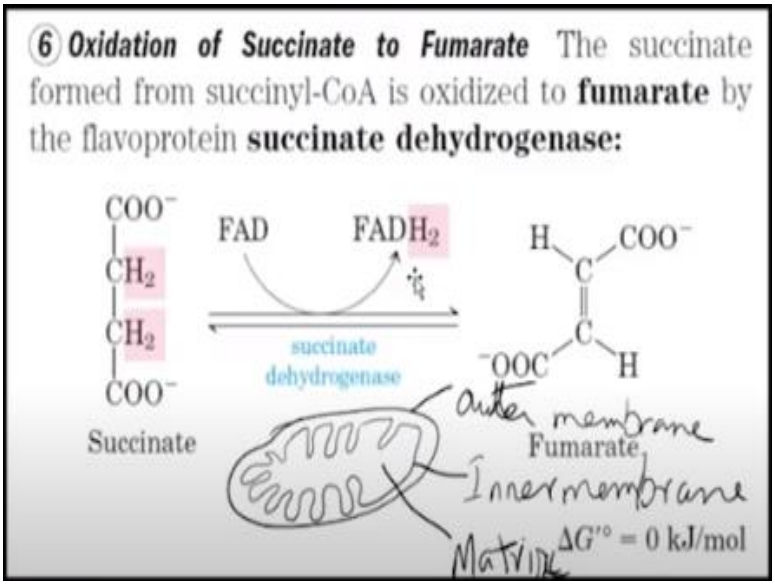
Introduction to Biomolecules
Prof. K. Subramaniam
Department of Biotechnology
Indian Institute of Technology-Madras

Lecture - 21
Citric Acid Cycle (Part 2/2)

(Refer Slide Time: 00:14)



So let us start with answering the question. So there is one question about substrate channeling. So substrate channeling is when you have multiple enzymes as a single complex. What I mean by this is you know the multiple enzymes let us say multiple polypeptides, when they are all bound together into a single complex through covalent or non-covalent interactions when they are present as a single complex.

And when you have a particular reaction going on in the active site and if the product of that reaction becomes the substrate for another active site and if this product without diffusing into the medium you know whether it is mitochondrial matrix or cytoplasm, without diffusing into the surrounding medium on the enzyme surface itself when it directly passes to the next active site okay, that is what is substrate channeling.

So in the pyruvate dehydrogenase complex that we discussed, so you have the first reaction where you have the pyruvate dehydrogenase, the product of that is the acetaldehyde carried on the thiamine pyrophosphate okay in the hydroxyethyl format

and the two electrons abstracted from the substrate. So these do not get diffused into the medium and then the next enzyme takes up from the medium.

That is in this case mitochondrial matrix. So instead directly on the enzyme surface itself from the pyruvate dehydrogenase active site it moves on to the dihydrolipoyl transacetylase active site where the acetyl group by the time it is oxidized to carboxylic acid and it is in thioester form and that linkage is transferred to coenzyme A.

Now this coenzyme A diffuses into the medium, but the two electrons taken there which is in the, you know sulfhydryl form the reduced lipoic acid moiety directly goes to the next enzyme okay, which is the dihydrolipoyl dehydrogenase. So this sort of product from one active site directly on the enzyme surface itself moving on to the next active site, this is what we call as substrate channeling.

So the advantage of this is the effective concentration is very high because once it gets into the medium then it depends on the its diffusion rate and the actual concentration in the medium, all of that matters while on the enzyme surface it is nearly like an intramolecular reaction, because enzyme itself is a molecule. So that is one.

The effective concentration is very high and therefore catalysis happens very readily. And second, once it is in medium then each enzyme competes for the substrates present in the medium. So if in that particular example, if the acetyl group is also used by another enzyme that enzyme also will take this acetate group. So that is another reason.

So these are the reasons why substrate channeling has evolved and that is favored for these kind of reactions. All right, so let us continue with our journey of the electrons from glucose all the way to oxygen. So we have now completed our path through glycolysis, pyruvate dehydrogenase complex. By the time we have come into mitochondria from cytoplasm and now we are going through the TCA cycle, okay.

So now the electrons are at the end of the TCA cycle. They are all now in the form of NADH or FADH2, okay. So right in front of us is the dehydrogenase reaction where it is the FAD that is the cofactor. And I want to constantly remind you these coenzymes are vitamins, okay. So that directly connects you to everyday life. You know it connects you to the food that you ate this morning or the lunch you are going to eat.

So these are all like completely unlike many other topics you learn, these are directly connected to your very existence and your very activities, okay? So it is one of the pillars of biology. So take the time and effort required to learn biochemistry thoroughly. The only other course, which is parallel in this importance is genetics. And then, of course to some extent cell biology.

So these three courses, the foundational concepts must be thorough in you for the rest of your life, okay? So make sure to have a good textbook on all three of these subjects biochemistry, genetics and cell biology. Read them thoroughly, regardless of to what extent they are covered in the syllabus for the exam or not, okay. That is important, you need to have good grades, I am not belittling it.

But at the same time, when you graduate with your degree in biotechnology and if these concepts are not clear that will be worse than you know not having gotten a good grade. So make sure you do that regularly. So this is flavin nucleotide, okay. So this is the one that is getting reduced here by oxidizing succinate to fumarate. And this enzyme interestingly is a membrane bound enzyme.

Other enzymes in the TCA cycle they are all in the mitochondrial matrix. So mitochondrial matrix, why mitochondrial matrix? I am not sure whether you have already had cell biology and how much cell biology you remember, but for this topic that is very important. We will see a good picture somewhere but for now I will draw a small cartoon. So let me take this one and try here.

So mitochondria is kind of an oval shaped structure, okay. Once upon a time, it was a bacteria freely living and it entered into a eukaryotic cell and then set up a symbiotic relationship with eukaryotes. So this is the outer membrane, okay. So this is the outer

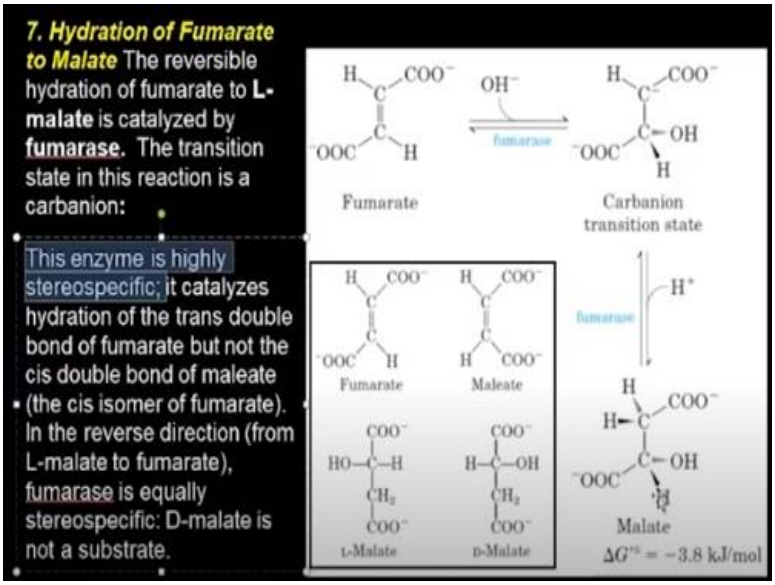
membrane. And then you have an inner membrane which is folded like this, okay. I am not going to draw for the rest of it in the interest of time.

But you understand the inner one is folded, so the surface area is increased. And this is the inner membrane. On that membrane is where all the oxidative phosphorylation we are going to learn are present, the electron transfer complexes are present. And this phase, inner phase is what is matrix, okay.

So the author of this book Lehninger and another scientist by the name Eugene Kennedy, these two are the ones who discovered TCA cycle, oxidative phosphorylation. They all happen in mitochondria, okay. So this is matrix and this is where TCA cycle is happening. So this succinate dehydrogenase enzyme is actually attached to the inner membrane.

So the importance of that will become clear when we go to the oxidative phosphorylation, where we will look at the electron transport chain. So this is the one that oxidizes succinate by a dehydrogenation reaction. So two hydrogen and two electron are transferred. So here the electron transfer is in the form of hydrogen atoms.

(Refer Slide Time: 09:00)



And that fumarate by the action of fumerase becomes malate. Essentially this double bond gets hydrated, meaning a water molecule is added, hydroxyl ion and then the proton here. And this (()) (9:14) is very peculiar, you know it is highly stereo specific.

It catalyzes the hydration only of the trans double bond. See the moment you have a double bond we have this cis trans or geometrical isomerism that we learned long time ago.

So that comes into picture. So only when it is in trans, this enzyme will accept it as a substrate. It will not take the maleate as a substrate. So this is the cis isomer of fumerate. And this is not a substrate for this fumerase enzyme, okay. And similarly, in the reverse reaction it will not take the D-malate and it is only the L-malate. So this is the hydration reaction by fumerase to produce malate. See we are almost there.

This is seven of eight steps and the next is a dehydrogenation of this malate forming oxaloacetate where we started. So malate dehydrogenase uses NAD, the oxidized form you know shown by this plus sign. This is not the overall charge on NAD. It is the sign that that particular nitrogen atom is in the oxidized form. So that is reduced and you have the oxaloacetate.

Essentially, this is a removal of two hydrogens. And here the mode of transfer is hydride ion, two electrons and one proton that is into the NADH. And the other proton goes into the medium. So this is how we get the oxaloacetate back.

(Refer Slide Time: 10:56)

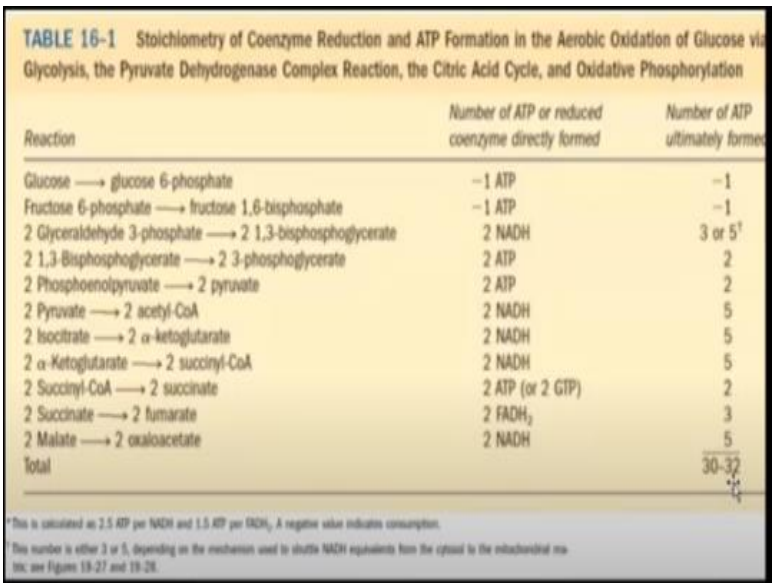


TABLE 16-1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed*
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30-32

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

†This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial ma... trix; see Figures 19-27 and 19-28.

So this table summarizes starting from glucose till completing one cycle of oxaloacetate, okay. In the process what have we done? We have done removal of 3 carbon dioxides, meaning 3 carbons are lost. One, when the pyruvate became acetyl-

CoA. The pyruvate dehydrogenase enzyme acted as a decarboxylase and removed carbon dioxide. Then second, when isocitrate became alpha-ketogluterate.

Then alpha-ketogluterate dehydrogenase reaction ending in succinyl-CoA. So essentially half of glucose has been converted into carbon dioxide by the whole step, glycolysis, pyruvate dehydrogenase complex and then TCA cycle. If you take TCA cycle alone acetyl group, acetyl-CoA entered, meaning 2 carbon atoms CH3CO entered and both of them are gone out in this form of carbon dioxide.

And these are not directly from the acetyl-CoA that enters. Eventually overall it balances. Two carbons entered and two carbons left. So that is the count of carbon atoms here and we see that it is oxidized to reach the highest oxidized state. Then in terms of the energy currency we need to calculate. So glucose to glucose 6-phosphate 1 ATP we spent to energize in the preparatory phase of glycolysis.

Then fructose 6-phosphate to fructose 1,6-phosphate another preparative step, one more ATP. So two negatives. And then we made 2 NADH in the glyceraldehyde. So once it is triose, the number increases to 2 to indicate we are talking with reference to glucose, okay. From one glucose you get 2 glyceraldehyde 3 phosphate.

So when this is cleaved, it is cleaved into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Then the isomerase converts the other DHAP into glyceraldehyde 3-phosphate. So it is 2. And here we produce 2 NADH and that could be equivalent to 3 or 5 electron depending on where the electron transfer happens. Then in this step 2 ATP. Then phosphoenolpyruvate to pyruvate 2 ATP.

That 2 substrate level phosphorylations we saw in glycolysis, okay. That time I told you, substrate level phosphorylation is from substrate the high energy bond cleavage is used to making ATP and the phosphate group is transferred from the substrate to the ADP. This is in contrast to what you will see in oxidative phosphorylation, where the proton gradient leads to the synthesis of ATP.

And there the phosphate comes from the medium in the form of inorganic phosphate. So that is oxidative phosphorylation, this is substrate level phosphorylation. So then

pyruvate dehydrogenase complex produced 2 NADH that is 1 per pyruvate. And then this isocitrate dehydrogenase. Then alpha-ketogluterate. Then this high energy thioester bond of succinyl-CoA its hydrolysis 2 ATP.

Then succinate to fumarate, succinate dehydrogenase, the one that we just saw, the first reaction today the sixth step, 2 FADH2 and that equals 3. Then malate dehydrogenase, again 2 NADH. Converting all this electron carrier loaded electrons in terms of ATP equivalent the total comes somewhere between 30 to 32, okay.

So this is the total amount of ATP available when glucose is completely oxidized to carbon dioxide and the electrons abstracted are used to reduce oxygen into water molecules. So this is the balance sheet as of now.

(Refer Slide Time: 15:33)

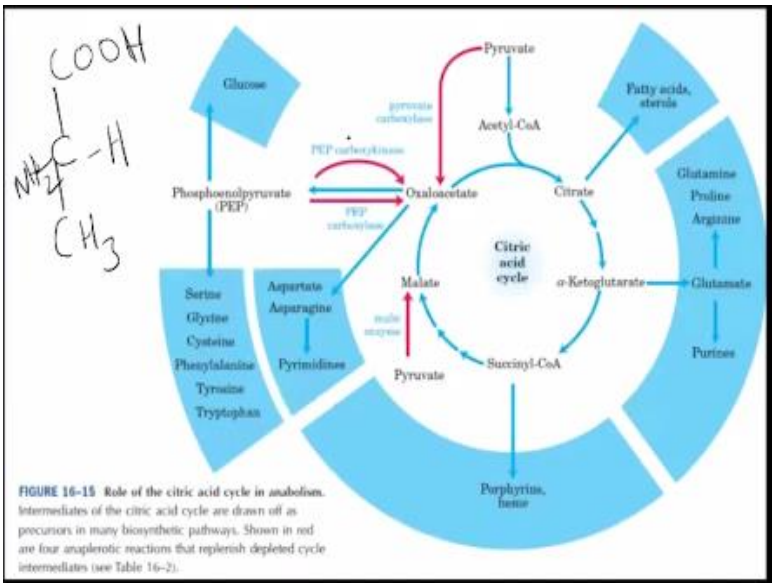


FIGURE 16–15 Role of the citric acid cycle in anabolism. Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways. Shown in red are four anaplerotic reactions that replenish depleted cycle intermediates (see Table 16–2).

All right. So now remember yesterday I told you nothing in biology makes sense except in the light of evolution. So we are going to look at that TCA cycle from this point. So I just summarize. The big thing that has happened in TCA cycle is acetyl group got oxidized into 2 carbon dioxides. Big deal, right. It is, just burn it away and it throws carbon dioxide.

Why all this complex enzymatic reactions you know decarboxylations, dehydrogenations and different kind of molecules producing? Why is all this complexity just to oxidize acetyl-CoA to carbon dioxide? So that is the kind of

question you will ask if you are an engineer designing the perfect energy efficient machine, okay. Biology is not a machine.

Living organisms, although their functions may have analogies to manmade machines, but they never meant to be machines. They are simply progression. Each one of the living organisms is an intermediate step in the progression of rearrangements of matter that is made possible by the sun's energy as permitted by the laws of physics and chemistry, okay.

So there is nobody here designing a perfect energy efficient machine. There is nobody designing the shortest route to convert acetyl-CoA to carbon dioxide. So here what is actually happening is whatever is possible keeps happening in that particular environmental context. And water process in that context helps that organism to survive to the level it can reproduce, okay.

Here success in biology means reproductive success. No organism right now exists whose ancestors were reproductively unsuccessful. Just think you know in an entire lineage of any organism or yourself or another human being you think of, can it or could it have had a reproductively unsuccessful ancestor? It is impossible.

So when we think of successful organism, the fittest to the environment, we are thinking about in terms of reproductive success. Did that organism manage to grow and survive to reach the age of reproduction, and did it successfully reproduce? Because only when it reproduces, its genetics, genes or genetic inheritance is passed on to the next generation and it perpetuates.

In the absence, every organism is a dead end of the evolution. So it is only based on that sort of a context, we understand why this complex pathway to convert acetyl-CoA to carbon dioxide. It is probably you know why these steps may have been happening, one step or two or three steps in an ancestral organism for a different purpose and the pathway could have been linear.

And eventually as oxygen accumulated through the action of the photosynthetic organisms like cyanobacteria, which was the first photosynthetic organism, then

organisms learn to live with the presence of oxygen. They learn to use oxygen for producing energy. And that is how this pathway would have been put together. So this is one.

So this pathway or this way of converting acetyl-CoA to carbon dioxide, provided the advantage for survival in during the course of evolution. So that is how you need to understand this. And second, the purpose of this pathway is not simply energy abstraction from burning a fuel. It is also producing precursors for many other biosynthesis of many other molecules.

That is what this slide focuses on okay, the role of citric acid cycle in anabolism. For example if you take, let us take pyruvate itself. So the pyruvate simply by transamination becomes alanine, okay. So just I will show you, the transamination we will learn at the very end of this course. So say if you take pyruvate. The way we have been seeing the diagram the same way I will draw, okay.

So now at transamination we are going to do here, so remove that and then you are going to have an NH2 and here again H. So that is the transamination reaction, the end product of it. So now what is this? This is alanine, okay. So this is an amino acid. You have a carboxylic acid group and amino group. This the alpha carbon then you have methyl group is the side chain, that is alanine.

So through transamination you convert pyruvate into alanine. And by a very similar logic oxaloacetate here can become aspartate and by the amide bond forms and ammonia group is attached then it becomes asparagine. And these go into making pyrimidines, which are the nitrogenous bases of you know thiamine and cytosine, uracil of our nucleic acids, okay.

Similarly, you make serine, glycine, cysteine, phenylalanine, tyrosine, tryptophan all of that from these intermediates. And citrate goes into fatty acids and sterol biosynthesis. Alpha-ketogluterate another transamination like oxaloacetate forming aspartate, this transamination forms glutamate and glutamate is precursor for purines you know adenine and guanine. And from glutamate you make these amino acids as well.

And succinate is important for porphyrin, which is the you know which is porphyrin rings attached is what is heme and heme is the prosthetic group in cytochromes and hemoglobin where it carries oxygen, okay. So like that the intermediates of TCA cycle are precursors for the biosynthesis of many important molecules, okay. So these so this is another reason why you want to go through this complex pathway of oxidizing acetyl-CoA.

So this is the role of citric acid cycle in anabolism, okay. So this red arrows, we are going to look at it in another probably in the very next slide. So the so all of this is happening in the mitochondrial matrix and glycolysis happens in the cytoplasm. So each all of this or these molecules the intermediates are in a constant flux.

So as we just discussed this alpha-ketogluterate, if glutamate is required and if this is getting siphoned off from the TCA cycle, then this needs to be produced, okay. So each one is connected to other pathways as well. So due to that, there is a constant flux through these networked biochemical reactions.

And therefore the enzyme activities are very tightly regulated to ensure the required concentrations of each of these intermediates to ensure efficient operation of TCA cycle as long as acetyl-CoA is available. Like for example, you do not want to be in short supply of oxaloacetate when you have lot of acetyl-CoA that needs to be oxidized. So therefore oxaloacetate must be produced by some other means.

And that kind of replenishing the TCA cycle intermediates is what is called anaplerosis or anaplerotic reactions, that is here okay, anaplerotic reactions. The noun is anaplerosis. And this is highlighted here. For example, pyruvate by the action of pyruvate carboxylase. Do not confuse with pyruvate decarboxylase, which removes carboxyl group from pyruvate.

Instead here you add another carboxyl group to pyruvate to make oxaloacetate. So that is one way of replenishing oxaloacetate concentration. This is one of the primary anaplerotic reactions, okay. So only the primary ones are marked here. Among them,

this is the most common one. So you can have pyruvate coming from multiple sources, the main one being glucose via glycolysis.

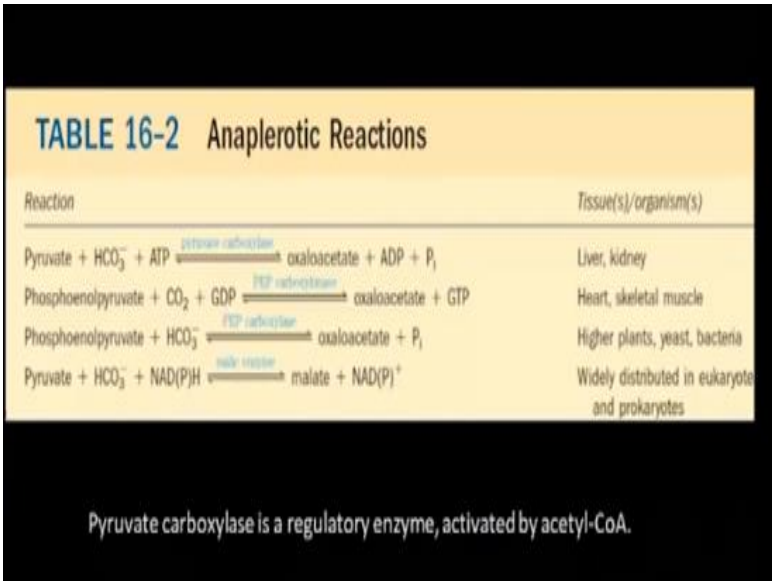
Another one transamination of alanine gives you pyruvate, in the reverse reaction as what I drew here and you get oxaloacetate. And similarly oxaloacetate can be made from phosphoenolpyruvate as well by the action of PEP carboxykinase or PEP carboxylase. And another intermediate that is replenished is malate, again the source is pyruvate.

So primarily anaplerotic reactions rely on PEP and pyruvate to produce either oxaloacetate or malate. So which one is the main one depends on the tissue. So in the liver and kidney, this is the dominant one and this is an example of an important theme in biochemical reactions.

So therefore, we are going to look at this pyruvate carboxylase reaction in some depth to the same level we saw how thiamine pyrophosphate functions in pyruvate decarboxylase to produce acetaldehyde in ethanol fermentation. So similarly, we will look at this as well. So there we learnt one vitamin which is thiamine. Now we will learn another B complex vitamin biotin, okay.

So this biotin dependent one carbon transfer is a common theme or concept in biochemistry and this is a good example and therefore, we are going to learn about this enzyme.

(Refer Slide Time: 27:40)



So these are the four main anaplerotic reactions. This is taken from the medium. This is carbonic acid which is the primary buffer in our blood and that provides the carbon dioxide and that needs energizing. It makes a carboxyphosphate by hydrolyzing ATP. So we will see that in detail now. And that is one, so that is primarily in liver and kidney.

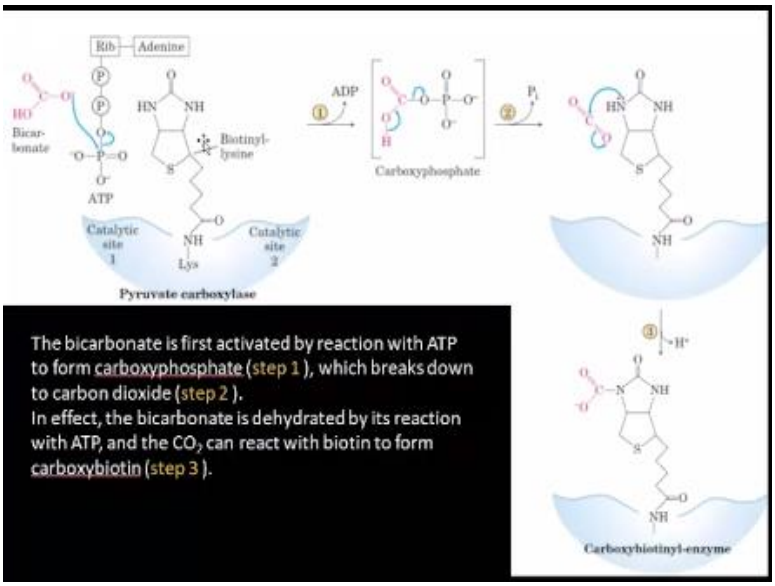
And from PEP this carboxykinase, this kinase because of this GDP to GTP conversion and that is in heart and skeletal muscle. Then phosphoenolpyruvate by another route like similar to this with carbonic acid you have carboxylase making oxaloacetate. That is in these tissues like plants, yeast and bacteria. Then a different way of converting pyruvate that is malic enzyme to malate.

So there is a dehydrogenation involved and so that is in widely in all organisms, okay. So we are not going to look at all the four of them. So our focus is for one main concept take one well known reaction and learn about it. So that is how an introductory group clause can be balanced. It is not totally superficial. We get enough depth but only to the extent we can handle in a 40-hour class.

So we are not going to gloss over all the things and instead we are going to learn a few things and some examples in some good detail. So in that sense, we are going to focus on pyruvate carboxylase alone. And this is a highly regulated enzyme. It is virtually inactive if you do not have acetyl-CoA, okay. So if you do not have acetyl-CoA what is the point of making oxaloacetate?

Because acetyl-CoA oxaloacetate only can combine to start the citric acid cycle. So it is activated allosterically when you have acetyl-CoA available so that enough oxaloacetate is produced and the cycle can start. So how does this enzyme carboxylase pyruvate?

(Refer Slide Time: 30:03)



So biotin is the conzyme here. So this is a prosthetic group. It is like lipoic acid. This is attached to lysine NH2 group, okay. So the remember lysine has a long side chain with an epsilon amino group and there in amide linkage this is attached. So like lipoic acid this also carries a carboxyl group and that is in amide linkage here. And this again has a long chain. Only thing is it has a ring like structure.

And main atom in it like the carbanion carbon that you know carbon 2 which is weakly acidic in thiamine pyrophosphate or the thiol group sulfur in pantothenic acid containing coenzyme A. Like that here it is this nitrogen which is the main atom that is going to participate in the reaction. So this is the biotin. And this again has a long arm just like what we saw with coenzyme A.

Or primarily that long arm's usefulness we learnt in the context of lipoic acid right, lipoic acid how it can swing from the active site of phosphopyruvate dehydrogenase all the way to the dihydrolipoyl dehydrogenase, the third enzyme of that PDH complex. So similar thing this also does, this long arm. So here first the bicarbonate you know is this hydroxyl group attached to the carboxyl group that is bicarbonate.

And that donates a carbon dioxide essentially in the form of carboxyphosphate. So that is the active or reactive form of carbon dioxide. So that happens in one active site of the pyruvate carboxylase and you know ATP is hydrolyzed and phosphoric acid is attached to this you know carbon this carboxyl group carbon here, carboxyphosphate is the intermediate.

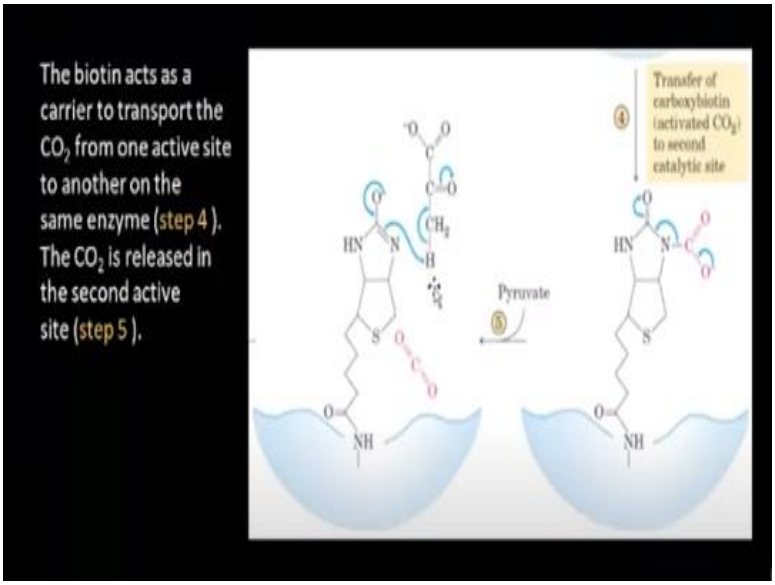
So then the phosphate is removed and you this generates the free carbon dioxide at the active site, so this is not fully diffusing away. And this nucleophile you know this extra pair of electrons and that reacts with this carbon which is you know electron deficient because of these two oxygen atoms. And now you have the carboxybiotin. So this is a biotin carries carbon dioxide.

And since it is one carbon we call this as one-carbon transfer, okay. So this one-carbon transfer is an important class of reactions, group transfers that happen in biochemistry. So these are acyl group transfer example. Phosphoryl group we saw so many of them. And this is an one carbon carrying step.

And so temporarily this one carbon in the form of carbon dioxide is carried by this carboxybiotin. Now it swings and takes this to this active site in the next step. And so we saw this step 1, which is actually activating the carbon dioxide, so we have that in step 2. This is the carboxyphosphate formation, step 1 and then the carbon dioxide.

So it is essentially dehydrated, okay. So it loses the H2O water from it. And then it is in the third step it is attached to the biotin.

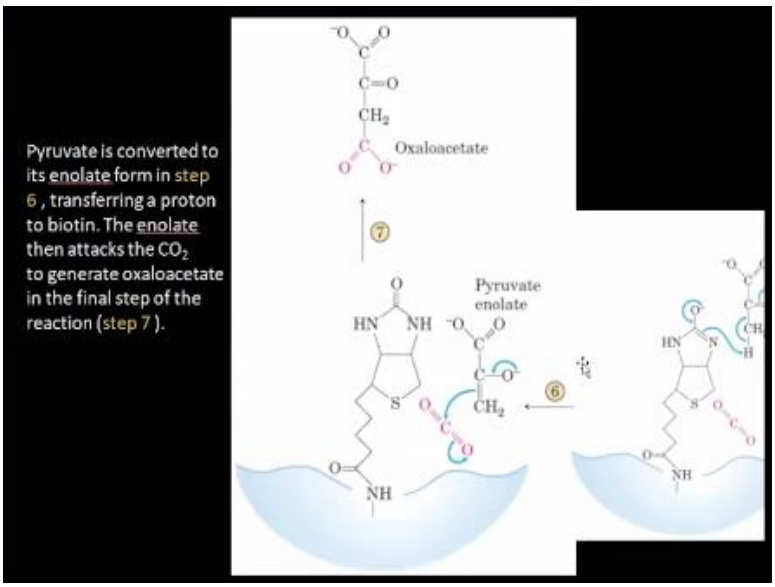
(Refer Slide Time: 34:26)



Then in the fourth step the biotin you know swings to the other side and in the other side you have the carbon dioxide in the other catalytic site, okay. So now in that site the pyruvate is converted into its enol form by abstracting a proton here by this nitrogen. So this ring, abstract this and converts C double bonds and this will be a hydroxyl group.

So the proton essentially goes here sorry the electron goes here, proton is abstracted by this and the enol form is stabilized. Remember pyruvate can exist in keto-enol tautomerism and that is why pyruvate is more stable than the phosphoenolpyruvate and that is why PEP 2 pyruvate **is** proceeds with large negative delta G, okay. So and in that enol form that carbon interacts with the carbon dioxide, okay.

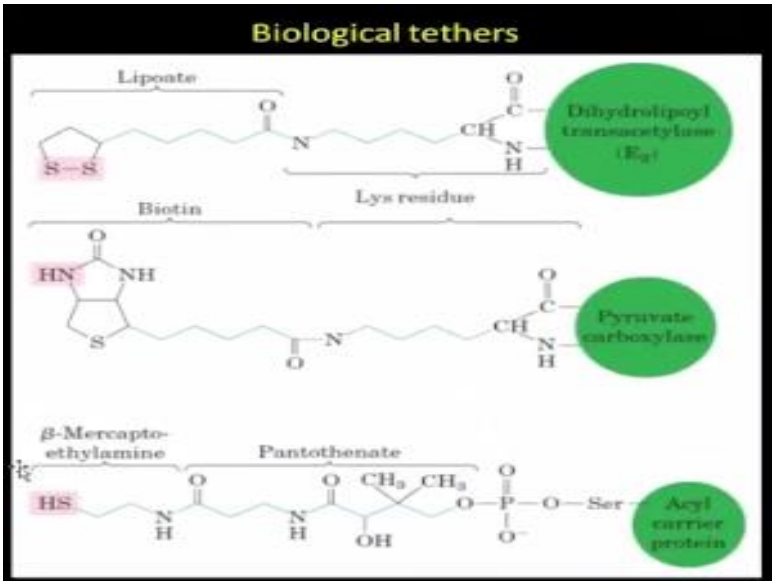
(Refer Slide Time: 35:36)



So this is the enol form here. So this is the same thing, for continuity I have put the same image once more here. So these electron flows results in the enol form and that enol form that enol is what interacts with the carbon dioxide and that gets attached and you have the oxaloacetate, okay. So remember, this is aspartic acid side chain. This double bond O if through transamination becomes CH NH2, this is aspartic acid.

So you get the oxaloacetate and the enzyme is back. So this is the role of biotin in carrying one carbon from one active site to another active site. So this is the pyruvate carboxylase reaction mechanism.

(Refer Slide Time: 36:30)



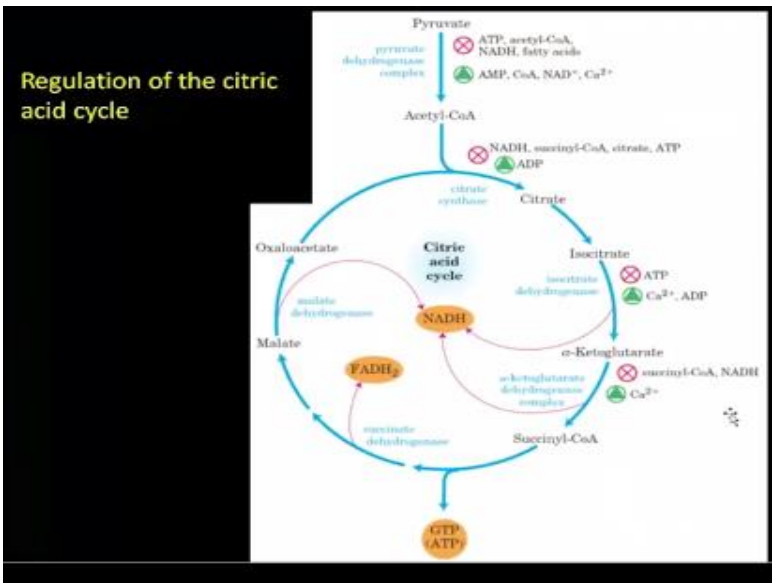
And this is sort of summarizing these long armed molecules that we have encountered. So so far let us count how many vitamins we have encountered. We saw niacin, flavin, then thiamin, then pantothenic acid as part of coenzyme A and now we have got biotin. So five vitamins we have learned. So along with biochemistry, you are also learning biochemistry of nutrition here.

So among these molecules, what is our focus here is this long arm, how that helps. So this we saw very clearly how this swings among three active sites, this lipoic acid. And this one biotin we just saw. So this is the biotin's part, this is the lysine. So this is lipoyl lysine, this is biotin L-lysine. Then pantothenic acid along with this mercaptoethylamine adding you know couple of more carbon and this nitrogen and extending this chain.

So these molecules, so this we will encounter in its, this tethering role in fatty acid biosynthesis, okay. Also these molecules all of them with due to the presence of this long arm they tether, tether meaning attaching you know connecting a molecule to another bigger molecule helping substrate channeling, taking group from one active site to another active site. That is substrate channeling.

You just saw an example of substrate channeling here. This is substrate channeling, from one active site to another active site. So this is the, you know highlight on the biological tethers because we have seen these vitamins just recently.

(Refer Slide Time: 38:41)



And the last topic for today is regulation of citric acid cycle. It is quite simple concept. Essentially what is happening is the important enzymes that proceed with large negative delta G, they are subject to regulation by the products of the pathway, not necessarily by the immediate product of that enzyme, okay. So immediate product of pyruvate dehydrogenase will be you know like acetyl-CoA is an immediate one.

NADH is an immediate one. But ATP is not, fatty acids are not. And so for example, when you have lot of fatty acids, you do not need to convert pyruvate to acetyl-CoA. These fatty acids through their own catabolism will generate acetyl co A. So the overall end product is what ultimately ends up negatively regulating these enzymes, the key enzymes. In this case, this is like a committed step.

And this is also rate limiting step, this is the slowest. The first decarboxylation step, the pyruvate dehydrogenase step is slowest. And though such an enzyme is subject to feedback inhibition, allosteric inhibition by the end products. And they are also positively regulated allosterically by the substrates like AMP.

You know you have lot of AMP and less ATP means and you need to make ATP then this enzyme is activated and so on. So this is one. And second the first step of TCA cycle that oxaloacetate acetyl-CoA producing citrate, citrate synthase again is similarly allosterically regulated by the pathway's end products, okay. So here there is no NADH involved.

But when you have lot of NADH you know all the NAD are in the reduced form. Why operate TCA cycle because the TCA cycles the main thing is producing this orange color here the electron carriers carrying electrons that is in the reduced form. And if they are already plenty, you do not need to operate this cycle. And therefore, the early steps you know are stopped. So this is the second such important enzyme.

And in addition to these two which are the principal enzymes regulated in TCA cycle operation, these two are also subject to regulation. So isocitrate dehydrogenase as well as alpha-ketogluterate dehydrogenase. So again remember this enzyme is very similar to this. Only the first step, that PDH dehydrogenase has substrate specificity for pyruvate while alpha-ketogluterate complexes first enzyme that is this dehydrogenase is substrate specific for alpha-ketogluterate.

The transacetylases and the next transacetylases of these two and then the dihydrolipoyl dehydrogenase, the third enzyme, they are identical in these two complexes. So the and similar complex works in branched chain amino acid oxidation as well. And these are thought to have evolved from common precursors, okay. Evolutionarily they come from a common ancestor. So this is again subject to regulation by the products.

So these are the four steps where you have allosteric positive activation by the substrates of the pathway, not that enzyme step itself and negatively regulated by the products. Sometimes the immediate product or substrate, and sometimes the overall

product or substrate of the pathway. So this is the main regulatory mechanism we need to remember.

In addition, there are hormones that regulate the gene expression like genes that encode these enzymes. They are subject to regulation at that level as well, okay. So do I have any, okay then we move to oxidative phosphorylation. Let us begin that in a new class like next Monday. **"Professor - student conversation starts"** What is this cross and green mark in this? **"Professor - student conversation ends".**

Yeah the cross meaning negative regulation and the green triangle meaning positive regulation. So these molecules activate this enzyme allosterically meaning positive effectors. And these molecules are negative effectors meaning they are allosteric inhibitors of this enzyme. Okay, what is the meaning of anaplerosis? It is basically anabolic replenishing, okay. So that is what is anaplerosis.

Anaplerosis is producing from other sources, these intermediate molecules here, you know primarily oxaloacetate and malate from pyruvate or phosphoenolpyruvate. So that is anaplerosis. That is replenishing the intermediates of the citric acid cycle from other sources. So here are the main ones listed. Okay.