Inorganic Chemistry of Life Principles & Properties
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Lecture - 37
Role of Nickel in life - Carbonmonoxide dehydrogenase (CODH)

Welcome you all to the next class in Inorganic Chemistry of Life Principles and Perspectives. In the last two classes we have been looking at nickel containing enzymes, and nickel as you have seen it is a very unusual kind of an enzyme, nickel containing enzymes where most of the reactions are gas based reactions either the reactant or the product.

So, today we will look at one another category of enzymes based on the nickel which is popularly known as carbon monoxide dehydrogenase, ok. So, this is a in the short form is referred as CODH carbon monoxide, CO is for carbon monoxide, dehydrogenase is DH, so CODH.

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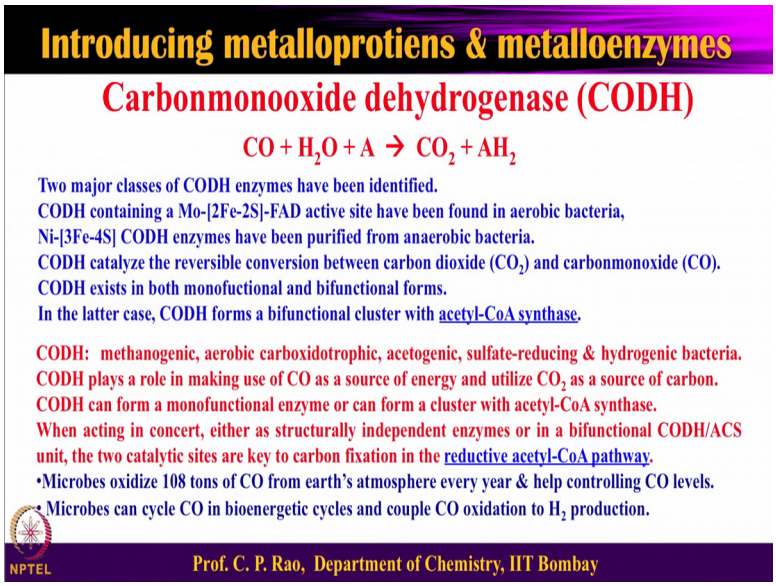

CODH carbon monoxide dehydrogenases are there even in the aerobic bacteria where molybdenum is possibly not the nickel, but here therefore, we are going to look at only the nickel containing one not the molybdenum containing. So, the nickel ones are there in the anaerobic bacteria. So, if you look at the earth creations life generation I am sure

you must have read again there it has started with a reducing atmosphere and then the life has started out of that.

So, it is believed that these CODH enzymes which are capable of converting carbon monoxide to carbon dioxide and back and along with other enzymes can convert into a methane do many more conversions. So, therefore, it is a strong belief that these enzymes would have turned the whole the reducing atmosphere of the earth to the current kind of a situation, ok. So, as I said that it can the CODH can catalyze the reactions of a CO 2 to CO and also you can have CO to CO 2. And the CODHs are having both mono functional types, bifunctional type.

So, when you say a bifunctional you have the activity coming from the carbon monoxide dehydrogenase and another enzyme may associate with itself to in order to make a additional reactivities. For example, acetyl coenzyme a synthase, so the CODH can combine with this towards the end of this lecture or end of this topic we will look at this multiple functioning of the CODH. So, as I said that this C CODH with the nickel is anaerobic. So, therefore, it is a part of the methanogenic, aerogenic, carboxidotrophic, acetogenic, sulfate reducing hydrogenic bacteria. So, it present in a variety of bacteria where it uses the CO as a source of a energy and CO 2 as a source of the carbon. And that is how the entire carbon cycles are being maintained by the CODH.

So, as I said the mono functional form and then bifunctional form. Bifunctional form along with the acetyl enzyme CO synthase a and that will couple with this CODH and we will see all those details as we keep moving across. So, it is a some kind of statistics is known the these enzymes tend to remove about 108 tons of CO from the earth atmosphere per year and then convert into other kinds of products, initially to CO 2 and then CO 2 by other enzymes into other kinds of things too. So, therefore, these enzymes help in controlling the atmospheric levels of at the earth's surface the CO levels, ok.

So, they can also utilize this CO and in the process it can generate the hydrogen 2. So, therefore, these are a huge set of enzymes where the carbon monoxide dehydrogenase is one of that which plays an important role, ok. Let us look at one of those enzymes carbon monoxide dehydrogenase, here on the left side on the top the panel we have the enzyme structure.

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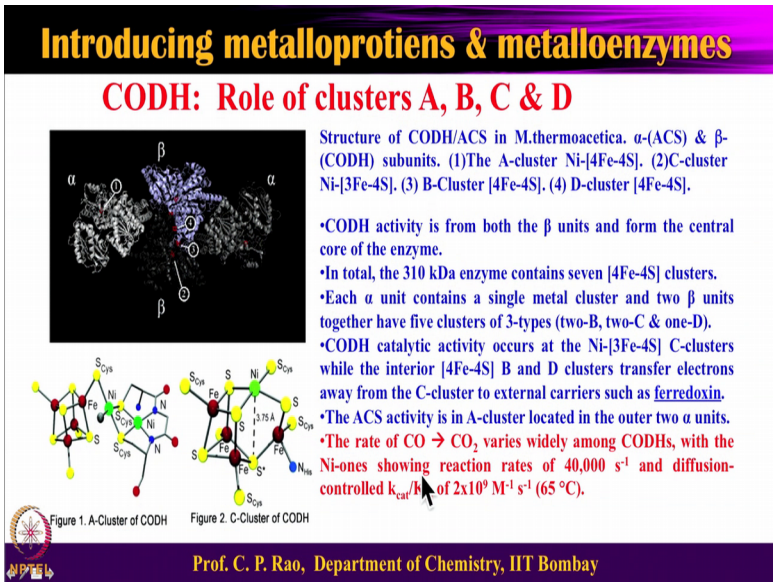

This particular enzyme structure is a from the bifunctional, it is C carbon monoxide dehydrogenase with acetyl coenzyme synthase. Acetyl coenzyme synthase is referred in short form as ACS. So, this is in thermoacetica bacteria and from these bacteria this protein has been isolated crystallized and the crystal structure what you are seeing here is a crystal structure.

As you can see there is something which are labeled as a alpha, there is something else is again here labeled as a alpha, and there is something labeled as beta and there is something labeled as beta. So, it is primarily it is the alpha units are the ones which are coming from the acetyl coenzyme synthase part and the beta parts are the ones which are coming from the carbon monoxide dehydrogenase. So, basically the carbon monoxide dehydrogenase enzyme is embedded into the 2 units of this acetyl coenzyme A, ok.

So, and these have got the nickel iron sulfur clusters called A, and nickel iron sulfur cluster called C and these are there and besides these nickel iron sulfur clusters. The nickel iron sulfur clusters should be taken as the reactive centers where the reaction occurs actually. In addition to that you find a several of the iron sulfur clusters. These iron sulfur clusters should be taken as those involved for the electron transfer we already have studied in this under the story of a iron the irons sulfur clusters electron transfer properties all of these that we know very well. So, as you know the CODH is in the

center part of that as a beta units and flagged by the alpha units into this, so into the central core of the enzyme.

And each of this have got the nickel iron sulfur cluster of different types that you have for the reactivities of this. And this total enzyme of this is 310 kilo Dalton and it contains 7 4 iron 4 sulfur clusters these can be seen in the later slides much more with clarity, of which one iron sulfur cluster is in this, other iron sulfur cluster is in this, remaining 5 are present within these between the beta subunits of the CODH, ok.

And among those 5 you have 2 are the B type, 2 are the C type, one is the D type. So, 2 B type, 2 C type and 1 D type of the iron sulfur clusters are embedded into this, and as I said in the later slides you will clearly see how they are and how they communicate the electron transfer to this thing, ok. So, these are all involved the catalytic activity as I mentioned is through the nickel iron sulfur clusters and the electron transfer is through the just simple iron sulfur clusters of these. And these iron sulfur clusters the interior ones been of the B type and D type. These clusters transfer electrons away from the C cluster and these carry towards the external reducing proteins redox proteins not reducing, but a redox proteins that is let us say for example, ferredoxin.

So, the in the acetyl coenzyme synthase the active center is in the alpha unit, there is one more in the alpha unit of this one. So, the rate of the conversion of the CO to CO 2 by these CODH kind of an enzymes of the nickel containing one shows the rates of 40,000 per second inverse and these are basically diffusion controlled because there is a lot of gas molecules are involved in this.

So, k cat by K M is around 2 to the 10 power 9 mole inverse second inverse at 65 degree Celsius. And as I was talking to you about a cluster looks like this part is the iron sulfur, this part is the nickel, these two are connected and this is C type cluster this portion is the iron sulfur, this portion is the is the nickel part of it is a green one nickel part of it. So, this is the iron sulfur. So, these two are integrated. So, these two are bit different in nature, and these are present accordingly in these enzymes, ok.

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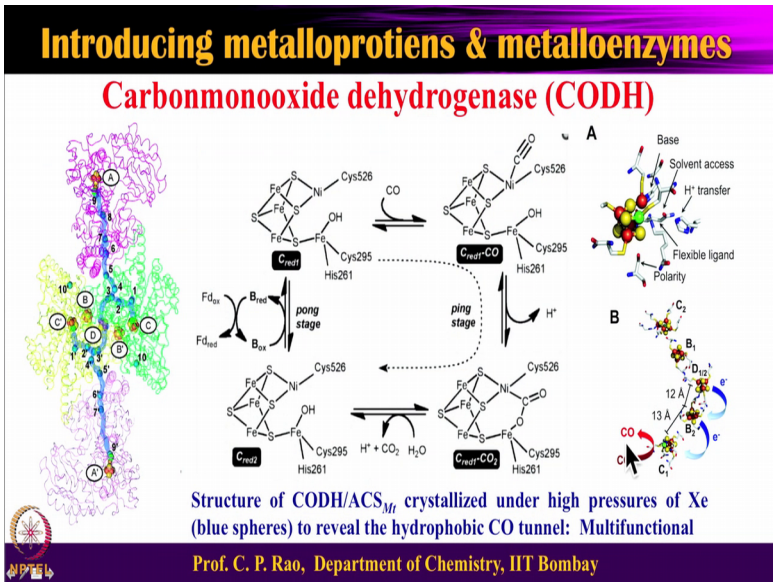

As I told you earlier that you can see that the 5 iron sulfur clusters you can see here 1 here, 2 here, 3 here, 4 here, 5 here that is how it is. So, these are all present in the CODH and one each is present in the acetyl CO enzyme a synthase. So, there that is that is how you can see that they are all separated by 10 12 13 angstroms from here to here, here to here, here to here, here to here.

So, these are the ones which couple the electron transfer and they too take electron from C to B and D and then finally, give it to external redox proteins like ferredoxins etcetera in these ones. This is one of the centers of the nickel iron sulfur cluster which is reactive center and you can see the surrounding how it is. Now, a crystal structure of one of these CODH is yes crystallized under high pressure and the pressure is used by the xenon atoms.

So that means, xenon atoms have got crystallized in the open space or the cavity. So, this blue ones is the one where you have a kind of a tube like structure where the gas passes through and in a enzyme that is a free space. So, this free space is filled by these xenon atoms when you pressurize and then you can see the path of the gas how it goes from one to the other. So, this portion is from acetyl coenzyme synthase here, this portion is a acetyl coenzyme synthase here and this part which is in the center is the one for coming from the carbon monoxide dehydrogenase. And you can see the 5 clusters here, the iron sulfur clusters which you have seen over here, ok.

Now, you understand the skeletal aspects of the structure of the CODH coupled with the acetyl coenzyme A. So, it is a very thing. Now, let us come to the reaction. So, the in the reaction of this you have talked about the C cluster of a iron nickel iron sulfur cluster with the nickel center here when you pass carbon monoxide ah. So, for a period of time then this one tend to attach the nickel center over here, and this the neighboring iron center has the hydroxyl which will act like a nucleophile.

So, the nucleophile will be acting on this CO or carbon monoxide which is bound to the thing and for that you require a deprotonation and the deprotonation is triggered by the binding of the CO here will bring some changes in the in the protein which will pull out this proton out therefore, you have a bromines and this bromine is will attack on this particular CO and then form an intermediate you see that Ni C O O Fe. So, you have a bimetallic intermediate is a bimetallic intermediate is there.

Now, this is due to a further entry of water and conformational changes and this will come out as CO 2 and the proton and that will lead to the cluster in a slightly different redox state of this one, ok. So, you have a different state, and this particular different state of the redox state as you can see over here. So, this is regenerated back to this by using a coupled reaction, ok. So, this is the B type oxidize going to reduced, the B type iron sulfur cluster oxidized will go to the reduced and then it oxidizes the reduced part. So, you have a reduced form of the nickel iron sulfur cluster here and this is reoxidized.

So, the oxidized one will get reduced and the this reduced one will get oxidized and that is given to ferredoxin. So, therefore, the B cluster of a iron B iron sulfur cluster is communicating the electron with the ferredoxins outside, ok. So, now, you understand, ok, you are written back. Now, this one as you can see the carbon monoxide binding carbon monoxide conversion this iron release is called like a ping. So, the protein going from this native state to a reduced state and then to bring back to the native state you have a pong mechanism and this is the electron is taken out by this oxidized cluster and the cluster becomes reduced and the catalytic center will get oxidized.

So, you know that always when there is some reduction it is always coupled with an oxidation you should see always that one. So, is a excellent way of. So, this part of the thing is called ping, this part of the thing is called the pong. And you know where the ping and pong referred I am sure you know in by various means or you yourself will be

playing a table tennis in the table tennis its referred as a ping and the pong. So, ping is your pushing the ball and there is and the back in the receiving from the other your neighbor. So, this is a kind of a ping pong reaction. Hope you understand the whole protein.

So, the protein has got the acetyl coenzyme on this side flank acetyl CO enzyme synthase A and then CODH and then you have a all these clusters 2 of the B type, 2 of the C type and a D type at the end 1 of the B type will revert it back. So, you have a an enzyme going to the reduced form and then reduced form is reoxidized and this is a kind of a cycle. And by putting these xenon atoms you could see the path through which the gas is going out. So, you need the entry here and the exit all these kinds of things are there and they can be funneled to the funnel.

So, therefore, you can have a gas molecule going from the acetyl CO synthase A to this CODH as well from CODH acetyl CO enzyme A. So that means, these two together can do a lot of things we will come to that in a while. So, is this clear? Carbon monoxide binding at the nickel center Fe OH providing a nucleophilic attack by deprotonation giving an intermediate between the nickel and a iron the CO O is bridged within them and this is released as CO 2. And the protein in the center is reduced and this nickel center is the reduced nickel center is reoxidized back to the normal by this B part of the oxidized iron sulfur cluster going to reduced and the ferredoxin oxidize goes to the ferredoxin reoxidized accordingly this is one part where we have seen CO O into CO 2.

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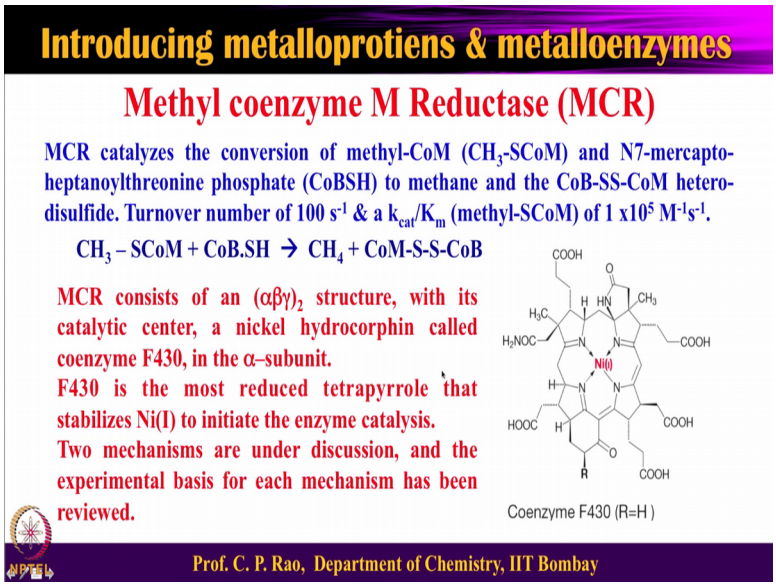

Let us look at another enzyme where the methyl goes to methyl, moiety goes to methane moiety the methyl going to the methane moiety and this is referred as methyl coenzyme M reductase. So, MCR this methyl enzyme coenzyme M reductase has got a CO enzyme part called F430. So, have a look at this F430. And this enzyme can do a turnover of 100 per second and with the k cat by K m of 1 into 10 power 5 mole inverse second inverse very interesting.

So, this looks in the first side as if it is a power frame and if you look at very close by no not a power frame highly reduced centers that you have. So, and this enzyme has a hexameric with alpha beta gamma twice structure. So, dimer of the trimeric protein, alpha subunit, beta subunit, gamma subunit twice of that the catalytic center of this nickel hydro korfin which is referred as the F430 this is present in the alpha subunits of this protein.

So, this particular is a thing is really is present in the alpha subunit of the coenzyme, ok. And by being this highly reduced form of this and this is able to stabilize the nickel 1 and nickel 1 is essential for initiating a reaction. If you do not have a nickel 1 here if it is nickel 2 no reaction can occur. So, therefore, the nature has designed this coenzyme F430 for the reason that it can maintain nickel 1 in the state and nickel 1 can initiate the catalysis. So, there are as you can see in the next slide there are two paths of mechanism

the path 1 is here and path 2 is over here. Before that let us come to this particular reaction of the enzyme to propose paths.

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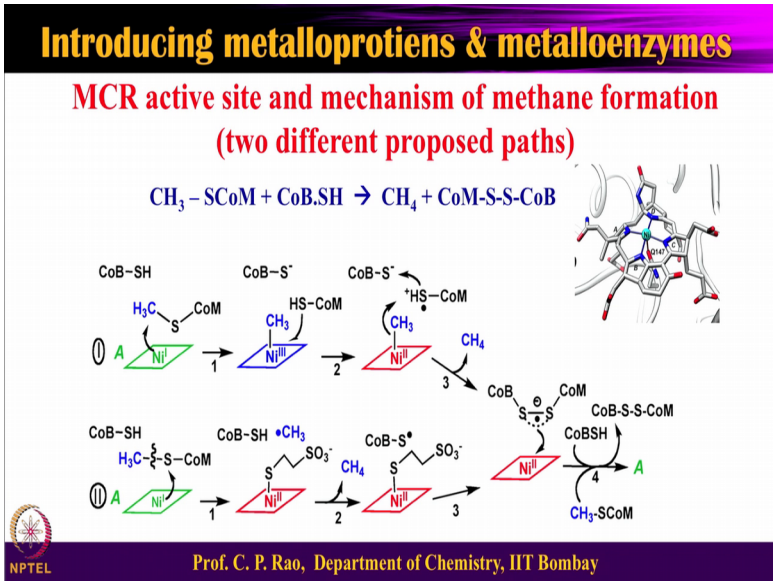

So, the methyl portion about the coenzyme M, there is another coenzyme B with the sulfidryl will assist the reaction to break down this SME to form SS bond in the process it gives a proton and the CH 3 radical will get take a proton radical or CH 3 minus will take a H plus and then form the corresponding CH 4, and then the corresponding disulfide. And this will be further broken down to bring back to the normal by other enzymes. So, as you have seen in the previous slide this this is the F430 structure over there.

Now, let us look at the mechanism in the in the this goes through the step 1, goes to the step 2, step 3 and then goes to the both of them step 4.And this also goes to the step 1 different type, step 2 different type, step 3 different type then common, so that means, they come to a common kind of an intermediate at this stage which leads to the product of this, ok. Nickel which is in reduced form is called nickel 1, nickel 1 now can act like a act like a nucleophile.

So, the nickel 1 can act like a nucleophile because it is electron reach you have lot of electrons. So, there this can attack at the methyl and the methyl is pulled out from this coenzyme to form a nickel methyl and the nickel 1 goes to what nickel 3. So, what does it mean? Nickel 1 is got oxidized to nickel 3. So, what does that mean? That means that it

has given away the two electrons. So, it has given away the two electrons to make this one and then bind into itself that means, CH 3 minus you are getting into this.

So, now, the nickel methyl moiety and then you get the other part the CoM S S H part of that where the proton is coming from the this counter part of the thio of part of this. And now this forms a nucleophile attacks on this particular thing and and then that will pick up the methyl as the methyl radical with the edge. So, therefore, you get the methane out.

So, once the methane out you get a intermediate structure like this and this intermediate structure is returned back to the disulfide. And this disulfide will further react to get back to the individual ones that we do not need to worry. So, pickup of the methyl from the coenzyme by nickel 1 convert into nickel 2 methyl nickel sorry nickel 3 methyl. And now this is being assisted by the other one CO co-enzyme with SH giving away the proton and that leads to the creation of this radical and then attack here to form the thy ill kind of a radical, thy ill disulfide thy ill kind of a radical and that goes back.

The other side here the nickel 1 can instead of taking away the methyl can attack on this to make a break into the methyl radical and this methyl radical is sort of initiates a radical into this part SoB S dot and that methyl radical will take a hydrogen. That means, the methyl is picking up the hydrogen outside with the other one.

So, these two are two different kind of a mechanisms in this case straight away the methyl getting to nickel 1 going to the nickel 3 and here you are not proposing nickel 1 and nickel 3 the kind of a nickel 2 both. And this particular thing takes up a proton and then from this iron goes into meth methane and that forms this. And this again attacks on this and forms this particular intermediate go back to that, ok, so in all these.

So, these are the kinds of a proposed mechanisms and we are not going to the details, both of these are possible to be to be giving the final product. At this stage we do not need to worry which of these are fully supported by the by the experimental or some computational which I am not going into the details, at this stage you take these two as the two mechanistic aspects of it. No. So, so you have already seen CO 2, CO 2 CO going to CO 2 conversion.

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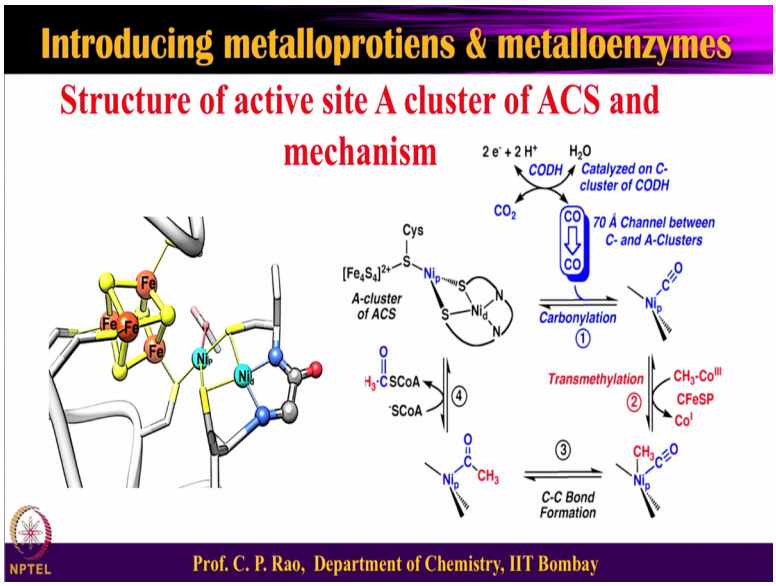

Now, here a coenzyme methyl going to methane conversion, and then let us look at the third part is a acetyl coenzyme how it functions. Acetyl coenzyme will do what? It will do acetyl moiety synthesis. So, what is a acetyl moiety?

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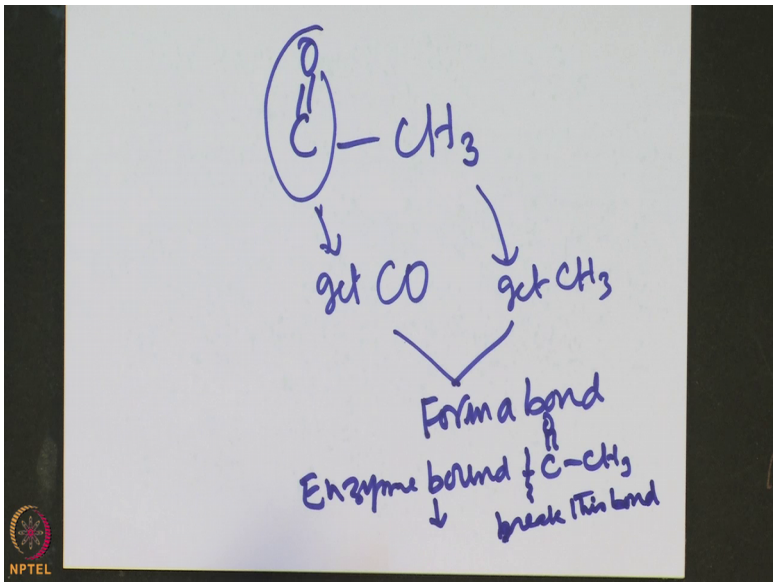

So, acetyl moiety is CO CH 3 so that means, you have to get CO. So, get CO, get CH 3 and then you form a bond to get enzyme bound CO CH 3 then break this one break this part of it, bound to release.

So, you have basically you have to bring from one side a CO the other side the methyl and combine them together. So, you can see that, you can see that that there is another enzyme CODH we have already talked about CODH enzymes can make a CO to CO 2 as well as CO to CO and therefore, from that enzyme let us assume that CO is coming. And this is the enzyme portion that you have and this is the enzyme a portion which is the acetyl coenzyme synthase A cluster not C cluster a cluster and that is way over here.

So, you see that iron sulfur cluster a nickel and a nickel and this is the active centers of this. And the CO which comes from this CODH will funnel through that particular channel which I showed you earlier. So, this will the CO that is coming from here will channel through and then go to the acetyl coenzyme synthase part, ok. So, that CO now is attached to the nickel center here. So, that is the nickel centre here and that nickel center which is shown as a P that is the one which is reactive which will make the carbon monoxide.

Now, the methyl has to come from somewhere where will it come it comes from some another and this methyl part will come from b 12, ok, which I talked to you when we talked about the cobalt enzymes, ok. So, therefore, it will come from a cobalt 3 methyl. So, the methyl is coming from another ends of coenzyme which is called the cobalt amine which with the in the form of a cobalt 3 CH 3. Now, this CH 3 is transferred to the nickel center.

Now, the CODH is transfered CO and the cobalt amine transfer the methyl and both of them transferred onto the platform of the acetyl enzyme CO synthase A ACS, acetyl coenzyme synthase see that. So, one part is coming through one enzyme other part is come through another enzyme and here you have the acetyl synthase CO enzyme. Now, now you have captured both the CH 3 and CO, ok.

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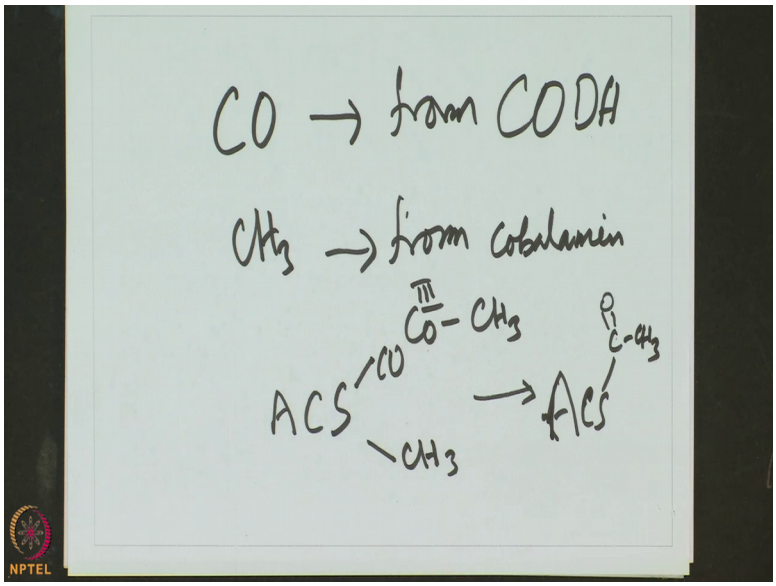

So, methyl CO is coming from CODH and methyl from cobalamin cobalt CH 3. So, the two joining together and this gives and both of these will give to the acetyl coenzyme synthase.

So, you will have CO, you will have CH 3 and this will go as ACS CO CH 3, ok. So, that is where the enzyme part is. So, you can see that it is formed the CO CH 3 and this will further undergo redox to get the cleavage of this and go back to that as CO minus A and then enzymes. So, you see that, ok.

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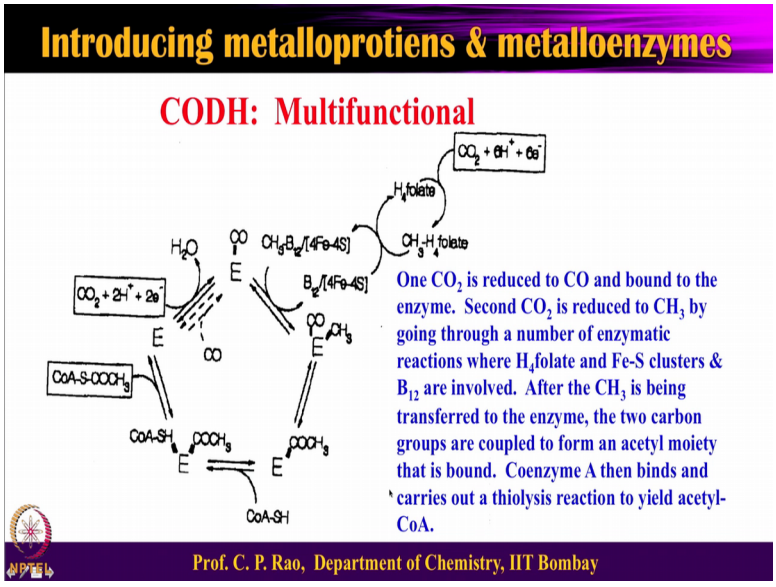

So, you have multifunction CODH is multifunctional as I said earlier. So, you can see this is very simple, but otherwise it looks very complicated.

So, this is the power on the right corner you have the folate kind of thing where you have the carbon dioxide and they convert it to the methyl and this is fun further funnel through the thing and given and you have an enzyme where the CODH gives the CO, and this folate enzyme even this one methyl and get on to the same as CO E CO CH 3 from just now I explained to you. And this is the other part of the enzyme this enzyme will come out as a coenzyme synthase and this will be further cleaved to give the acetyl part and this will go further reduction to the CO 2 part of it water and then CO goes back.

So, multiple there is an enzyme working, enzyme working, enzyme working in this, so all this. So, this is a multifunctional kind of an enzyme platform multi functional enzyme kind of a platform now you have seen. So, you have a CODH, you have a acetyl coenzyme synthase A, you have a other enzymes based on the folate etcetera bringing the CO 2 to CH 3 to CH 4 CH 3 sorry adding to this particular enzyme CODH adding the CO and together forming this one.

So, you see that whole thing is a complex mechanism of this. This is how the nature in the early days when we have a reduced atmosphere has been able to remove all the carbon monoxide from the earth atmosphere or try to reduce.

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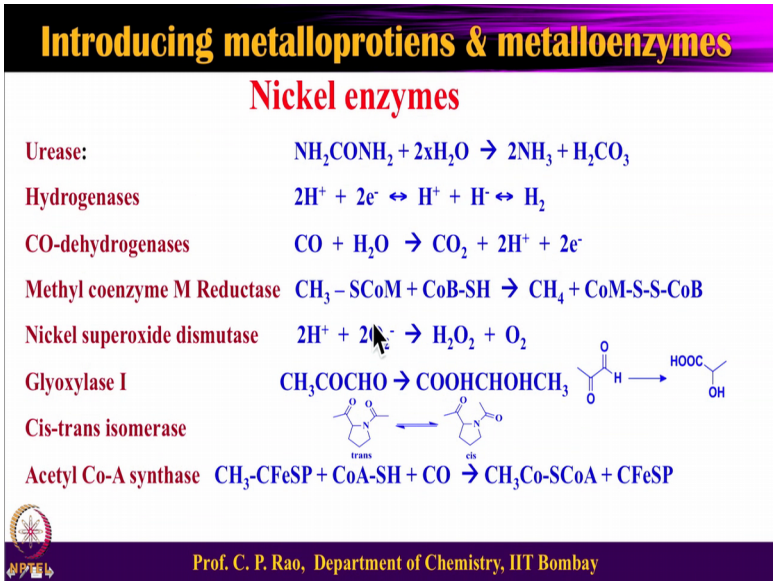

And now although it maintains the minimum levels of the carbon monoxide anything. So, essentially under this nickel title. What we have learned? We have learned an enzyme where the urea gets hydrolyzed as you can see 2 ammonia 2 CO 3 we have looked at the hydrogenases. Now, we have looked at just now the carbon monoxide carbon dioxide carbon dioxide carbon monoxide carbon monoxide dehydrogenase we have looked at the methyl coenzyme reductase.

We also have looked at the acetyl coenzyme A synthase, and we have looked at prior to that methyl superoxide dismutase all of these and glyoxylase how the conversion C strands in all these things and so only. So, we have more or less convert, covered entire thing that we have here a galaxy of the enzymes present under the nickel. So, and they are all except the glyoxylase one and they are all containing gas based, either gas is used and gas is produced reactions, so very marvelous kind of things that happen with the nickel enzymes.

Thank you very much.