one, glucose to acetyl-CoA. So this we have not yet discussed, we are going to do it right now and they probably begins in the next slide, pyruvate acetyl-CoA. Fatty acids come to acetyl-CoA, amino acids to acetyl-CoA. So this is the acetyl-CoA production, the stage 1.

Then in stage 2, the acetyl-CoA is oxidized. You see the two carbons in acetyl-CoA go as carbon dioxide via the TCA cycle and the electrons abstracted and the electrons abstracted from this. Remember the glyceraldehyde 3-phosphate dehydrogenase step where we have NADH plus H+ produced. So that is what is this and this we will see soon. And these we are not going to see right now.

And all these electrons are now stored temporarily in these electron carriers. Remember this niacin, vitamin B; flavin, riboflavin vitamin B. So from these ones through the electron transport chain or respiratory chain in mitochondria, these electrons finally you know go to oxygen and during that a proton gradient is set up and that proton gradient drives the ATP synthesis.

So this is how all these oxidation and the energy is now converted into this universal energy currency in the cellular metabolism. So in this process, if you look at it, then you realize why this is a hub. All of them flow into this and from this is where

electrons go to the electron transport chain for ATP biosynthesis, okay. So there is oxygen in this process.

It is reduced to carbon dioxide sorry water, I mean to say water and every time I earlier also told carbon dioxide, okay. So to give you a preview of what we will see later in photosynthesis, in photosynthesis, this reverse happens. Water is split and usually 4 water sorry 2 water molecules are split and you get 4 electrons and they go into the photosystem and releasing oxygen. So water is the one from which electrons are taken.

And now you do all of that and make food and the food is oxidation of mitochondria and the electrons go back to that oxygen converting back into water. So it is simply looking philosophically as long as sun's energy falls on earth, this electron flow from water to oxygen or oxygen to water keeps happening all the time, okay.

So this sort of clinically summarizes what happens in living systems. So now let us focus on this, pyruvate to acetyl-CoA.

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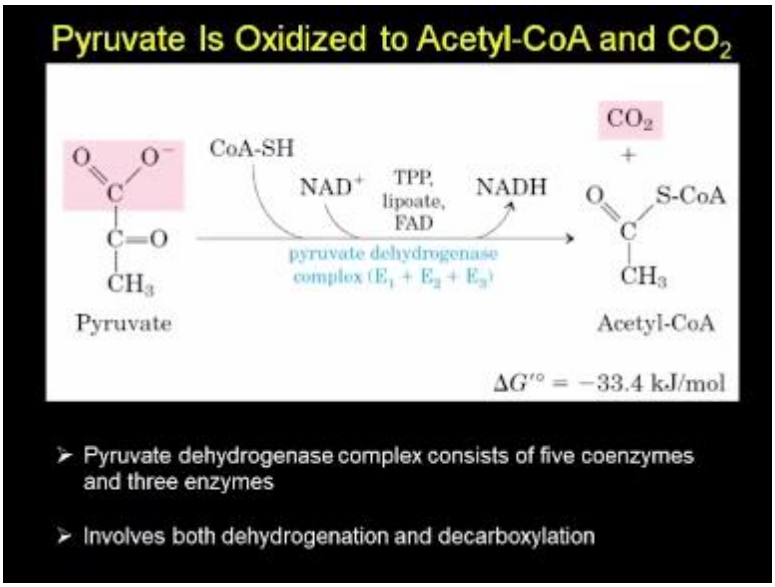

So this is like a humungous enzyme complex, okay. This enzyme complex is bigger than a ribosome. So we will see the complex in all its glory in the couple of slides later. First, let us get a bird's eye view of what is accomplished here. So what is accomplished is a decarboxylation and dehydrogenation, okay. Electrons are removed as well as carbon dioxide is also released from this molecule.

So this carboxylic acid part is where the CO2 comes from. So that is a decarboxylation step. And second, this aldehyde carbon is oxidized to carboxylic acid and that is in ester bond, a thioester here. This is an SH and this carboxylic acid group, okay. Carboxylic acid, in carboxylic acid the carbon's oxidation number is lower, meaning it is more oxidized than the aldehyde carbon that is here.

So the electrons obtained in this is gone to reducing NAD+. So you remember it is two electrons and a proton, it is hydride transfer. So this is what is accomplished. So electrons are taken into oxidizing NAD. So we have temporarily electrons captured here and there is a decarboxylation. This is what is accomplished in this. And to accomplish this, we have three enzymes; we will see them in the next slide.

And second, there are five vitamin sorry four vitamins and another coenzyme. So five coenzymes are involved in it. So I will list them all one by one as we go through. So this proceeds with large negative you know free energy chain. So this is irreversible as we saw that go in the opposite direction you need separate enzymes, okay.

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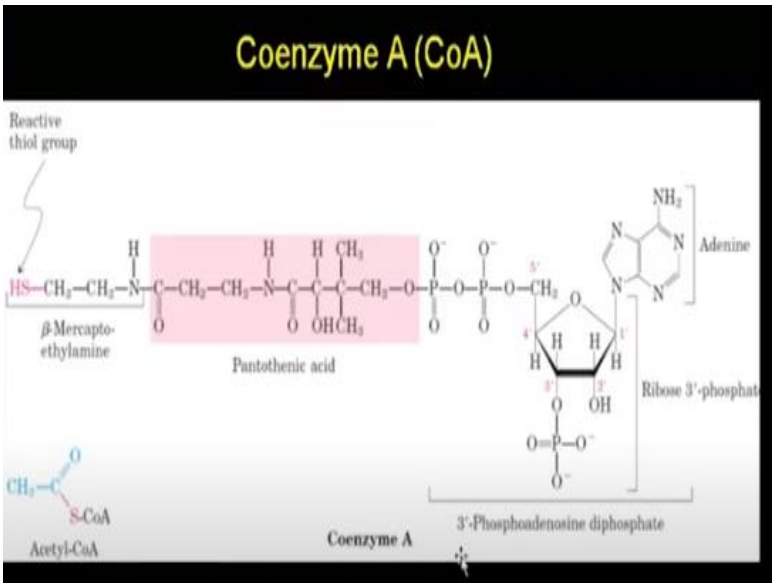

So the five coenzymes, the first one we will begin here. So in the B complex vitamins we have seen so far niacin as part of NAD. Riboflavin as part of FMN and FAD. Then we saw thiamin as part of TPP involved in pyruvate decarboxylase in yeast. And this is the fourth one, pantothenic acid okay, so the highlighted in pink here. So this is an important B complex vitamin.

So just like the thiamin's absence will be very, you know have serious consequences often death, very similar thing will happen if you do not have pantothenic acid. So these are readily available in regular you know a mixed plant diet. And as a result, we normally do not suffer these deficiencies. So that is how evolution has selected these molecules, okay. So nothing in biology makes sense except in the light of evolution.

So you need to remember the unifying principle in biology is evolution. Regardless of which branch of biology you are going to learn, how narrow specialization that is, if you are unable to fit that in the context of evolution, then you have lost the big picture. So always you need to view in that angle. So why these molecules are used and why you do not suffer the deficiency readily? Because that is how the life has evolved.

It has evolved by using what is ubiquitously available around. And remember, again if we go to periodic table, we have more than 105 elements, but we have encountered only a few of them. All of them are probably in this molecule S. You have carbon, hydrogen, oxygen, nitrogen, and sulfur and phosphate. That is it. This is what we have repeatedly seen in the last 20 lectures okay, the only these elements.

So the pantothenic acid we are seeing here as part of this coenzyme A. So this coenzyme is that cofactor participating in the pyruvate dehydrogenase. So it contains a familiar nucleotide here, adenine to ribose. And only difference here you have is a 3-prime-phosphate, and then 5-prime-diphosphate. And to that you have the pantothenic acid to which you have a sulfhydryl group in the form of beta-mercaptoethylamine.

So this is the business end of this whole molecule, okay. This sulfhydryl group is what is important for its function. So that is where the acetyl group taken from pyruvate is going to be in ester bond. So this is an alcohol group, thiol you know sulfur based alcohol. To that, that aldehyde carbon which is oxidized to carboxylic acid that is going to be in ester linkage.

So that is the end product that is going to form. So end product is actually the two carbons of the pyruvate attached to this sulfhydryl group of this coenzyme A. So this whole molecule abbreviated simply by these three letters here, okay.

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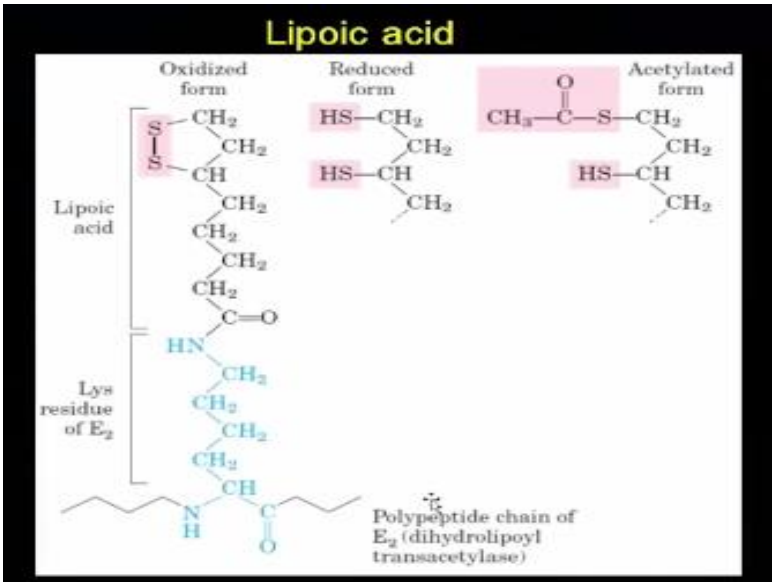

And the next coenzyme which is not a vitamin, which we are about to encounter, which is very critical for this pyruvate dehydrogenase complex is this lipoic acid. So lipoic acid has the ability to do two things. One, it can carry this or you know acetyl group as a thioester on it just like what we saw with coenzyme A. And this disulfide bond like 2 cysteine can become, they now can form disulfide and become a cysteine.

Like that it can be oxidized into this or reduced into this. Meaning it participates in reversible oxidation reduction. In turn means can take up and donate electrons, okay. So it can do these two things and both are important for pyruvate dehydrogenase temporarily carrying the acetyl group as the thioester at the same time temporarily carrying the electrons in them.

So when it is going from this to this two things have happened. One this is reduced to thiol groups. And two so thereby electrons have been taken up and two, one of them forms an ester with the acetyl group. And this has a long arm. So you have 1, 2, 3, 4, 5, 6, 7 carbon. Only the last three you have this disulfide bond and it is a carboxylic acid here. And this is an amide linkage with the amino group in the side chain of lysine.

So this is the epsilon, okay or epsilon NH2 of the lysine, to that it is attached. So this is called lysyl lipoyl group. And this lysine, where is this lysine coming from? Obviously it is part of a protein and that protein is one of the three enzymes in the pyruvate dehydrogenase complex. I will name all three one by one when we go into it. So it is to an enzymes amino acid side chain covalently linked.

And you see is the long arm and this long arm allows it to swing from one active site to another active site, merely a 5 nanometer distance it can swing this acetyl group and electrons from one to the other. So coenzyme A, now we have seen lipoic acid.

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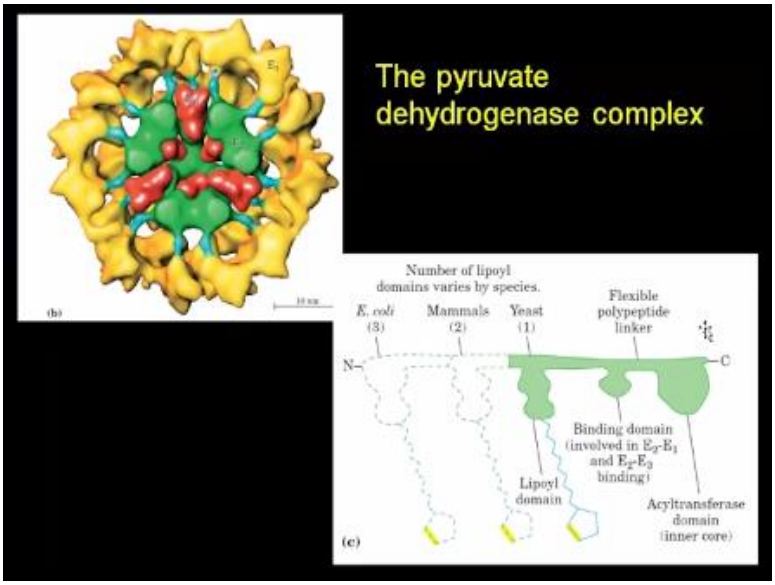

And the third is thiamine pyrophosphate. I have not labeled here because we have already learned in detail about thiamine pyrophosphate. And the fourth one is FAD, flavin riboflavin we already know. Fifth one is NAD you know niacin based. So that is the fifth one. So since TPP, FAD and NAD we have already learnt, I am not going into the details here. The two new ones we have seen are lipoic acid and coenzyme A in which the vitamin is the pantothenic acid.

So essentially we have seen two of them with sulfur as a major thing in them. So now let us look at the enzyme itself. So this structure comes from you know several studies involving cryo electron micrograph as well as microscopies and crystal structure solving and all put together this is a summary model, okay. So here this enzyme I told this enzyme complex has three enzymes.

And those three are in three different colors here. So one is in the outer. So that is the pyruvate dehydrogenase. And then this green one is the lipoyl transacetylase, dihydrolipoyl transacetylase and the third one the red is the dehydrogenase, dihydrolipoyl dehydrogenase. So all three we will see when we get into the reaction itself. But for now we will look at the enzyme structure.

So this enzyme has sixty polypeptides of the second enzyme labeled E2 here okay, arranged in this fashion. So this is supposed to be a pentagonal arrangement of dodecahedron. So I do not even know how to imagine that structure and that structure is cut in half here to reveal the inner structure. This is like a cross section. So the main thing we want to focus is this E2.

So this is the one that is really responsible, which plays a pivotal role using the lipoic acid moiety. So I will come back to that and explain how all of this works after we look at the reaction sequence, okay. And one detail about the structure itself is shown in this cartoon. So this cartoon shows you this E2 enzyme, this transacetylase, meaning it takes the acetyl group from one and gives it to another one, okay.

So that is the transacetylase job. So far that the lipoic acid plays a major role and the lipoic acid I told you is attached to a lysine residue. And that lysine residue is in one domain of this E2 polypeptide. That is this green domain. In yeast there is only one of them. Mammals have two. E. coli have three. So that is why these are shown in dashed lines.

And in that domain, this lipoyl domain is where the lipoic acid is attached via a lysine residue. So the lysine side chain plus lipoyl chain together is this tether like long structure and this is the disulfide this portion and this whole thing is this tail. And this portion okay, so this portion is what is this blue that you see here. So the three domains, this comes from the E. coli enzyme.

And this asterix here where I am putting the cursor, and that is where the lipoate or the lipoic acid is attached. And you see that is in good contact with the dehydrogenase where the acetyl group is going to be taken from pyruvate on to thiamine

pyrophosphate very similar to the pyruvate decarboxylase that we learnt in a ethanol fermentation in yeast.

The acetyl group is taken as acetaldehyde in activated form hydroxy ethyl structure that we saw in the last class. And that is what this yellow enzyme is going to do. Yellow enzyme is called pyruvate dehydrogenase. That is the one that gives the name for the complex itself, which behaves more like pyruvate decarboxylase.

In that it takes the acetaldehyde group on the thiamine leaving to the carbon-carbon bond cleavage leading to decarboxylation. So that we saw in the last class. And that acetyl group is taken up by this when this gets reduced, and it can readily swing to the next enzyme. So this is the main function of this long arm. It can swing from one active site to its own active site to then the red enzyme's active site.

So and like this, it happens in multiple places here all around. So that is why it is a major complex. So the three functional things are one molecule of E1, one molecule of meaning one polypeptide of E1 or one polypeptide E2, one polypeptide E3. But they do not function as a trimer, instead they exist as a big complex. In that E2 itself is 60 subunits okay, 60 polypeptides of E2 are present.

And that is why this whole structure is bigger than a ribosome. So you look at the scale for it, 10 nanometers. For a molecule this is a whole lot. Normally we talk about bond distances in angstrom units, but this is about 100 times that dimension. So it is so big that in an electron microscope you can view it, you can actually visualize it or you can take a picture of it using an electron microscope.

And okay, what are the other domains and the second domain of E2 is the one involved in interacting with E1 as well as the E3 the red one and the third domain is its own active site acetyltransferase domain. So that is the inner course. Therefore, it has three important domains, okay.

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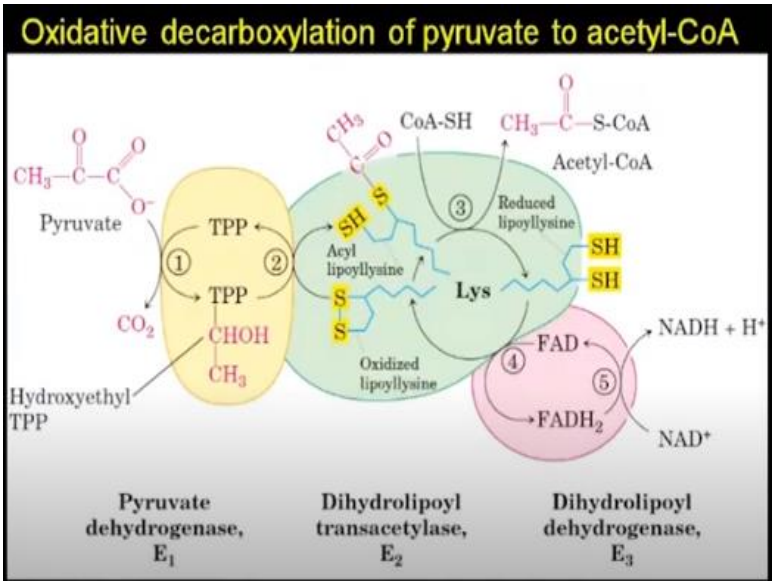

So this is the reaction itself. It looks complicated, but it is exceptionally simple if you look through step by step. This is something we are already familiar with. This thiazolium ring has one carbon that is carbanion, which is that carbon is weakly acidic. Therefore, it readily becomes carbanion.

And that has an you know nucleophilic attack on this carbonyl carbon and that is how this group these two carbons are taken in the form of an activated acetaldehyde on thiamine pyrophosphate that is this hydroxyethyl TPP. So this we already know in that pyruvate decarboxylation reaction mechanism. The pyruvate dehydrogenase, this E1 is identical to that. The only thing now we are adding is this.

So there this they got transferred, this got further reduced into ethanol okay in the ethanol fermentation in the yeast. So here and there it was alcohol dehydrogenase. That is the second enzyme. So here from the pyruvate dehydrogenase this group is transferred in the form of acetyl, meaning this aldehyde gets oxidized into acid group carboxylic acid.

And the electrons transferred have gone into reducing this disulfide into two thiol groups. And one of the thiol group now becomes an ester linkage with the oxidized aldehyde carbon here. So this is done by the second enzyme, which is this huge complex that 60 subunit this green stuff. And that is the dihydrolipoyl transacetylase. Transacetylase because it takes the acetyl group from here and then in the next step it transfer to coenzyme A.

So remember this coenzyme A we just saw here. So essentially it is given to this in this form and from here it is transferred to this. And that is what is happening here. So this coenzyme A is that big structure. So this is simply a transesterification from one thiol group to another molecule's thiol group. So this is an oxidation step aldehyde to carboxylic acid and as well as electron transfer.

So that is why this is called dehydrogenase instead of being called decarboxylase. And that high energy conserved here is what is used for this transesterification to make acetyl-CoA and acetyl-CoA is released. Now so we have seen the first step, second step, then the third transfer to this. And fourth and fifth are primarily to bring back to the original shape of the enzyme.

Remember, it is a catalyst. It does not undergo permanent change. It is only a transient change. So when acetyl-CoA is released, what you end up getting is a reduced this lipoic acid. So this is sulfhydryl group, thiol groups and that has to be oxidized again. And here the electrons taken from here that gets transferred to temporarily to FAD. So FADH2 is produced. It is reduced to FADH2.

That is by the third enzyme dihydrolipoyl dehydrogenase because it is dihydrolipoyl it is dehydrogenating it. And from that so it is a flavo protein, flavin is the tightly bound prosthetic group here. So here we are seeing the fourth coenzyme. So one okay, so then two, then the third, then fourth. And from there it is then transferred to NAD and that is reduced to NADH.

So the end product is pyruvate has become acetyl-CoA and then NAD is reduced to NADH. So this is the pyruvate dehydrogenase complex reaction steps. We have not actually gone into detailed mechanism. We only got some superficial understanding of the mechanism that gives us some feel for what is happening.

So one important thing in this kind of a major enzyme complex that we need to understand is this concept of substrate channeling, okay. So what is substrate channeling is, now which is the substrate here, which is the product. The very original

substrate is pyruvate and the ultimate products are carbon dioxide, acetyl-CoA and NADH. And another substrate is NAD itself.

So these two are going in and 1, 2, 3 are coming out, right? But if you look at the individual enzymes, so for this enzyme the substrate is this. And the next step substrate is these two and so on. So in this kind of a complex the product of one enzyme is the substrate for the next one. And in between the product is not released into the surrounding media meaning the cytoplasm or wherever this is happening.

We need not, it happens in cytoplasm we do not need to take that seriously here. So wherever it happens, it does not get, it does not diffuse away from the enzyme surface. Because they are all tightly bound with single complex from one directly to the next one and next one and so on. This sort of direct transfer of substrate on the enzyme surface from one active site to another active site without ever releasing or diffusing out of the enzyme is what we call a substrate channeling.

This ensures very high concentration of substrates for subsequent enzymatic steps because it is never released into the open solution. It is on the surface of the enzyme molecule itself. So the effective concentrations are very high. So the reaction very readily happens. That is one.

Second, the other enzymes that could use this intermediate substrates, like for example, this acetyl group activated acetyl group can be a substrate for another enzyme that uses this activated acetyl. And that enzyme does not steal from this complex, because it is never released into solution. It all happens, all the transfers happen on this clustered enzyme complex itself on the surface of the complex.

And that is what we call as substrate channeling. It is an important concept so remember, make sure to refresh this discussion and try to remember again. This occurs in other enzyme complexes as well. So this is probably the most important and biggest enzyme complex that we will read at this detail, learn at this detail and that is why I took you know plenty of time to go through this slowly, you know just one step.

So essentially what we have done is just this one step. So we did not breeze through it in a minute and that is primarily because to give you a flavor of what actually happens in biochemistry or in your cell, okay. So now that we have produced acetyl-CoA, so let us revisit and refresh our memory again.

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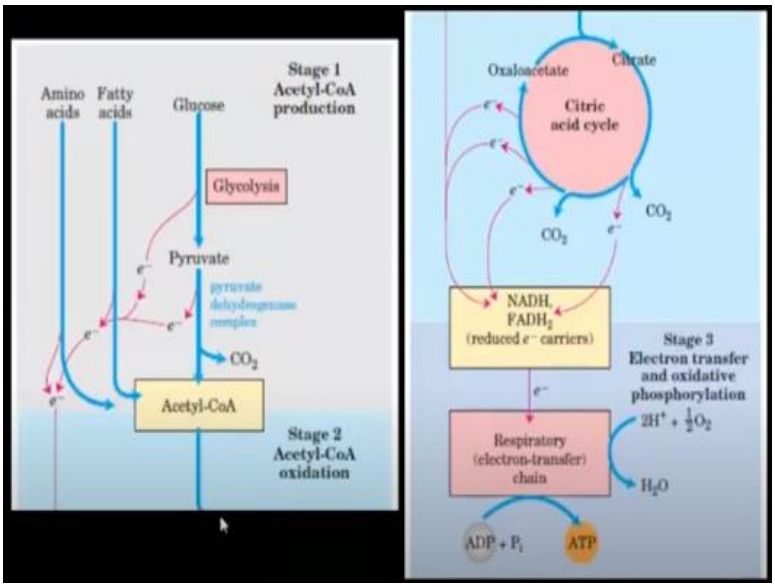

So we have produced acetyl-CoA from pyruvate. So we have slowly moved from glucose to acetyl-CoA. So that is going to enter into citric acid cycle. So now we will actually see the citric acid cycle.

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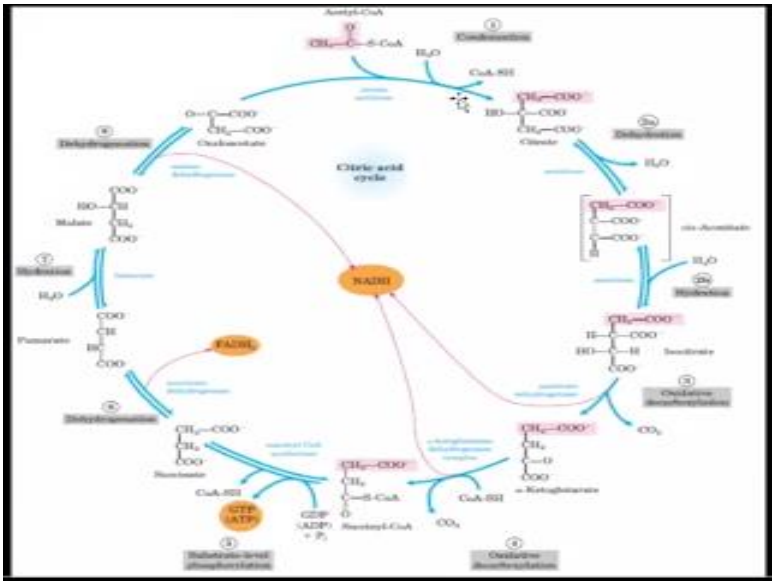

So this is the cycle itself. We will of course be seeing one step at a time. So this slide is here just to show you the overall picture. And this is little shorter than glycolysis, not 10 steps it is only 8 steps. So the when you have learnt it for quick refreshing of

your memory you can always look at this slide. It has all the names of the reactions what is actually happened in the grey shaded text.

Then it also tells you where the electron has gone from three steps you make NADH, one step you make FADH, FADH2 and then there is one substrate level phosphorylation again. So substrate level phosphorylation is another important concept that we have learnt already in glycolysis, okay. So this gives you a quick overview and the enzyme names are also there.

So before the exam you can quickly look at this slide instead of going through all the slide individual slides of this TCA cycle.

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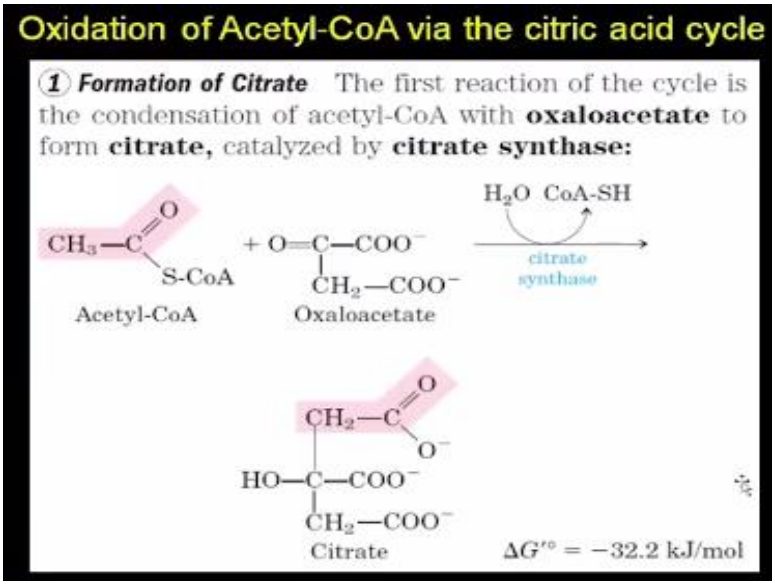

So the first step is acetyl-CoA combines with a molecule called oxaloacetate, okay. So this is the, this step oxaloacetate. So this is what we are looking at right now. So at the end this oxaloacetate is, you know generated again and that is why it is a cyclical reaction. So it is like a cycle. So this so it is attached to oxaloacetate at its this carbonyl carbon and it is the methyl group carbon that gets attached, okay.

So this is really made possible by the high energy available in this thioester. Remember, thioesters do not have the resonance that is possible with oxygen ester and as a result this high energy molecule and that enables citrate synthase to produce citrate from this. And you will count the carboxylic acids here 1, 2, 3. That is why it is a tricarboxylic acid cycle.

And since citrate is the main molecule produced here, in the very first step, it is also called citric acid cycle. So the coenzyme A is released. Basically this is hydrolyzed and that this carbonate group is what is attached here.

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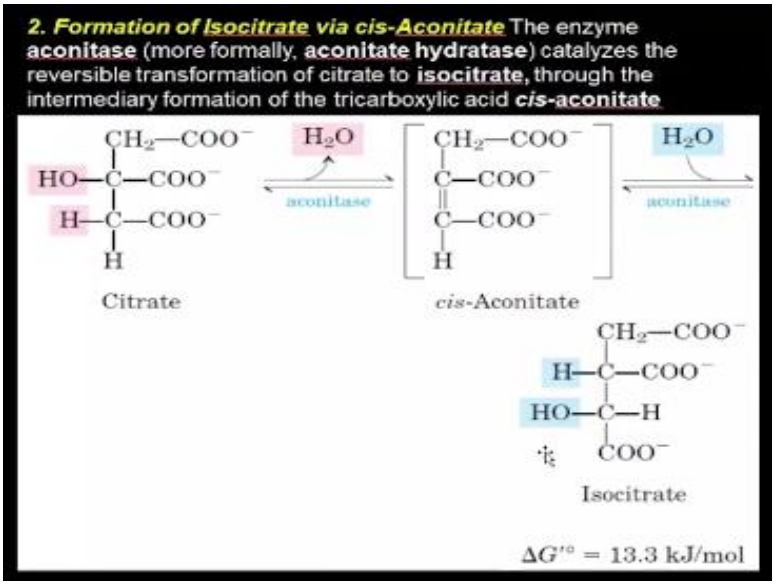

Then the next step is citrate undergoes internal rearrangement like the shaded hydrogen and hydroxyl group get swapped between these two carbons forming isocitrate via this cis-aconitate intermediate. So I promise you, you only will need to learn the reaction mechanisms of glycolysis. I am going to skip the reaction mechanisms here even if they exist in the slide, okay.

So Lehninger expects you to learn all the reaction mechanisms, but I understand you know this is biochemistry one course in four year program, so I do not want to get into the details of every reaction mechanism. So aconitase is the enzyme that makes this rearrangement, citrate to isocitrate. You will realize why this rearrangement is important when we go to the next two steps.

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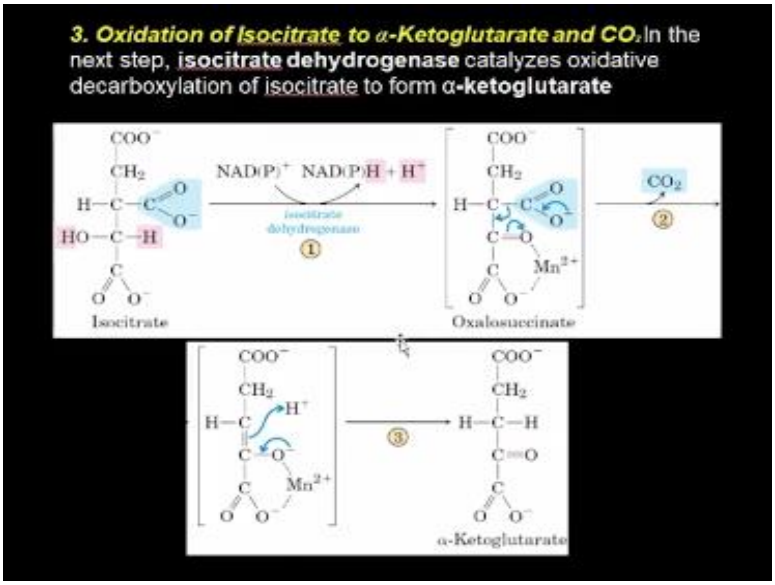

So the next step is isocitrate to alpha-ketogluterate. So two things happen here, one is dehydrogenation. So the enzyme name is dehydrogenase, isocitrate dehydrogenase. And so the electron transferred is used to reduce the NAD or in some enzymes in some tissues or organisms it is NADP. So one of these two cofactors, they get reduced. And then you have a decarboxylation as well.

And that is why so when you lose this you have the carbonyl and then when the double bond switches then you have this carbon dioxide released and then you have this carbonyl carbon and this is alpha. So this is the carboxylic acid, alpha, beta, gamma. So it is alpha-ketoglutarate because if you add an NH2 across this, this will be glutamate, okay.

So that is why this is called alpha-ketogluterate. So that is the end product of isocitrate dehydrogenase or dehydrogenation decarboxylation step.

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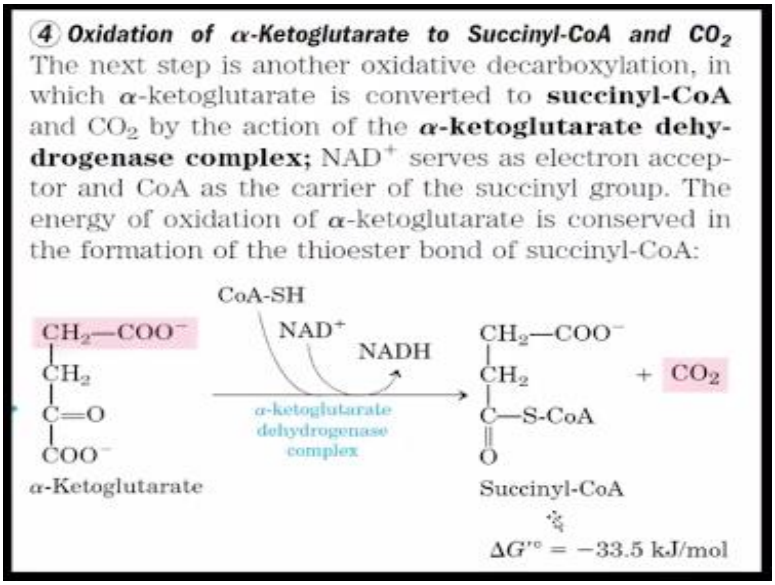

And the next again is a dehydrogenation decarboxylation. Quickly we have lost both the carbon dioxides you see. But they are not directly coming from the right away from acetyl-CoA. So for that we have to go through one full cycle. So here has alpha-ketoglutertae to succinyl-CoA is identical to our pyruvate dehydrogenase complex, okay. So there again we had an alpha-keto acid.

Now the pyruvate had a carbonyl group and a carboxyl group. And there again this after decarboxylation of this carboxylic acid group, this is an aldehyde which gets oxidized to an acid group which is in ester linkage in the coenzyme A. And ultimately the NAD is the one that takes up the electron although we have 5 cofactors. So it is very similar.

This alpha-ketogluterate dehydrogenase complex is very similar to pyruvate dehydrogenase complex that we just learned. And that is how alpha-ketoglutertae become succinyl-CoA. So there the end was acetyl-CoA. Instead it is succinyl-CoA. So the part of that oxidation energy is conserved in making this high energy thioester bond here, okay. So that is how succinyl-CoA is produced by alpha-ketogluterate dehydrogenase. So fourth step is perfectly fine.

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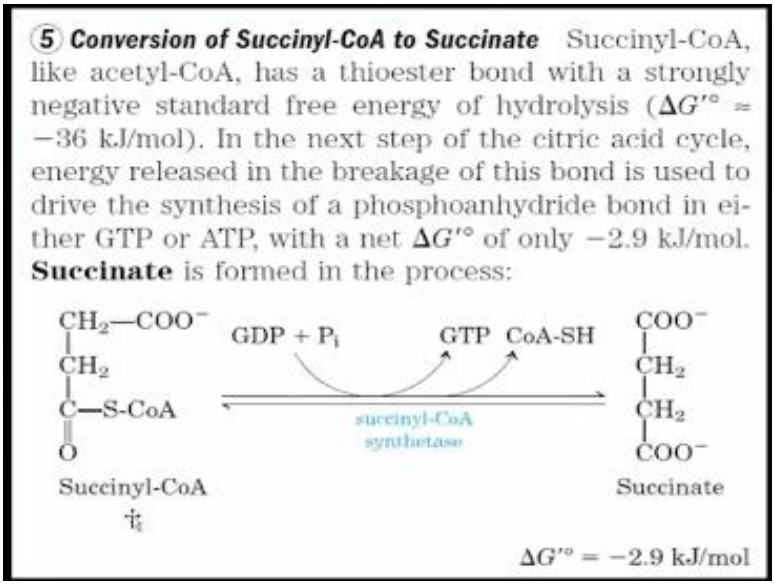

And the next one is a substrate level phosphorylation, where that high energy stored in the thioester is used for making an anhydride between the inorganic phosphate and the phosphoric acid that is part of GDP in this enzyme but depending on the which enzyme is used it can be ADP as well. So here GDP is converted into GTP and coenzyme A is released. And in the process succinyl-CoA become succinate, okay.

So this is the fifth step. So tomorrow we will continue from succinate back to oxaloacetate and then we will see some other aspects of citric acid cycle also and tomorrow we will complete citric acid cycle. So if there are any questions, feel free to ask.