Indian Institute of Technology Kanpur

National Programme on Technology Enhanced Learning (NPTEL)

Course Title
Bioenergy

Lecture-14
RUBISCO Enzyme

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Welcome back to the lecture series on my energy, so we are in module 3 which is by files and the third lecture of that section where we are supposed to cover15 lectures talking about how the by files are made in valued ecosystems especially in the plants algae and all other green forms and next step will be how they are being converted. So as a part of it in the last two classes we have talked about the beginning of the dark reaction well have talked about carbon dioxide single carbon molecule, attaching to a five carbon molecule and then making a fixed carbon intermediate which breaks down into two three carbon molecules this is where we you concluded the class.

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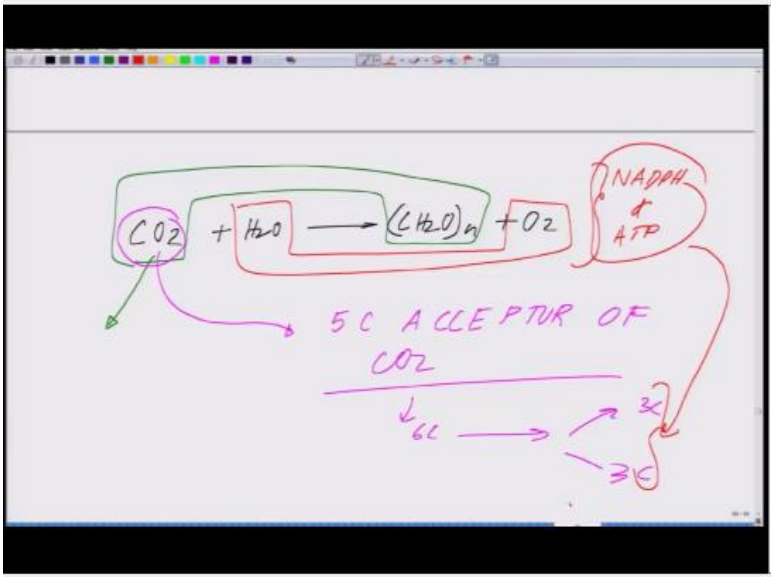

And the enzyme which is orchestrating this whole reaction is called Rubisco, so today we will briefly talk about Rubisco and the structure of Rubisco and different aspect of Rubisco to start off with let us take a recap where we ended the last class so this was the last this was the last drawing I made in the previous class where it can see C Phi plus C making a C6 and C3 and C3 okay.

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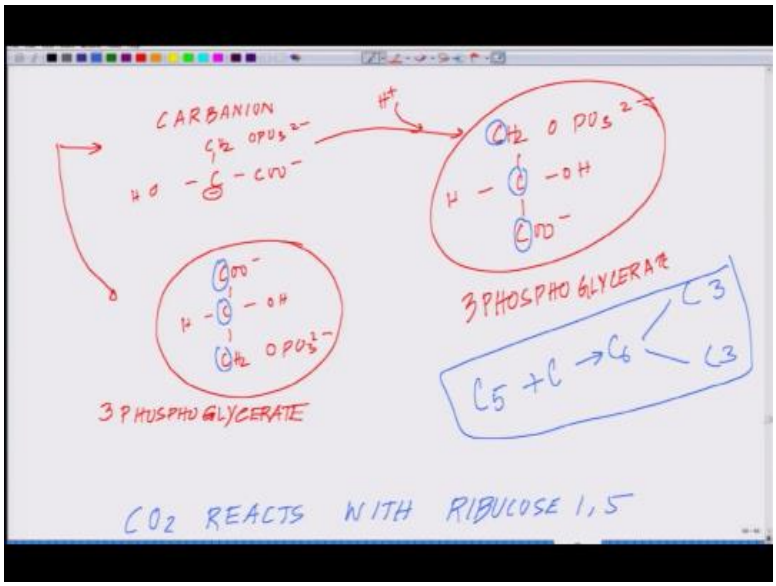

So interestingly if you look at it so what Rubisco is doing it is getting out ah I forgot to mention the energetic of it if you look at it so basically it is getting out a very high very high X organic reaction Oh Delta G look at it is around minus 12.4 kilocalorie for moles okay.

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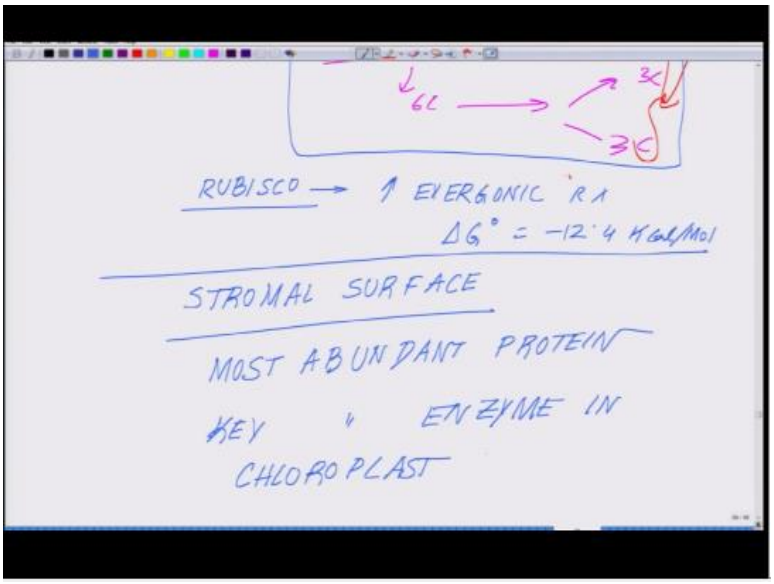

And in the last class we talked about Rubisco is located on the stromal surface and as I have already mentioned this is one of the most abundant protein and one of the key abundant enzyme in chloroplast. And Rubisco and chloroplast is consists of, chloroplast consists of eight large subunit eight large su which is a subunit each consisting of 55 kilo dal ton each L is equal to 55 kilo dal ton that is the molecular weight of it and eight small subunit where eight small so when it is each subunit consists of 13kilo dal ton of proteins involved on it okay.

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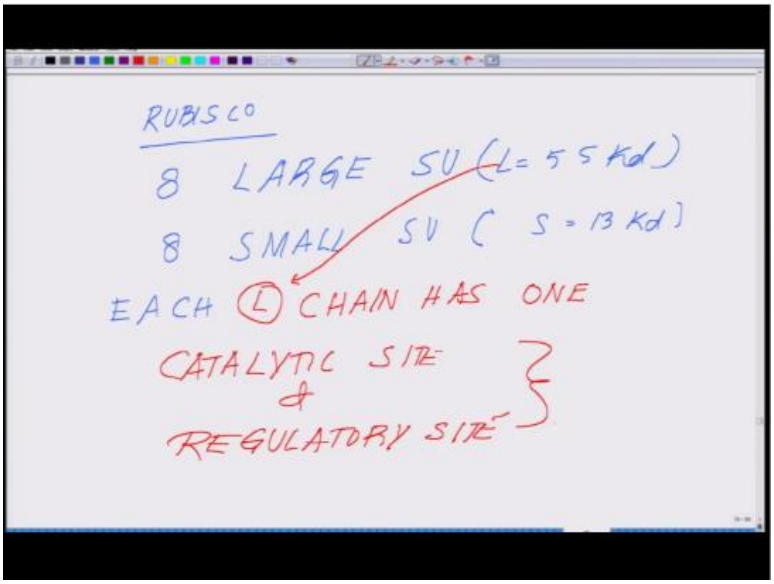

And each L chain contains a catalytic site and a regulatory site so each one second as of this L chain has one catalytic site and regulatory site this is critical as we will talk about how they get activated and another important thing which is what mentioning here is that the S chain enhance the catalytic activity of L chain, and hence the catalytic activity of L chain in other word S chain is helping the in chain L chain to carry out its catalytic activity and the enzyme is very abundant in chloroplast comprising almost of sixteen percent of the total proteins which are available in it.

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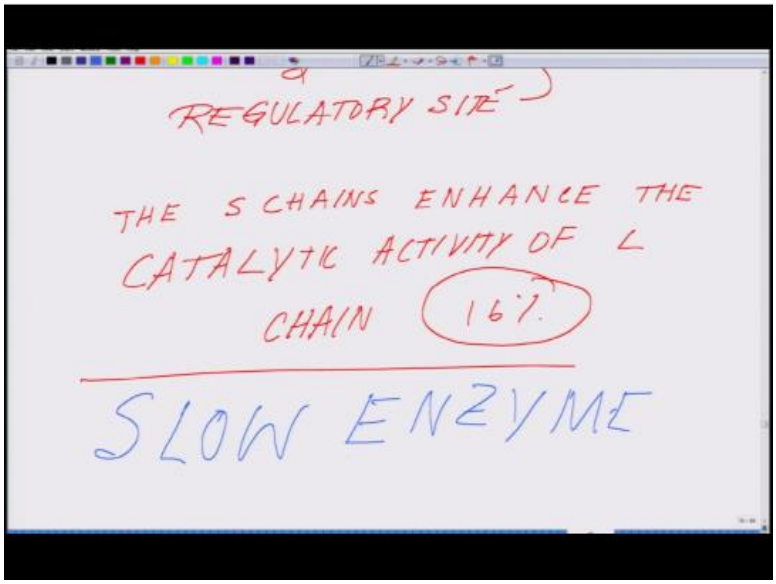

And in fact too viscous the most abundant in Xiamen probably the most abundant protein in the biosphere large amount of it is present in but it is very interesting here it is noteworthy it is a very it is called as a very slow enzyme why is it called a slow enzyme it is called a slow enzyme because its catalytic rate if you look at its catalytic grade is very stunningly slow it has a catalytic rate of three per second this is the catalytic rate of Rubisco choose amazingly slow.

Now that it is very interesting to note that though Rubisco is helping there is something you have to understand though Rubisco is helping in attaching one carbon dioxide to a five carbon ring it is very para toxically that Rubisco itself need a carbon dioxide to get activated so realizing the situation so just think of it so you have co_2 of your reaction plus water this is the basic reaction we are talking about CH_20 n which is your carbohydrate and oxygen okay.

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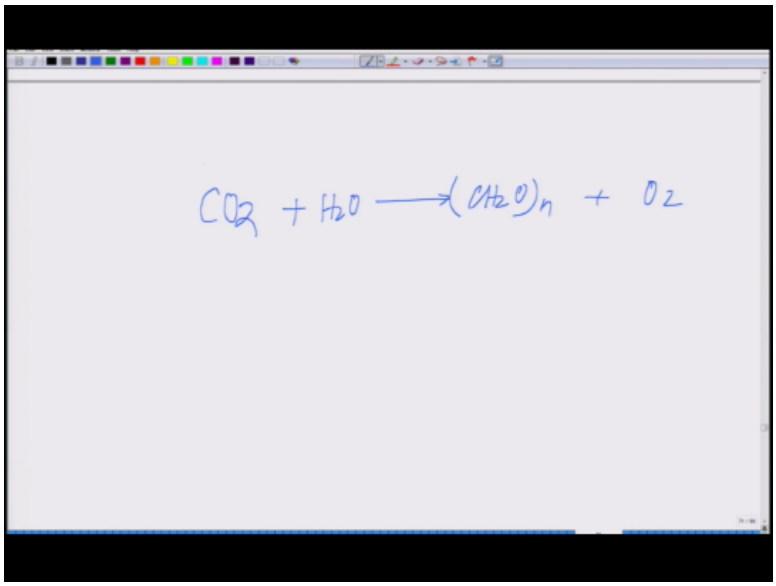

Now this is the reaction what we get now dealing with interestingly this carbon dioxide not only forming this ,this is also playing a role in activating the enzyme Rubisco which is leading to this co_2 reduction to CH_20 okay.

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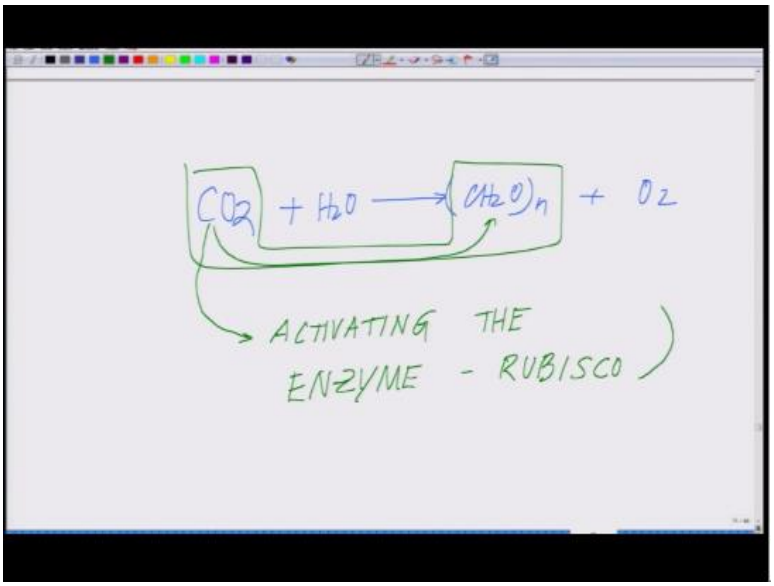

So it has a dual role but then how it does now what we will talk about is how Rubisco actually functions and what are those acts active site activation of Rubisco. So let us move on and write a little bit further down here, so talking about Rubisco so this is how the Rubisco looks like so the enzyme is converted into catalytically active form by the addition of co_2 so, so it has a uncharged amino acid residue epsilon amino acid of Rubisco okay. So let me put it all together for your basic understanding okay.

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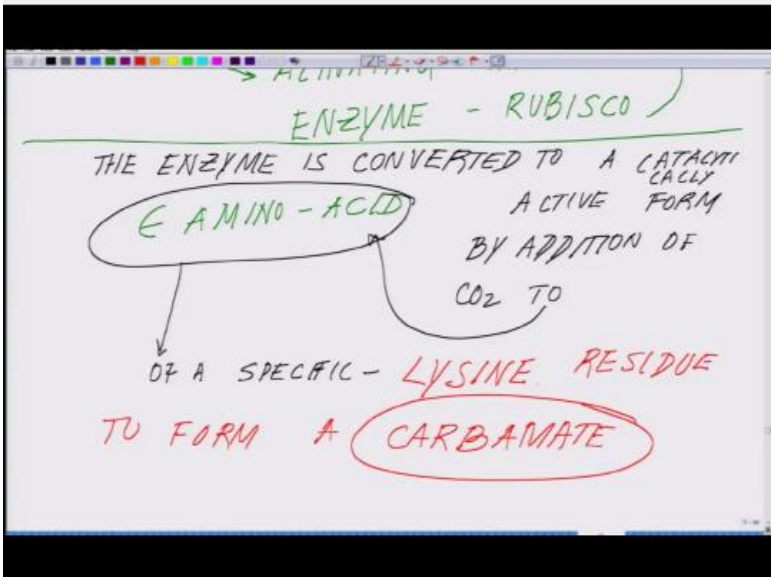

So the enzyme is converted to catalytically active form by addition of keep an eye on this by addition of co_2 to epsilon amino acid of specific lysine residue okay I will put the reaction together but before I put the reaction for you this is very interesting for you to understand to form a carbamate so this carbamate is formed and this carbamate is negatively charged attack so basically carbamate is negatively charged attacked this negatively charged adapt then binds.

This is the third set of reaction which happen then binds a divalent magnesium (Mg^{2+}) or divalent manganese(Mn^{2+}).

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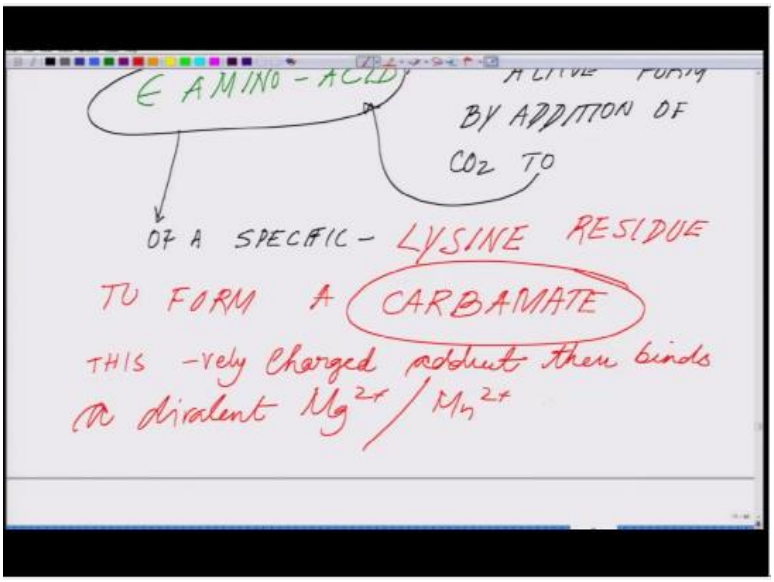

To form a positively charged metal Colette positively charged center or metal chelate okay. The bound metal ion so this is the most critical thing and I will wish you guys kindly take a close look on this the bound metal ion serves as an electron sink this is critical during as electron thing during catalysis so there are two kind of co_2 which are involved one co_2 which is forming CH_20 n and there is another kind of co_2 which is activating Rubisco.

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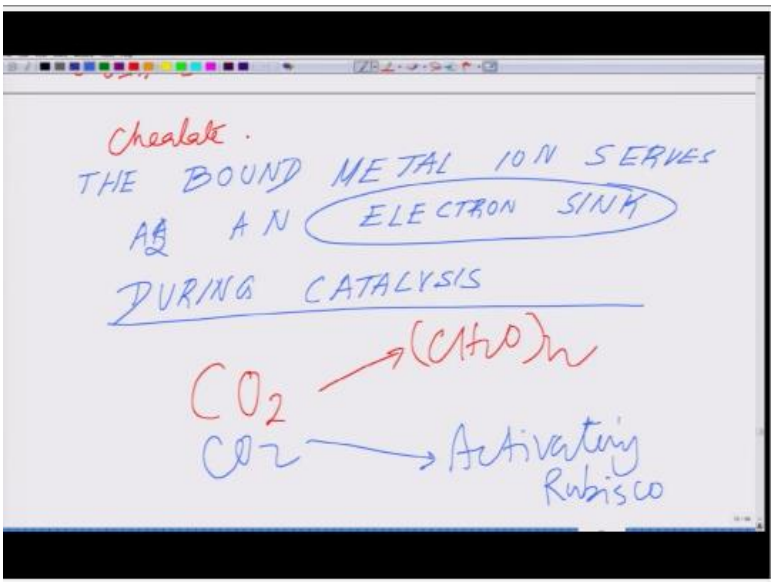

So in this whole equation of things there is two forms of carbon dioxide and they are totally different from each other one carbon dioxide which is involved in its own reduction whereas the other carbon dioxide is activating Rubisco and if you put this reaction together in one screenshot so the reaction will be something like this so you have CH_3. So this is that epsilon lies in residue so this is getting activated by that co_2.

So this is part of the Rubisco okay soon addition of co_2 this particular epsilon lysine ch_2 then you have your NH the other H is replaced by here is that you have the carboxyl group or this is called a carbamate moiety okay.

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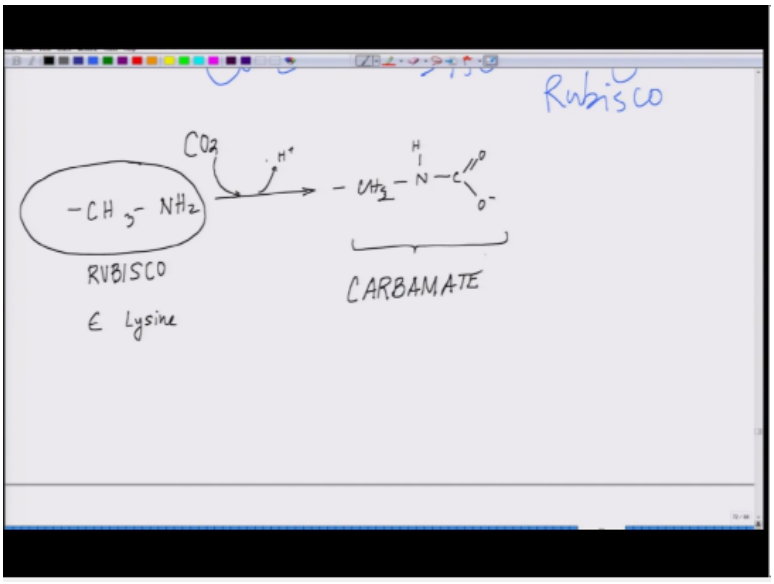

So this was trying to explain that before I get to the reaction so this carbamate moiety is getting added out here. So this is negatively charged on this you have addition of positively charged either magnesium or manganese coming through you can represent by Me 2^+ okay. What you are getting is this CH_2 sitting there at the n^H and you have the carbon carboxyl ate group O and on that other O you have 2^+ so this is the metal collette.

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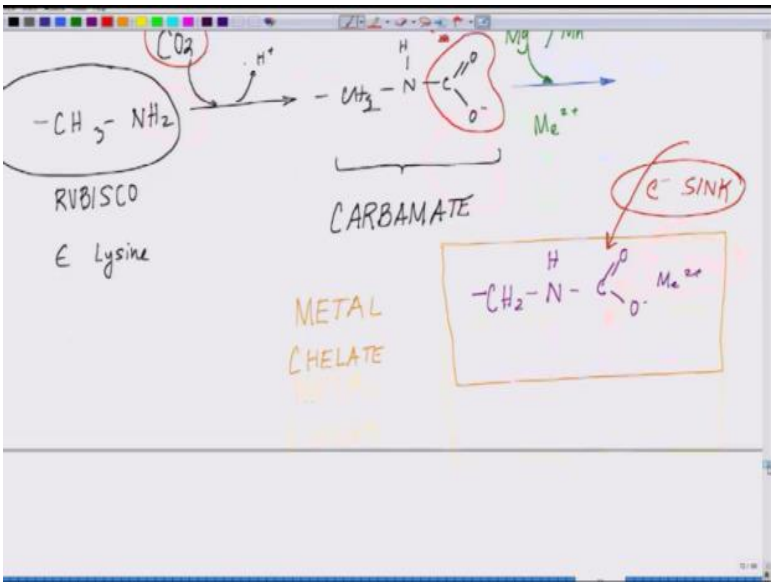

Metal collette I which is the electron sink in this reaction, so it is this is how the Rubisco which is involved. In fixing carbon dioxide also gets activated by another molecule of carbon dioxide there are two different molecule of carbon dioxide one is taking part in attaching to a five carbon molecule to form a six carbon molecule and then getting dissociated to 3 carbon3-phospho glycerate. Whereas the other carbon dioxide is attaching to the Rubisco molecule itself but not for forgetting converted but to activate the Rubisco.

So essentially you need carbon dioxide also for this reaction to take place, now having seen this in the last class I highlighted one very interesting point if you remembered what is the name of the Rubisco. Rubisco is carboxylase and oxygenase carboxylase and / oxygenase is and I told you that this is this second part oxygen is, is what is very critical allowed through the Rubisco.

What does that mean, so we have talked about the carboxyl a spot in terms of its addition of carbon dioxide to 5 carbon chains it assists the process by getting activated by another carbon dioxide. So you have two carbon dioxide one which is getting converted after adding to a five carbon forming a six carbon is getting split up into three phosphor glycerate whereas there is another carbon dioxide molecule which is activating Rubisco itself.

But this Rubisco which binds to which converts carbon dioxide can also at the same site binds to oxygen what does that mean it means where we are talking about as of now that Rubisco is the enzyme which is involved in taking the carbon dioxide and allowing it to bind to a five carbon ring and making it a six carbon so just follow up so this is what we are talking about.

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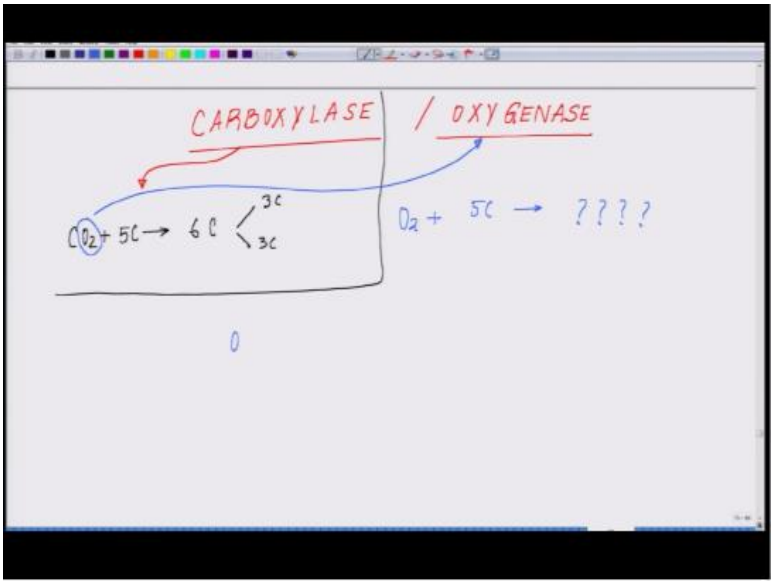

So you have CO_2 + 5 carbon making six carbon and then splitting up into 3C, 3C this whole thing is Rubisco carboxylase activity now think of it just put your imagination if it is an oxygen is activity same o 2 plus the 5 carbon and then what happens this is exactly another set of reaction which Rubisco does because it has oxygenase is activity. Now we will talk about that reaction so it means now if I put it in the broader perspective thing if you remember the first reaction.

When we talked about H_2O + CO_2 forming CH_2O which is carbohydrate plus oxygen it means this oxygen is also getting funneled out, some way or other because if this is because of the Rubisco, Rubisco is doing both things Rubisco can convert carbon dioxide and what does that

mean and what is the significance this is something very, very important okay. So let us today explore that reaction.

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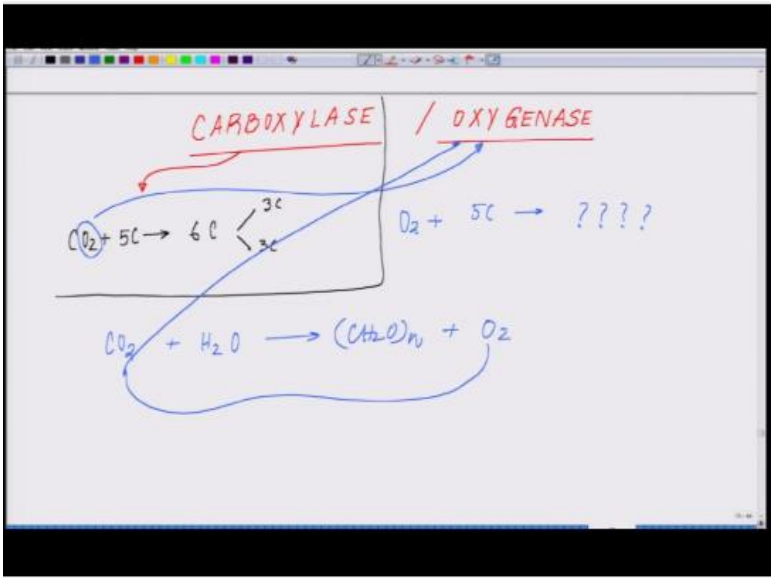

How Rubisco is actually doing it we have already explored the reaction in the previous class let me go back to that slide so this is where we have talked about how this, this edition is taking place okay. Now all of you recollect so this is that reaction where you have the C5 and making the an indie all calm play intermediate and followed by and this in Indie intermediate is helped by basically the Ruby squared cells. Now from the N endiol complex we talked about forming the sixth carbon hydrated intermediate.

Once again hydrated intermediate and of course then splitting up into two different 3-phosphoglycerate now we will talk about a different reaction in the same line ok now this reaction is very interesting.

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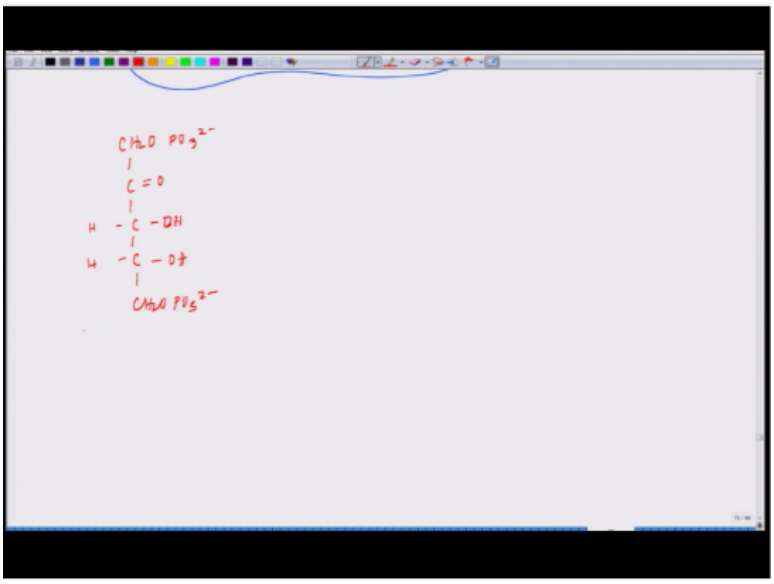

So again let us start off with the first molecule $CH_2O\ P0_3^{2-}$ C there is a ketone here second position hydroxyl in the third position again a hydroxyl on the third position and then you have $H_2O\ PO_3^{2-}$, So this is your ribulose 1,5 bisphosphate, okay. Now, again the same formation the endiol whole complex which his $CH_20\ P0_3^{2-}$ C-0H so the change is happening out here on the second carbon complex and then you have C-OH okay. Then similarly OH-H and you have $CH_20\ PO_3^{2-}$, so this is your endiol complex endiol intermediate okay.

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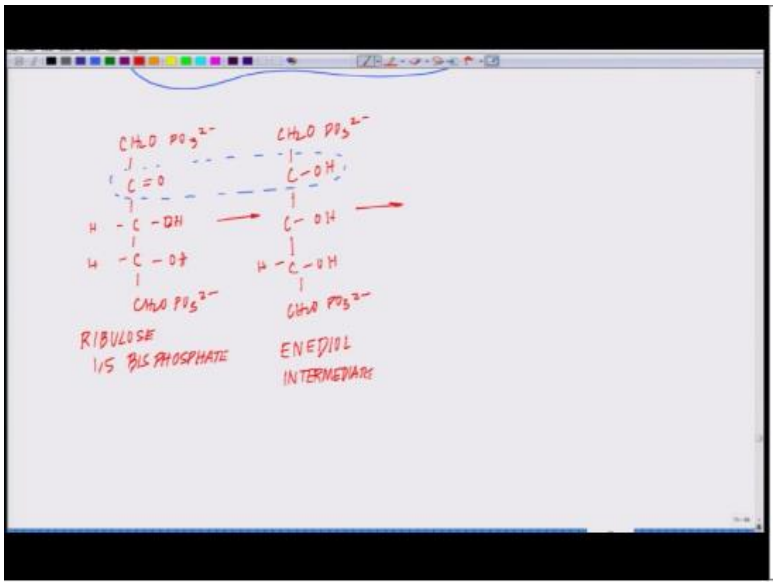

Next now instead of carbon dioxide we are introducing oxygen because this is what Rubisco can do now how the reaction will proceed reaction will proceed like this $CH_2O\ PO_3^{2-}$. Now keep an eye what is happening here O-O-H okay OH is the ketone group C-OH-H is H out here.

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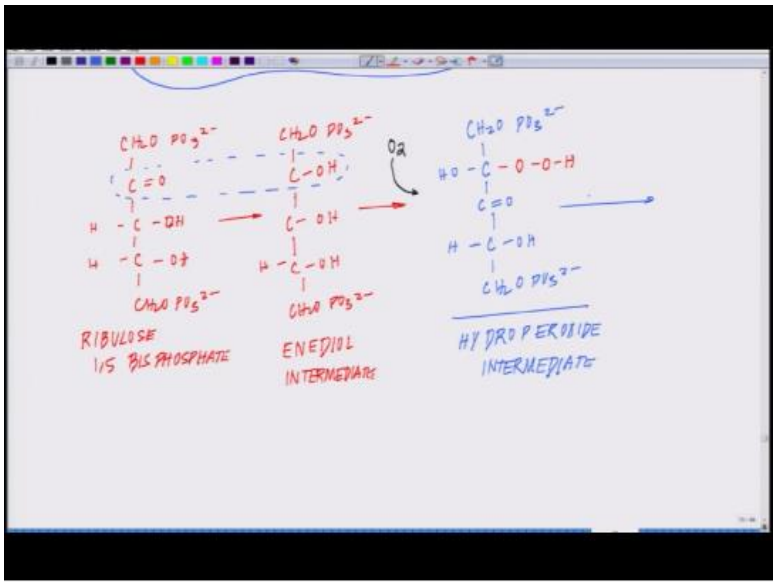

And then of course you have two three four five and then you have $CH_20\ PO_32-$,so this one is basically hydro peroxide intermediate and this hydro peroxide intermediate eventually breaks down to H2O 2H + H2O okay. And what you get is two different kind of molecule from here coming underneath it okay what you are getting is something called a phosphor glycolate, which is $CH_2O\ \ CH_2\ PO_3^{2-}$ and plus you have carbon oxygen, oxygen C-0-H-H $CH_20\ P0_3^{2-}$ and this is your original molecule 3 phospho glycerate.

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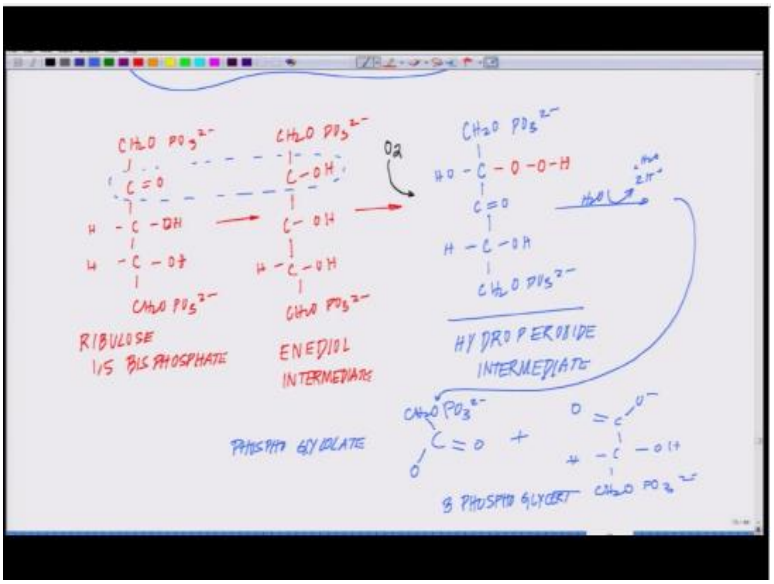

But if you look at it you realize that you are no more getting 2 phosphor glycerate what you are getting is 1phospur glycerate molecule and one phosphor glycolate what is happening to it and what is the significance of this reaction, this reaction is essentially is what we call as a process which is kind of negates the carboxylate effect of Rubisco and you can also termed it as something like this so it is something like in as it is being said it is an catalytic imperfection.

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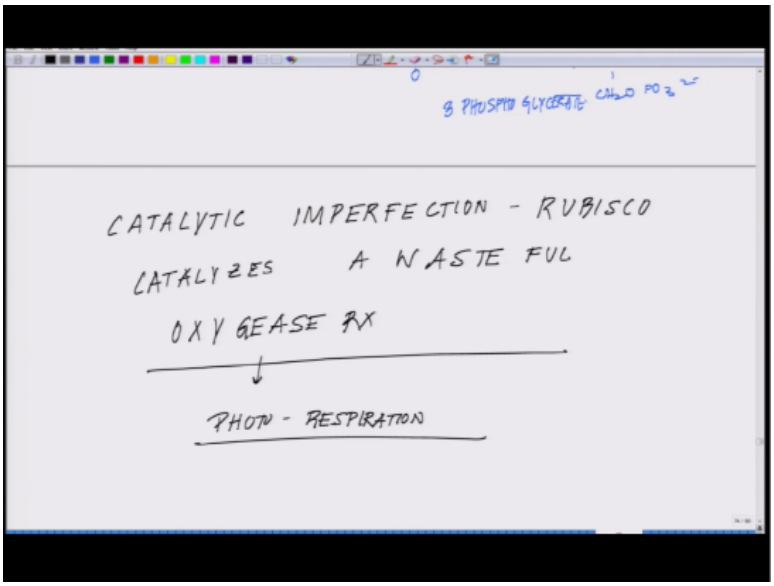

Were Rubisco catalyzes a wasteful oxygenase reaction and which is the reason for photo respiration ,so this imperfect nature of Rubisco reduces the efficiency of carbon sequestration so I will close in here. I will expect you guys to fonder over it think over it how the same enzyme which is doing such a wonderful thing of converting carbon dioxide into long-chain carbon more moieties by reduction process is also doing a reverse thing by attaching.

Why nature made it like that, what are the implications of it and from here how what are the salvage pathways so from here what will move. So now since I have introduced you to the Rubisco as I promised you to the bid 3rd class will introduce you to the Rubisco and we have talked about the carboxyl is already have carboxylase reaction you have already talked in the previous class, today we just kind of went through it and then we talked about the oxygen is reaction and we have introduced the concept.

There is a respiration also happening so and technically if you think of what is happening is CO_2+ H_2O is $(CH_2O)n$ +O_2 and this oxygen is further getting converted to some something so this getting converted is what we are talking about the respiration is also happening here, and whereas another interesting thing what we have talked about today is that whereas CO_2 is getting

converted into carbohydrate this there is another CO_2 which is activating Rubisco and it is the same Rubisco which is consuming oxygen.

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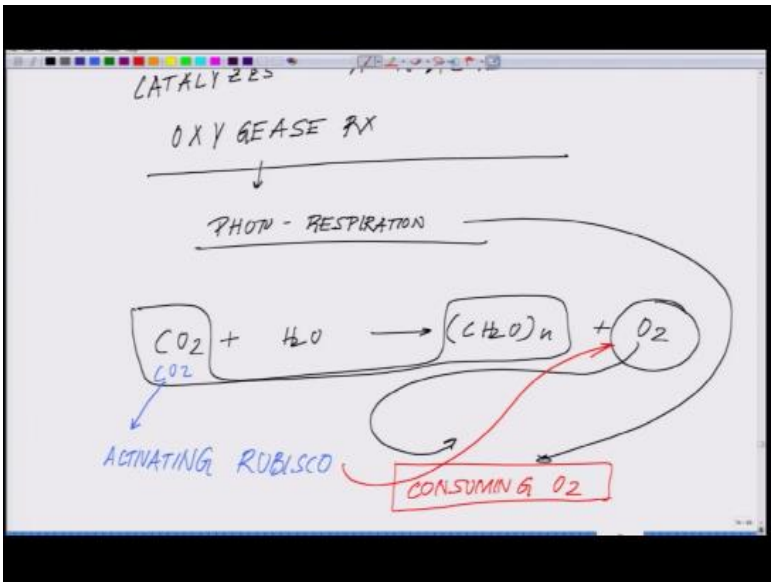

So it is a very complex equation Nature has developed on one hand it developed Rubisco which converts carbon dioxide to carbohydrate on the other hand for the activation of Rubisco itself it needs carbon dioxide up to that it is all fine but the Rubisco is also an oxygenase is why nature did it what made nature to follow such an intricate thing we do not know or is it nature is perfecting itself. Maybe through ages it is perfecting itself maybe it has reached to this point we do not know we do not have an answer to this but what are the implications we will talk about it in the next class.

Where we will talk about how this photorespiration and what are the cyclic process of Calvin will come back to the Calvin cycle the whole cycle will talk about how the three carbons are getting converted into X O's and the starch and all those things the formation will start C3 and C4 path ways okay, I will close in here thank you.

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