Indian Institute of Technology Kanpur

National Programme on Technology Enhanced Learning (NPTEL)

Course Title

Bioenergy

Lecture – 15

Photo Respiration & Calvin Cycle

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Welcome back to the lecture series in bio energy, so in the last class we talked about the catalytic imperfection of Rubisco. So what we realized is that the Rubisco is a very peculiar enzyme on one hand it needs carbon dioxide to get activated by the formation of carbonate followed by the positioning of the divalent ions, metal ions manganese or magnesium then upon activation it can bind to carbon dioxide and convert it into phospoglycerate, whereas at the same site where it binds to carbon dioxide it can also competitively bind oxygen and led to a molecule of what we have shown in the previous, a molecule of phosphor glycolate.

So and this process is because of this dual nature of rubisco of binding to carbon dioxide as well as a competitive binding to oxygen it leads to when it binds to oxygen it needs to photorespiration. So and that add up to the inefficiency of the system of converting carbon dioxide to high chain or long chain carbon moieties. So today what we will do in today's lecture will talk a little bit more about the photo respiration process what is the fate, so coming back to the slide if you look at the slide where we ended so these are the two products, so this is what is happening when instead of co_2 the oxygen is activity is being observed when the oxygen is activity of rubisco happens.

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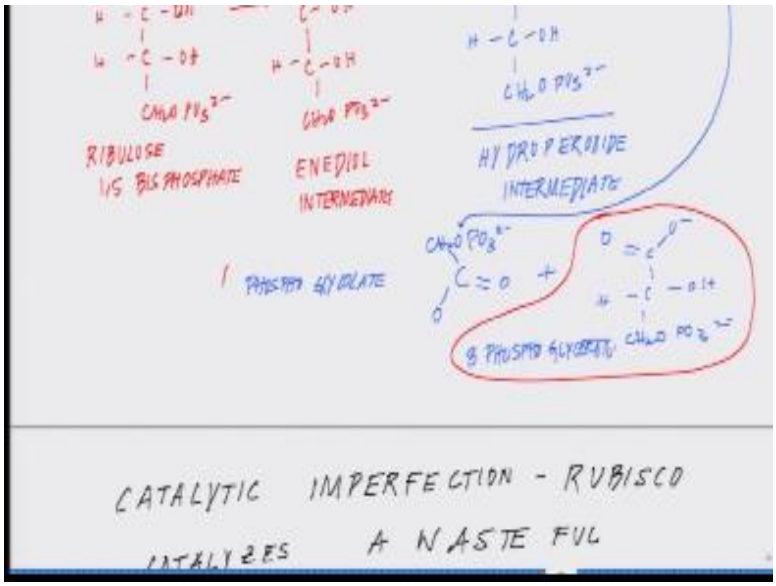

This is one of the product of course the 3-phosphoglycerate which is forming but apart from it you have a product called phosphor glycolate, so what is happening to the surface for glycolate is a two carbon so this is one carbon of phosphor glycolate, this is a second carbon of phosphor glycolate okay. So phosphor glycolate what is happening what is the fate of the fog likely so there is a path way so, let me kind of explain the pathway to you so phosphor glycolate through different steps will enumerate this step gets converted but before that you just kind of get an understanding, get converted into the simplest amino acid called glycine.

And essentially if you take two molecules of glycolate which sums up to 4 carbon because each glycolate moiety has two carbons right, if you take these two molecules then what you in result in what you get you get to glycine molecules and you eventually ended up with two glycine and there is one carbon dioxide which goes out, so let me put the reaction that will make more sense to you so this is what is happening so we have started with in the previous reaction we talked about the ribulose15 bisphosphate this was the precursor okay, the 5 carbon chain on which the carbon dioxide is added but in this situation what you are heading is an oxygen and what you are getting is one molecule of 3-phospoglycerate we have already talked about it.

But the second molecule is what needed to be got rid of which is having coo and CH_20 Po_3^{2-} which is a phosphor glycolate, phosphor glycolate these phosphor glycolate gets hydrolyzed and form a glycolate which is having ch_2oh, so what you are getting rid of is the phosphorus group out here which you can see the phosphorus is coming out so, what you are having the molecule is a glycolate. Now this glycolate moiety eventually gets oxidized where it gets oxidized so it enters into the paroxysms, so it is getting oxidized and in that process it perform h_2o_2, and this h_2o_2 is being further decomposed by an enzyme, inside the paroxysm call by catalyst.

And this is all happening inside paroxysms and you ended up with glyoxylate so which is coo-C-O-H, so what you are getting rid of is that you are getting rid of one of the hydrogen out here and based on that this is after the oxidation which has taken place because of this ox is oxygen out here glyoxylate this is where you have started with this molecule which is phosphor glycolate and you land it up with glyoxylate, now glyoxylate is so if you see it is essentially this whole process is this is not a very versatile molecule, okay.

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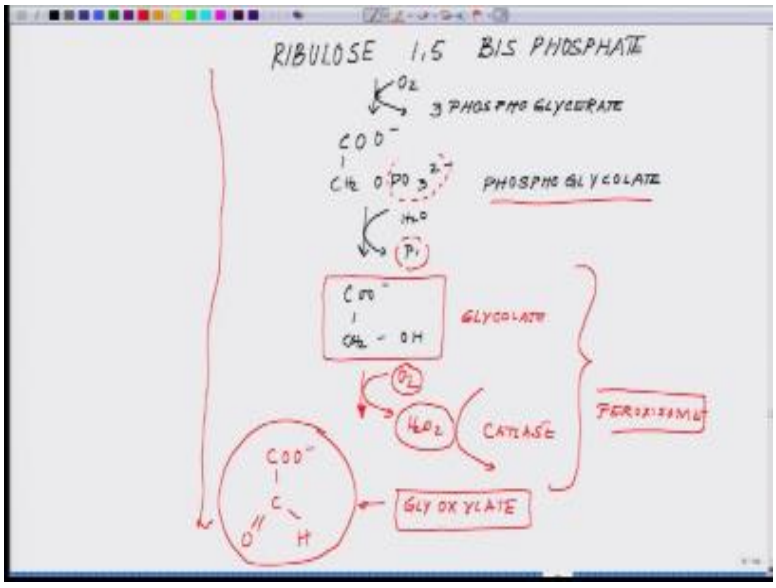

So this is basically it's called a salvage pathways where the carbon skeleton is kind of changing and the specific phosphates convert the glycolate which enter the paroxysm as I have shown you it enters the paroxysms okay and glycolate is an oxidized to glyoxylate , by glyoxylate glycolate oxidize. So that the enzyme which is doing this reaction is called glycolate oxidize where oxygen is converted into h_2o_2, and this h_2o_2 is further cleaved by catalyses to form h_2o + o_2 okay.

And then this glyoxylate is then so this moiety what you see out here gets tranceaminates, so this is what I was telling you in the beginning there is a tranceamination process in other words there is an amine group which gets transferred and the subsequent result is you get two molecules of from two molecules of course I am starting two molecules, two molecules of glycine which is the most simplistic amino acid plus what you are getting is so next what happened these these two molecules of glycine is then converted in the mitochondria, this second reaction takes place in the mitochondria where the two glycine moieties, so if I made glycine plus lysine this forms our serine.

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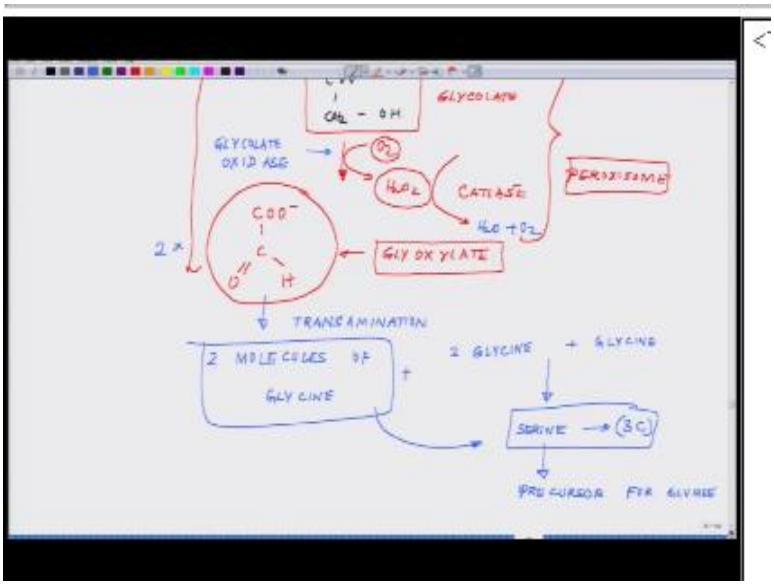

And serine is a three carbon molecule and this serine is further is basically it is a precursor for glucose for glucose, whereas if there are three carbon and out here glycine you are having two carbon so, two carbons but in this process while the serine is formed there is few other things which are formed, what you are getting is the form of co_2 is being liberated and NH_4+, so we started with four carbon, four carbon is coming from two of these molecules so, one and two so there are two molecules, then and at the end what you are getting is you could Salvage three carbon in the form of serine and you lose one carbon in the form of carbon dioxide.

And of course there is another product which is lost is NH_4+, amino group so, this pathway what you see this pathway serves to recycle three of the four carbon atoms of the two molecules of glycolate however, one of them is lost as co_2 and one of the two amino groups donated in transamination reaction is lost as NH 4 plus this process what we explained just now is called photo respiration, because this is one process where oxygen is being used up, oxygen is consumed and co_2 is released whereas as of now what we are talking about is co_2 is consumed to form carbohydrate whereas oxygen is released.

It's just the reverse where oxygen is consumed you see oxygen is consumed here, oxygen is consumed earlier, if you follow it there are several steps where oxygen is being consumed oxygen is consumed here first the next oxygen is consumed out here, so it's multiple steps oxygen is getting consumed. So photorespiration is essentially it's a very wasteful process so, fairly wasteful process which happens in the system and the organic carbon is converted into carbon dioxide without the production of any form of like ATP, NADPH or any other energy-rich molecules.

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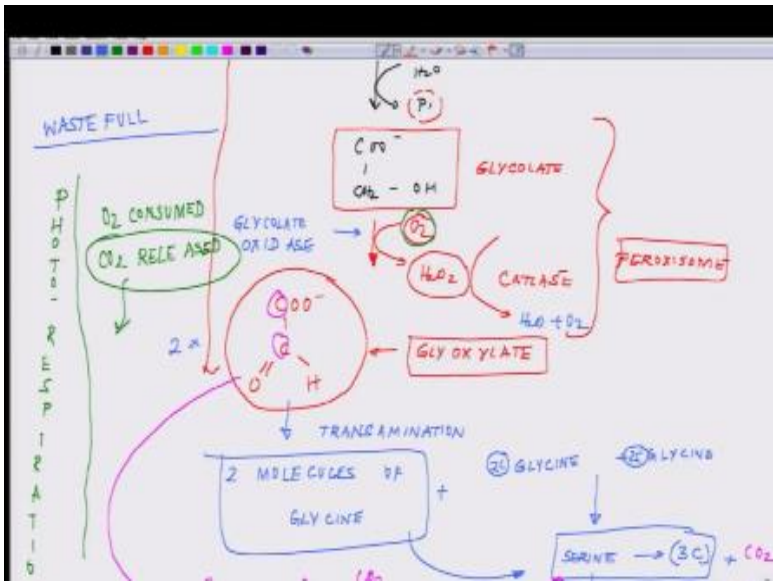

And because of this reason there are a lot of efforts which had been undertaken for decades together now to improve the catalytic efficiency of rubisco, some of the people who work all evolutionary chemistry or evolutionary inorganic chemistry they see that possibly rubisco has evolved in a environment which was rich in carbon dioxide. So rubisco really doesn't do very good in the presence of oxygen but as the earth from anaerobic condition started to move towards aerobic conditions rubisco get on this creeping defect where carbon dioxide is started to compete with, the oxygen started to compute carbon dioxide at the same binding site.

And so forth and there's one more thing I wish to highlight for those of you are thinking beyond it it's actually it's not the carbon dioxide, which is kind of the carbon dioxide in a form of bicarbonate is what is competing, because of the formation of the bicarbonate so, carbon dioxide molecule becomes much more accessible to the site so, this is what in summary in sum total if you see through this reaction from the beginning is what we talked about the photo respiration process.

So now keeping this in mind we will and by the way here I should highlight that this reaction what we are talking about is all what happened in mitochondria, where glycines are attached together condensed together to form a three carbon amino acid serene okay, so now from here

we move on to the calvin cycle so the title what we'll be talking under the heading will be Hexose or Hexose phosphate which are 6 carbons phosphates are made from phosphor glycerate. Whenever we talk about this Hexose those are the first commonly and readily available sugar and ribulose this phosphate is regulated and we will talk about that how that gets regulated is regulated.

So having put this in front of you now I wish to recall the beginning I told you that we still haven't accounted for the NADPH and the ATP that same, NADPH which was formed in the photo system 1, now we will recall it where that NADPH actually comes very handy okay, so now what I will do I will put the so again just sticker let's do a recap photo system 2 p-680 photo system 1 p 700 system 2 and system 1, at the same time absorbs light shoots an electron add different redox potential, so one of our multiple chlorophyll molecules are now devoid of electrons those chlorophylls which are devoid of electron in photo system 2 are brought back to their ground state by splitting of water and the electron subsequent electron which are generated bring them down back to their ground state.

Whereas in photo system 1 the electron has brought back to their ground state by the electron which are hopping from photo system 2 to photo system 1, whereas the electron which has moved out of our system 1 reduced form a very strong reductant called NADPH and in that process there is a proton gradient which is created which leads to the formation of ATP which is a both a week and strong, weak and weak reductant as well as a weak oxidant and while water is getting a split it generates form of the strong oxidant o_2.

And we have talked about with NADPH is responsible for supporting that calvin cycle now we will talk about that what is the role of NADPH and ATP. So we are almost coming there where we left these two molecules from the beginning of your number these are the two molecules we haven't touched, so now let me go back and put the cycle which was developed by Kelvin and his co-workers. During 1945 and 1965 and after that also the work continued until this day there are so many mysterious things which we really do not know okay.

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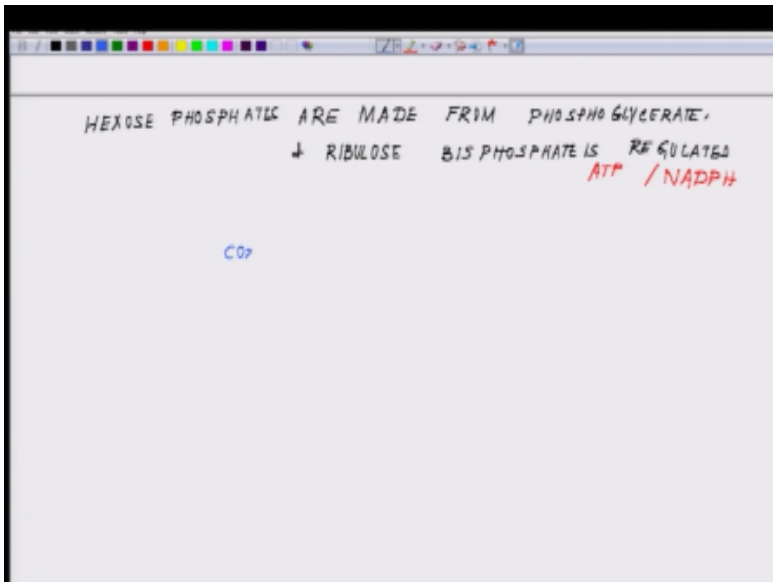

To start off with on the slide so we talked about the first molecule which is entering here is co_2 so, co_2 as a single carbon, one carbon reacts with ribulose 15 bis phosphate, 15 bis phosphate which is five carbon okay so now this reaction, this attachment in the presence of rubisco leads to the formation of3-phosphoglycerate okay, so you are having two such molecules of phosphoglycerate so which is three carbon moiety so you are having now 2 x3 6 carbon. At this stage you need ATP to play a role and this leads to the formation of TP of course the phosphate is donated and having a ATP what you are getting is 13 bis phosphor glycerate okay.

Now 13 bis phosphoglycerate with the help of now the second molecule comes into play in ADPH a strong reductant donating its hydrogen coming NADP+ leads to the formation of glyceraldehyde 3-phosphateokay and glyceraldehyde 3-phosphate could change it structure to die hydroxyl dihydroxyacetone phosphate the reason for this will come later okay, then this died higlyceraldehyde-3-phosphate could form call as fructose 6-phosphate and this is the first molecule fructose 6-phosphate.

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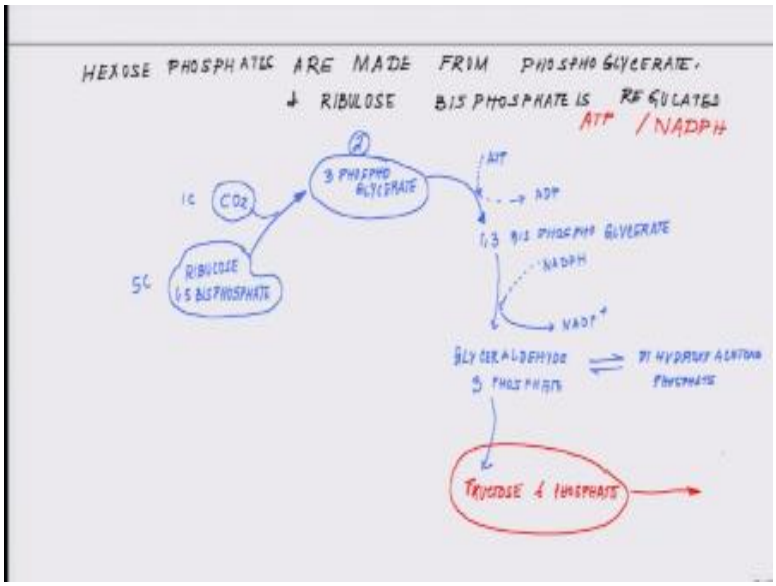

So when you talk about fructose now we are talking about sugar and if this molecule polymerized further this is not part of the circle then what we talked about formation of starch and sugar will come later under that so fructose6-phosphate there is another pathway by virtue of which fromglyceraldehyde-3-phosphate through a series of enzymes will come later about that, it could form what you call as ribulose 5-phosphate and the enzymes which are involved making this happen includes from trans titolays aldolase and there are other enzymes which are involved in it.

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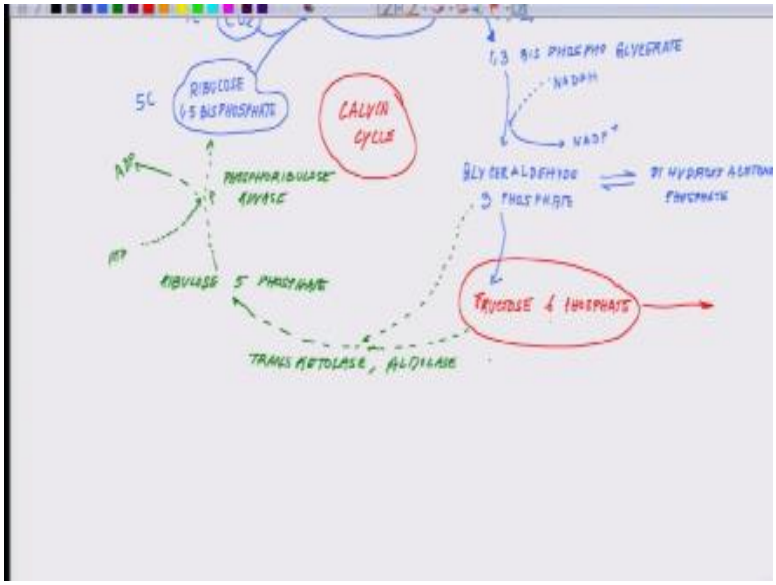

At this stage there is another molecule of ATP which comes into play which is donating the phosphate and that brings it and the enzyme which is involved in it is called phosphor ribulose kinase, so that makes it right below s1 save this phosphate so, now this is what we talked about is the famous calvin cycle and out here there are a few other things which i am going to highlight now, okay what is happening to fructose so, you saw that fructose 6-phosphate out here okay fructose 6-phosphate so, what is what are the other fate of fructose6-phosphate so it's one second okay so, if you take this fructose 6-phosphatehere and react it with glyceraldehyde-3-phosphate as you could see glyceraldehyde-3-phosphate the product you will get will be using transketol as e as an enzyme.

So enzyme involved is transketolase, so what you are getting is ribulose 5-phosphate plus erithrose 4 phosphate so, further there is erithrose 4 phosphate which is this compound if you take this compound and add this with you see this compound dihydroxyace one phosphate what you will get is in the right action of aldolase you will get seudohefilose 17 bis phosphate, so these are the different form of sugars which are getting formed different kind of carbons okay now this Seder have to loose bis phosphate if you react this one again with glyceraldehyde-3-phosphate which is what you will get is in the presence of transketolase, what will we get is ribulose 5-phosphate, ribulose 5-phosphate plus value those 5 phosphate.

So in other words what is happening is that if I had to summarize these three reactions so what happened here is that c6 with c3in the presence of trance keitel is trans Keitel is the transcript or less what you are getting C 4 plus C 5 when this c4 chain, what you took again add up with anotherc3 in the presence of aldolase stand for a you get a c7 4 plus 3 then you take this C 7 plus another c3 in the presence of Trance Keitel is shown by TK what you get is a c5 plus c5, c5 plus c5. So you are having five carbon so you realize that what is the origin of this fight carbon molecules there are always five carbon molecules which are playing this game.

And in order to have this process functional what you needed is but there are few other enzymes which are involved in this process so the four additional enzymes which are involved in it are called as 17 bisphosphate phosphatase phosphophine to epimerize and phosphopintos, I summaries and phosphorylase kinase you do not have to worry about these things but what is important herein this whole process what is happening is that you are consuming 2 ATP plus 1ATP if you see the cycle and there are two NADPH this is what you are consuming in this process of so if you now go back to the cycle and add up if you look this is where you are you have the NADPH what you are using here 2 NADPH okay you have your having two ATP which are getting confused and consumed and here you are having another ATP which is getting consumed.

So now let's again summarize so from four to stem one you get this NADPH which is fed hear that NADPH, this NADPH is fed here into this system you have the other substrate carbon dioxide which is fed into the cycle out here to a five carbon ribulose for bits of it 15 bis phosphate then inform 3-phosphoglycerate and you have this ATP the first series of ATP is fed here and the second line of ATP is fed out here okay. so now we account for all the things which are involved in it we talked about where the carbon dioxide is fed into the system we talked about where NADPH pays the role and especially ribulose 15 bisphosphate in the chloroplast it exclusively once we bisphosphate bliss rate is exclusively acts in the presence of NADPH okay.

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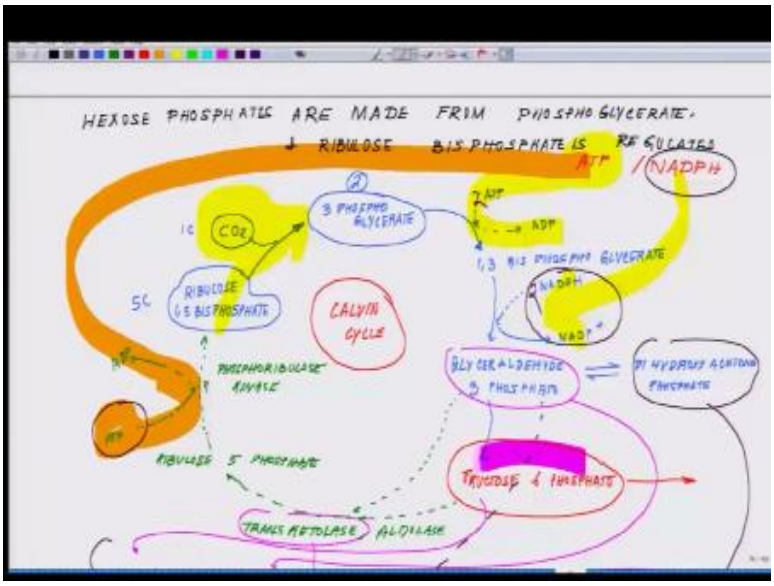

So overall what we see in this scheme of things the most important the first sugar which is formed is out here, so now if we summarize what we talked about we talked about the dark reaction where it is the carbon dioxide is converted into carbohydrate the simplest sugar and that process is executed by an enzyme called rubisco. Rubisco has both carbon attachment property as well as oxygen binding property. So when it binds to oxygen it leads to photo-oxidation, whereas when it binds to carbon dioxide, it leads to the formation of hydrogen carbons and then we talked about when it follows the photorespiration it leads to the formation eventually of the glycine which eventually converted into serene.

But end of it we liberate a carbon dioxide by absorbing oxygen and we lose the amino group and out of four atom of two molecules we could only salvage three carbon and one carbon is lost as carbon dioxide. And now we talked about the whole calvin cycle of what all the products which are formed and how this is in a cyclic fashion so you always form a five carbon moiety which along with the carbon dioxide in the presence of rubisco as you could see out here in the presence of rubisco and the presence of rubisco the critical reaction taking place followed by this.

So this fructose bit sucked or 6 phosphates is the starting material for all the biomass which is formed because these fructose 6-phosphate moieties then attached with each other we will talk

about it in the next class from the long chain of starch and sugar. So our next goal will be to you know to see this and what it needed is these so we'll be talking about now fructose 6-phosphate to starch and sugars which are source of all the bio fuels and while we talking about this we'll also talk about c3 verses c4 plants this is something which and this process what are the regulatory features.

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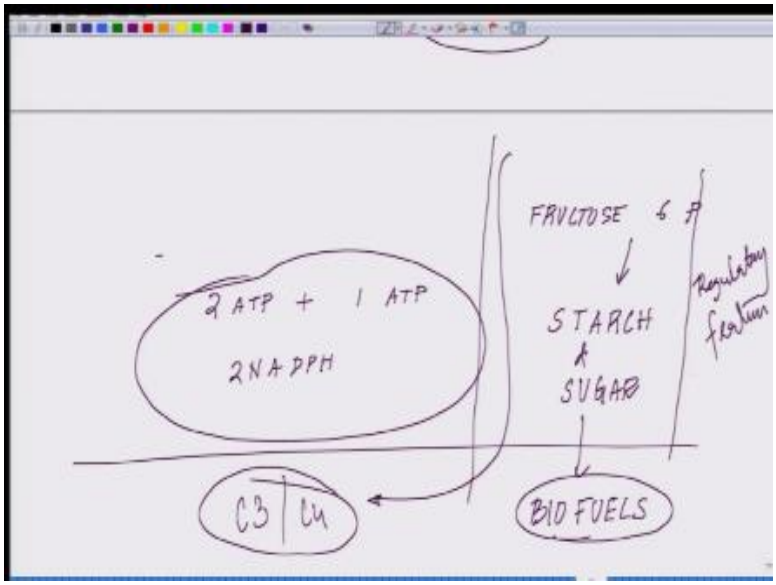

So our next class will be dealing with some of these topics how the first set of molecules are formed and of course, now we have already talked about the first set of molecules now we'll talk how they form the long chains and how the starch and sugar other sugars are being synthesized and how this process becomes fairly efficient at high temperature in crops like sugar cane other others where there is always a danger of photorespiration because of high temperature because, photorespiration level goes up at high temperature what are the mechanism which has been adopted by plants to create a technique to bypass photorespiration, okay.

So we'll close in here with this lecture so will carry over from this point how the starch and sugars are formed so this is the whole scheme of things now we know how the starch and

sugars are formed it will be easy for you to realize how we are going to transform them to different other viable product. Thank you.

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