Inorganic Chemistry of Life Principles & Properties
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Lecture – 34
Role of Cobalt in life

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. Immediately prior to this particular lecture we had completed the iron over a period of 10 lectures or so. All different types of enzymes, stories transport as well as the oxidative type, reductive type, hydrolytic type more or less a good range has been covered.

So, let us now get into the next one in the periodic table of the iron what comes is the cobalt unlike the iron cobalt is involved in only one particular case and that is vitamin B 12. We all know that a vitamin B 12. Actually vitamin B 12 is just a coenzyme it is not an enzyme and therefore, a coenzyme containing adenosylcobalamin is acts as a coenzyme with a host of enzymes and does lot of lot of reactions.

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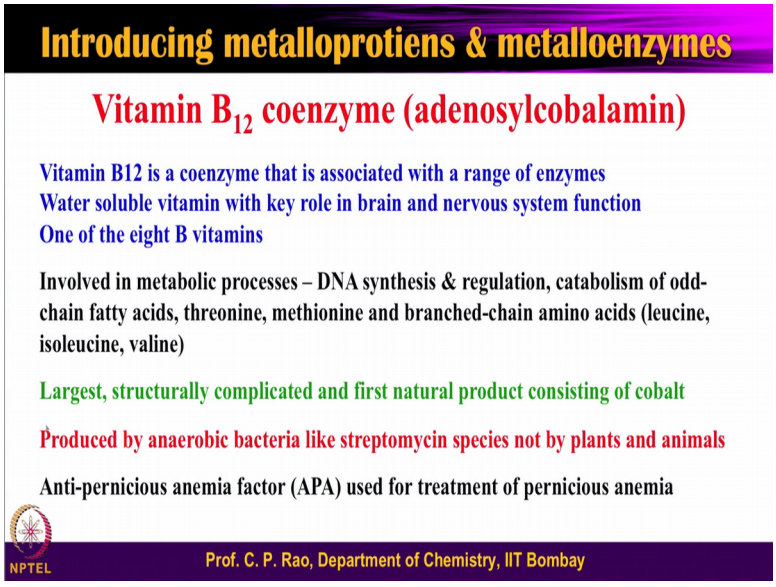

So, therefore, you can see that a water soluble vitamins has a key role in brain and nervous systems and functions very well. And it is one of the 8 vitamin B vitamins it is involved in a variety of metabolic processes particularly DNA synthesis regulation catabolism of fatty acids

Some amino acids like threonine methionine and branched to ones like leucine isoleucine variety of these things are involved, but only one enzyme has got the cobalt in the entire thing whereas, iron is present in more than 200, 300, 400 kind of different enzymes that you see the different.

Also cobalt is not present directly in the enzymes present in the form of adenosylcobalamin which is associated with a few host of enzymes and does the function. How it does we will learn in this particular class, ok. So, it is one of the rarest one that we have for the cobalt and that is what we standard ok.

And it is a produced by the anaerobic bacteria like streptomyc, streptomycins species and not by the plants and animals. So, this is also having entire pernicious anemia factor used for the treatment of pernicious anemia. So, people use you know very well vitamin B 12 they take for whenever you feel somewhat weak you take that one, that is how you supplement the cobalt in the form of a cobolamin and some kind of a supplements in that too, ok.

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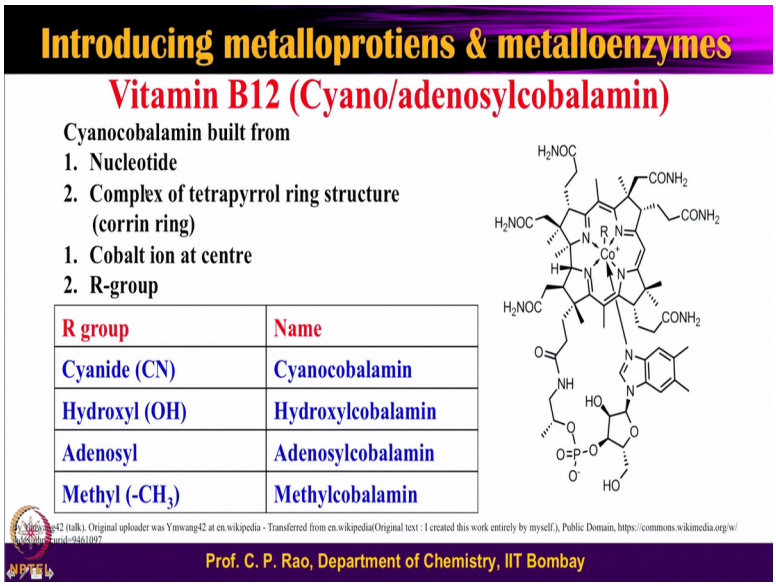

Now, let us look at some these different forms of this, we can built in the forms it has see that this is looks like a porphyrin ring. But if you look at closed by it is not because there are some saturated centers are there and this is known as a corrin ring, this is known as corrin ring.

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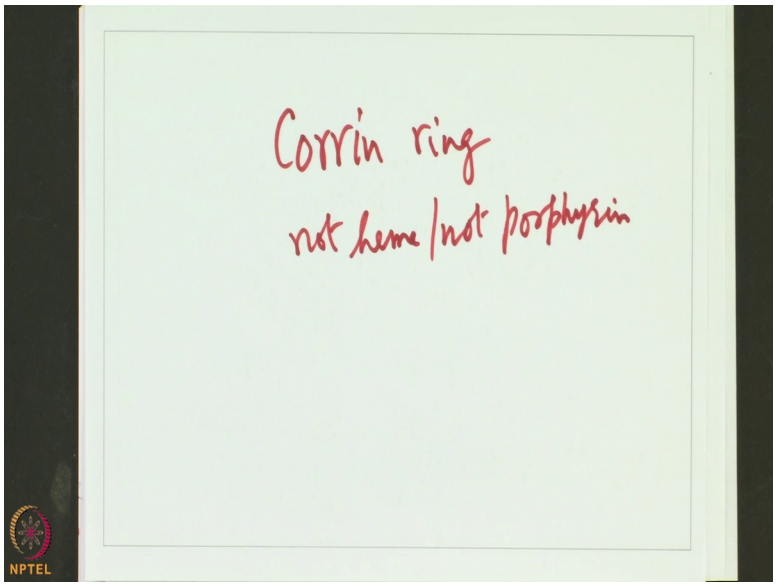

As you can see that not heme, not porphyrin it is a somewhat like reduced porphyrin or something we can see. Now, so that is bound through the 4 nitrogens over here and of course, it has it shown and then the one more group is possible the and this is what is this you can see that this is connected through this. And if you look at here this is the benzimidazole and this is the glyco kind of a moiety and the phosphate.

So, this is more or less like a nucleotide. So, it is its bound through a nitrogen of a nucleotide, ok. And then this side you have the some groups depending upon during the during the different kinds of an enzymes most of the enzymes will work with adenosyl, but some enzymes will work with methyl and some enzymes some during the isolation process can cyanide other kinds of things are also hydroxy all this things can form ok

So, there is a nucleotide it has a tetrapyrrol ring which is corrin it is a cobalt of course, at the centre and is an R group. If the R group is cyanide then you call cyanocobalamin, if a R group is a hydroxy then you call it is a hydroxycobalamin of course, you can say acrocobalamin and when you have a adenosyl moiety which I will show in the later slides then I will adenosylcobalamin and if it is a methyl methylcobalamin.

So, I will show some examples most of the examples are by adenosylcobalamin and one example I will show by the methylcobalamin two, how they are all involved along with the corresponding enzyme and function. So, this is whole thing is called the cobalt based cobalamin coenzyme. Primarily when R is adenosyl I mean adenosyl then it is called

adenosylcobalamin that is the one which is involved, in some cases methyl some cases cyno etcetera, in in different forms it will change.

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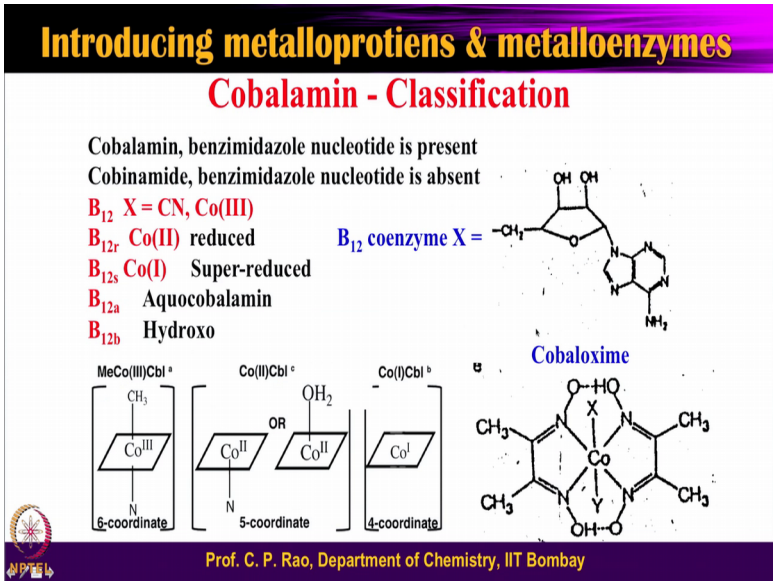

So, we have looked at this then we have we can also look at the cobalt center, the cobalt can be in the cobalt 3 form, cobalt can be in the cobalt 2 form, cobalt can be in the cobalt 1 form. When it is in 3 then it is referred is a normal, then is 2 reduced when it is 1 super reduced. So, how will it happen? It depends upon what kind of a binding how that changes it is depends on the binding and the X group both it depends on the both the binding.

So, the binding in this case is the is through the that nucleotide benzimidazole based nucleotide as I showed in the previous slide and X could be various things. So, this particular thing is basically is referred as the adenosyl, ok. So, that is a adenosylcobalamin when it is bound. So, the the whether the cobalt is 3, 2 or 1 also depend on the X and its bulkiness etcetera I will explain another thing.

When you have water it is called B 12 aquo, when you have hydroxo its called hydroxocobalamin this B 12a, B 12b and B 12r is cobalt 2, B 12 s is super reduced cobalt 1, B 12 is cobalt 3. So, when you write B 12 is cobalt 3 and this bound can change depending upon the X that you have and you can see this the cobalt 3 is with the perfect normal binding with a 6 coordinate the cobalt 2 with the 5 coordinate one of this is basically looking kind of thing.

Cobalt 1 both of them are broken because it is a very strongly electron dense kind of a center binds that. So, for a 6 coordinated 5 coordinated and 4 coordinated. So, from that also one can try to identify this is a. This is a synthetic kind of thing synthetic kind of thing, where X and Y can be varied and then people have made all these kinds of this these are called cobaloximes, cobaloximes. So, like other kinds of oximes cobaloxime.

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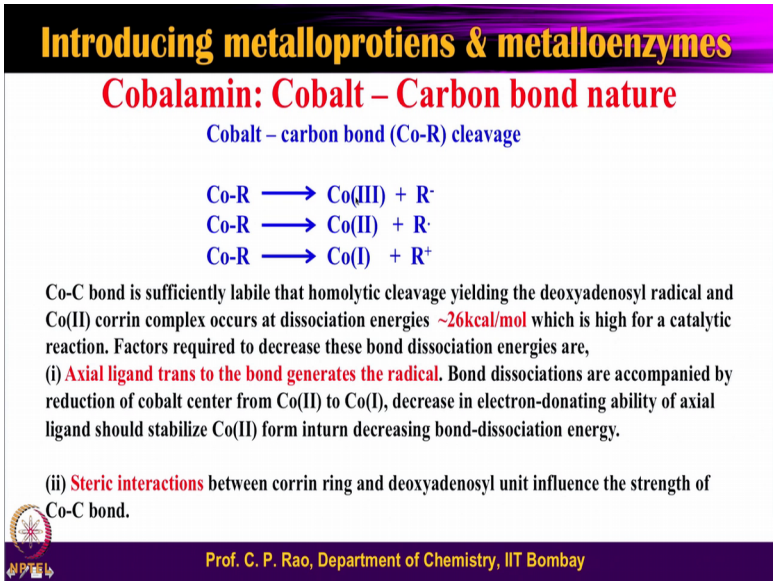

So, let us come to the situation of the cobalt carbon bond. As I told you earlier that the cobalt can be in 3, 2 and 1 and so cobalt R bond if it is cobalt 3 the R will get a characteristic of R minus ok. And if the cobalt is cobalt 2 characteristic then the R will be like R dot and the cobalt is cobalt 1 then R R be R plus. So, in this case it is the cobalt 3, cobalt 2, cobalt 1 R minus R dot R plus.

What is that depend on? First of all what is the cobalt carbon bond cobalt carbon bond in these things requires energy for about 25 to 30 kilocalories which is generally higher than for the catalytic kind of thing. So, therefore, one needs certain kind of a reorganization rearrangement which is also dependent the X group that you have.

So, the air one is the axial ligand and the bond dissociation energies are accompanied by the reduction of the cobalt center from cobalt 2 to cobalt 1 and decrease in electron. If you do if you decrease the electron donating ability of this fifth coordination of the axial ligand then you can stabilize the cobalt 2. If more electron electron donating property it

will become cobalt 1 and R plus if it is much lower an electronegative electron donating then it will be cobalt 3 and R minus. So, these are the things. So, therefore, all these will.

And the second important very point is the X group its steric parameter, so because this whole thing is going to interact with the enzyme. So, therefore, the R group that is present, X group that is present on the cobalt center between the corrin ring and this one. So, in the case of adenosyl adenosylcobalamin then it influence the strength of the cobalt C bond. So, whatever you have in the 6th coordination it is steric bulkiness, its interaction with the when it interacts with the enzyme how the conformational changes will influence the bond these all will decide the breakage.

So, essentially whether it will break as cobalt 3 R plus, cobalt 2 R dot, cobalt 1 R minus, cobalt 1 R plus sorry cobalt 3 R minus, cobalt 2 R dot, cobalt 1 R plus. So, these are the kinds of things. So, having looked at that let us try to understand this different electronic states I mean you have a cobalt 3 3, ok.

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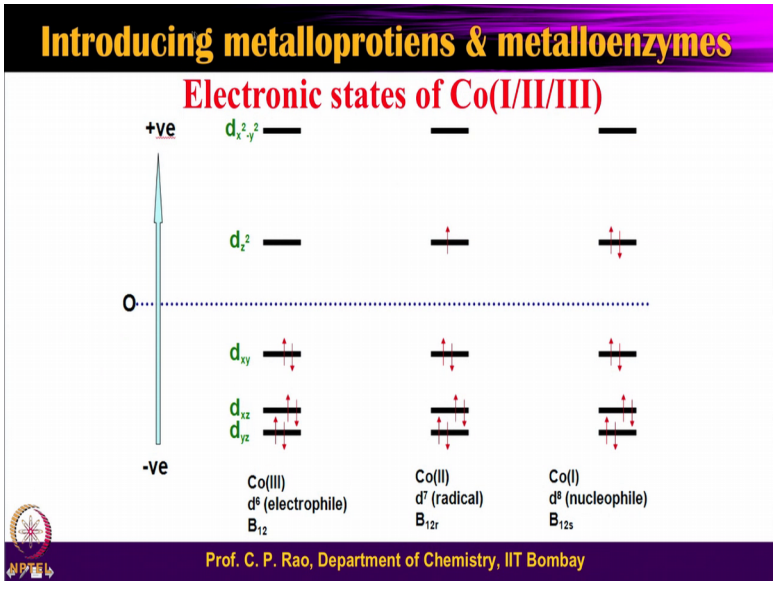

Then you have 6 electrons and the cobalt 2, the 7 electrons d and cobalt 2, 1 8 electrons. So, if we look at this and try to fill this and they are all filled early and there is a empty orbitals and the ones which you have these empty orbitals, that means, it is capable it is like a Lewis acid. So, it is capable of accepting an electron pair low lying on the empty orbital.

Now, you come to this the 7th electron is here therefore, this electron only one single therefore, it is a radical kind of thing. So, radicals can be very reactive too and if you look at this one you have both the electrons are filled. So, it is a pair. So, it will be a nucleophile, so electrophile radical nucleophile. This is B 12, this is B 12r, B 12s. So, these are there. So, B 12 is cobalt 3 B 12 or it is cobalt 2 B 12s is cobalt 1 and that is what we.

Now, having looked at the characteristics of the cobalt center oxidation state binding from the benzimidazole binding from the X point of view and whole thing interacting with the protein and what kind of reactions these guys are involved the cobalamin based enzymes.

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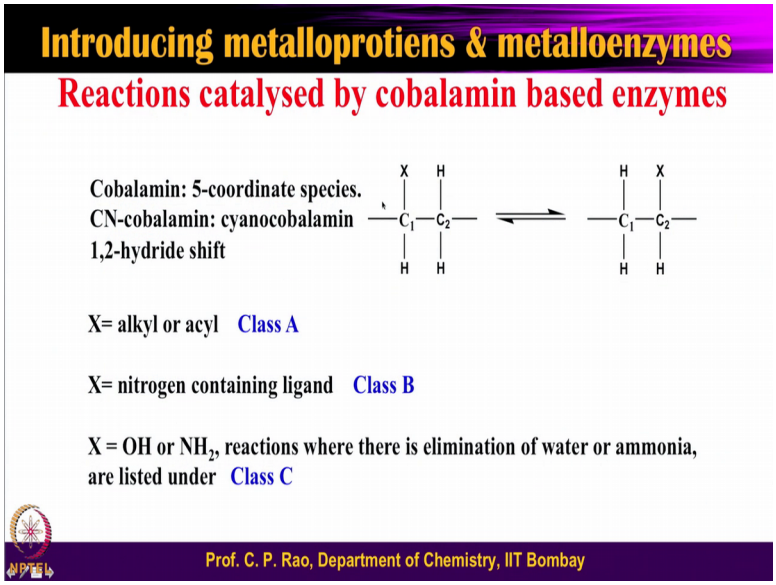

Basically they do a kind of a breakage and makeage break a bond and make a bond. So, you can see this example C 1 C 2, the C 1 has X and on this side C 2 has X.

So, it is because there is a hydrogen and shift is called a hydride shift in this case 1 2 hydride shift, 1 2 hydride shift. And if the X is a acyl or alkyl group it is called class A kind of reaction and this is again different enzymes are associated. And if you have a nitrogen containing ligands it will be class B if you have O H amine and at the end the water is eliminated or ammonia is eliminated then such a kind of class of reacts in the class C, no elimination in A and B there is a water or ammonia elimination in class C.

So, overall the cobalamin enzymes cobalamin coenzymes are associated with a host of enzymes and does the reaction of A type, class A type does the reactions of class B type does the reactions of class C type ok. And that is clear.

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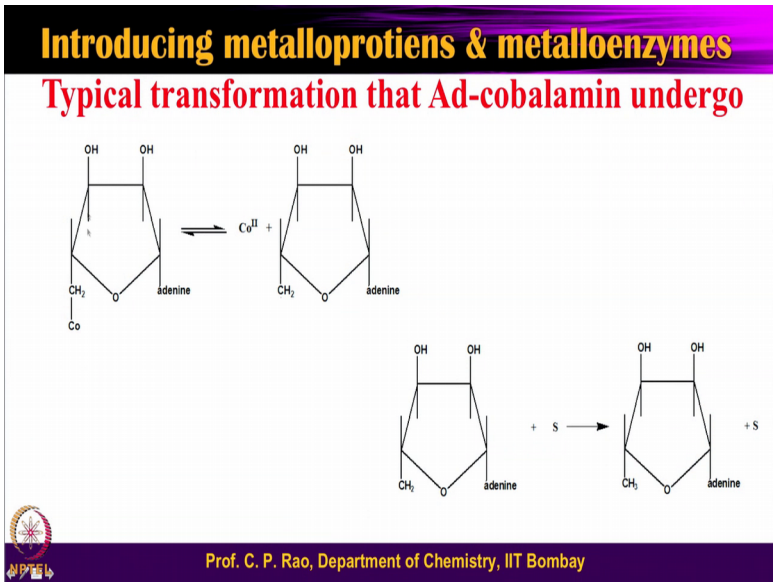

So, what would happen at the adenosyl center, that is let us look at. This is a part of adenosyl and the cobalt carbon bond. So, through this from, this is not from the other side this is from the adenosyl side and this in are just in such a way because it is its bulkiness in such a way it always goes in the cobalt 2 with a this particular radical here actually CH 2 is a radical. And such a radical can induce a radical in the substrate and transfer the hydrogen to this and become CH 3. So, the CH 2 radical can become CH 3 and then radical is associated here and then that radical can rearrange etcetera etcetera.

So, you generate a radical by breaking the cobalt carbon bond as the cobalt adenosyl and this radical is transferred to the substrate and substrate gives the hydride, and becomes methyl and again goes to substrate radical, and substrate can make any kind of a changes and become a product.

And now this is transformed to methyl then there is another set of enzyme which will remove this hydrogen as a H dot and then go back to the cycle kind of thing ok. So, this whole thing occurs at an interface of the vitamin B 12 which is a coenzyme and the corresponding enzyme. So, is that understand?. We have explained what are the different

kinds of things then we have looked at that what different types of O cobalts 1, 2, 3 and what kind of an X group and adenosyl how does that break.

So, I said the reactions of the A type, reactions of the B type, reactions of the C type. Let us look at some of the reactions for example, glutamine mutase, if you look at this and one of these carbon, there is a shift 1 2 hydrogen that becomes methylene.

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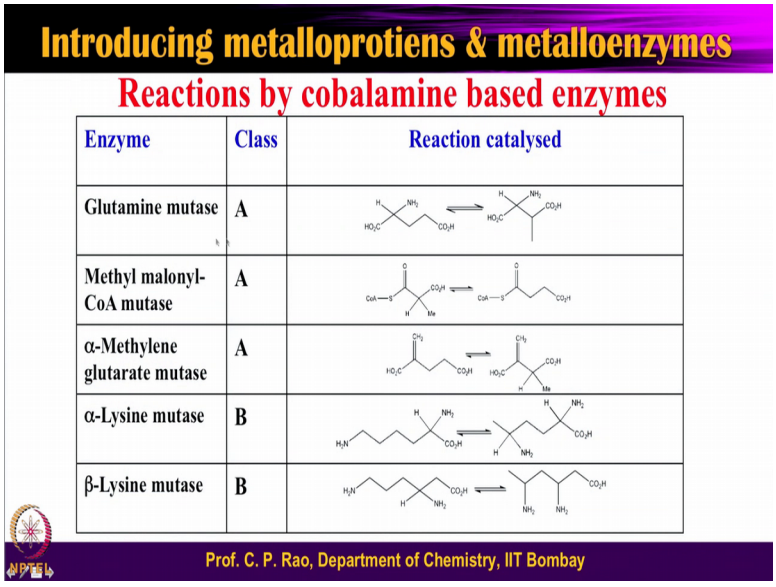

So, this goes and connects here and this becomes CH 3 and that is why the 1 2 hydride shift. Here you have this methyl that will get convert in to methylene and then get inserted into this. So, it is the 1 2 hydride shift, again here another 1 2 hydride shift. So, these are all A type kind of things where you have the groups of A the mentioned there. So, these are mutases, mutase, mutase. So, all these mutases is obviously, are involved in 1 2 hydrogen.

Then B type where nitrogen kind of ligands are there, but no elimination takes place and you see that and this shifts over here shifts over here, so NH 2 and methyl group; and shifts over here, so NH 2 and methyl group. So, this is lysine mutase, alpha lysine mutase, beta lysine mutase. There is NH 2 group nitrogen containing, but no elimination of anything happens. So, class A type 1 2 hydrogen, class B type hydride shift, but having a nitrogen. So, that will become amine group and that will rearrange.

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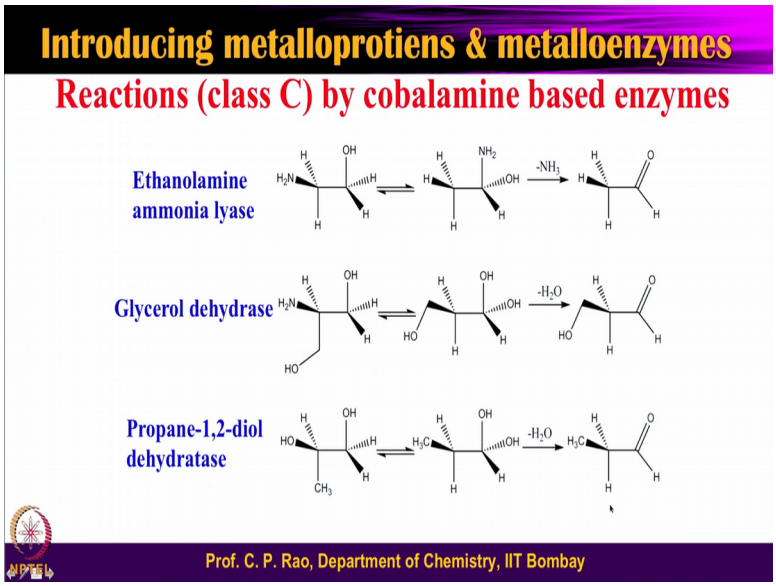

And then you have a class 3 type class C type where it is after the 1 2 hydride shift. There is an elimination of water or ammonia. And look at the glycerol dehydrates and this one this will become the 1 2 that shift that will go into two hydroxyl groups, on the same which is called gem dihydroxy and this is we know very well from the organic chemistry. This is kind of situation most often leads to the elimination of water and then gets use this one; obviously, and another example propane 1 to diol dehydrates again you will go to the (Refer Time: 16:28).

So, this kind of reactions are type C and in this case it is the amine which you have, so therefore, it is the NH two plus h that is ammonia is lost and then. So, either the ammonia loss or the water loss any of these things will happen with this too this is the class 3.

So, now, I explain you the class A type of reactions, class B type of reactions, class C type of reactions in all these things and let us look at their mechanistic aspects. Then let us look at the last example we have taken diol dehydratase, diol dehydratase means water kind of thing.

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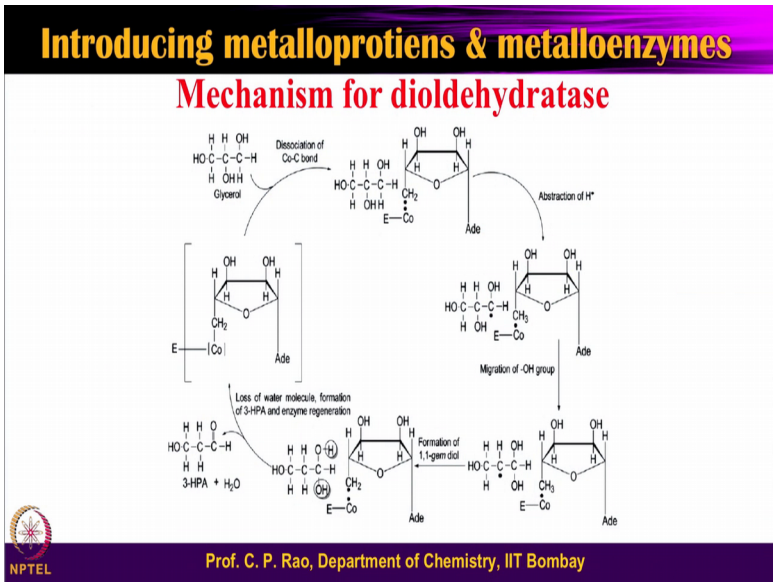

So, the enzyme let us take in the normal situation cobalamin adenosyl. This is the adenosyl cobalt this is the rest of the enzyme. So, this enzyme cobalamin enzyme these are associated this is the coenzyme.

Now, when the substrate comes into comes in contact, obviously, substrate substrate has to bind in very close proximity of this the adenosyl group because the radical has to be transferred without any other destroying or other kinds of things happening. So, therefore, it will bind very close proximity of the cobalt center, cobalt carbon bond center from the enzyme side.

When it binds there is certain kind of a the conformational change that occur that will trigger the cobalt CH 2 bond to weaken this bond. So, when it is weaken then this will hydride abstraction of H and that will lead to the this kind of a things, you see that and starts breaking and then you get the radical part and this radical of ; obviously, has gone to this.

So, the rupturing hydride and therefore, it becomes the radical. And this radical is transformed or racemized to the next carbon that that is also same as saying that the hydra hydroxyl is shifted to this, migrated to that. So, that is again hydride shift 1 2 hydride shift.

So, now you find a situation where these are all on the on the on the substrate of and that substrate is binding very close to this you see that very close to that that brings a lot of conformational change. And that confirmation change is the one which is responsible for weakening this bond and make a the radical and the radical abstracts a hydrogen or hydride hydrogen dot and kill the radical on the substrate radical. This is a substrate radical there is the radical shift happening because of the hydride shift.

Now, this particular thing is not stable and this will go by removing the OH and H as a water. So, when you remove the water you get this obviously, the raised ok, now you can see that. And now this one again has to bind because it is the C CH 3 has to make the form on the binding by binding should take place a normal. So, therefore, cycle is regenerated. So, you understand.

The cobalt adenosyl bond breakage, and then the radical that radical is transferred to the substrate and the hydride is transferred to the adenosyl to become CH 3 and then that radical is racemized; And then the in that process you have a hydroxyl shift and then 2 hydroxyl come on the same carbon and loses the water and then makes this one, ok.

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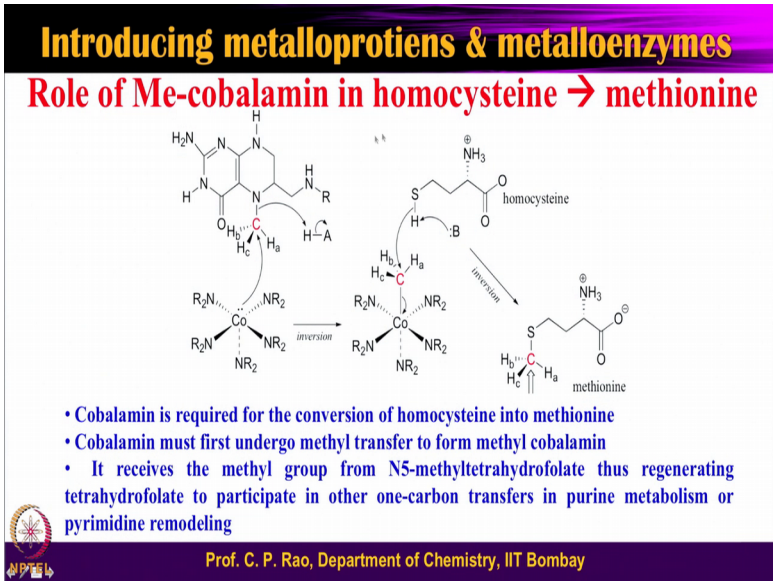

Now, not only the adenosyl even the methylcobalamin in the also involved in the homocysteine to methionine, ok. First you will have a biologically important thing is a not methyl one it is a adenosyl one, adenosyl one will lose this one and get the methyl group from this particular moiety this is called N5 methyl tetrahydrofolate from here the

methyl comes here. As you can see the methyl of course, in an inverted fashion, and this is the one which is interacting with the homocysteine and the homocysteine this gets inserted and therefore, you have the whole thing going into this.

So, I am not shown all the other parts. So, it will convert into the methionine, so homocysteine to methionine. So, cobalamin is obviously, required for the conversion of homocysteine to methionine methyl methionine and it is not the normal cobalamin is a methylcobalamin. So, the normal cobalamin is adenosylcobalamin is loses the adenosyl and methylation takes place cobalamin must first undergo the methyl transfer to form methyl cobalamin.

So, it receives the methyl group from N5 methyl tetrahydrofolate thus regenerating the tetrahydrofolate to participate in other one carbon transfers in purine metabolism or pyrimidine kind of a thing. So, these are all kind of things are happen that is why DNA synthesis and other things now.

So, we have seen one diol dehydrogenase dehydratase, then we have looked at the homocysteine to homocysteine to this methionine conversion and for which you require the methylcobalamin, and now we have another example ribonucleotide reductase.

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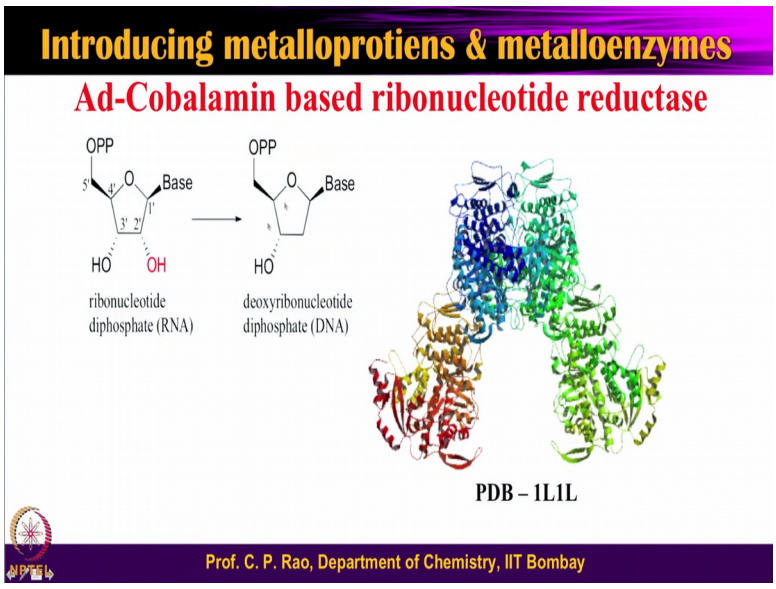

We have already seen under the iron what are ribonucleotide reductase is. A ribonucleotide reductase is the enzyme which converts the ribose into the deoxyribose,

but the entry point is a nucleotide of the ribosyl and then goes into the deoxyribosyl ok. So, this I have fully explained to you under the ribonucleotide reductase under the iron completely.

As I told you that there is the center or the di ion center the iron with the tyrosine radical this radical is transferred to the second portion of this particular enzyme, to the through the cysteinyl to the to the center of the ribosyl to the C 3 prime then converts into C 2 prime. And then finally, d d hydro change kind of water then giving there that we have seen already.

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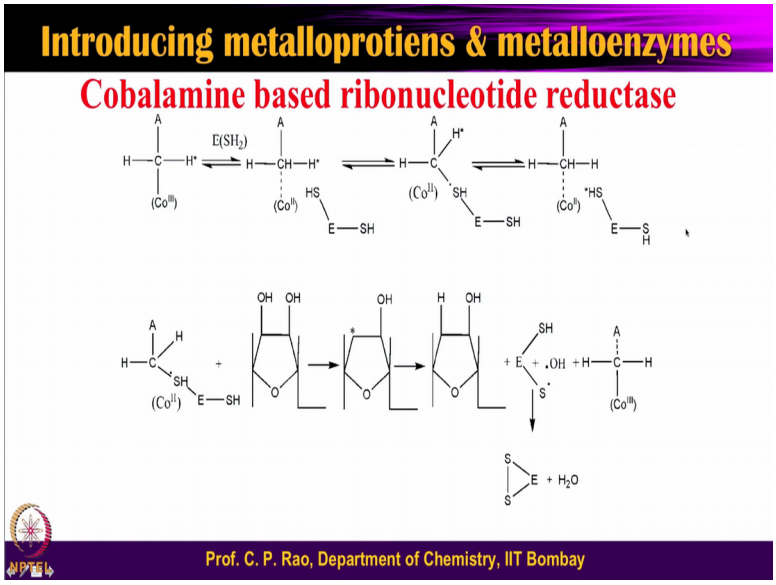

So, C 2 OH and C 2 H and this is the ribonucleotide reductase activity. And this is also done by adenosylcobalamin based enzyme and you see enzyme is very complicated you have so many lobes etcetera are involved in all of these. And then you can see that, yeah. So, how does this happen? Happen very similar to what I have mentioned in the previous case the where the dihedra the the diol dehydratease in some context.

Now, let us look at in this enzyme. Again you have a coenzyme vitamin B 12 coenzyme, in the enzyme you have something more very close where it reacts where it interacts with the cobalt cobalt this enzyme there is a di sulphide (Refer Time: 24:13) functions, dithiol functions are involved. The protein based dithiol functions are involved.

So, therefore, it is somewhat analogous to that what you what we talk in the case of the ion though in the case that ion the radical is transferred via the cysteine via the glycome via the ribosyl moiety to the diethiol function, but here it is very close proximity, ok.

When this particular substrate binds to the enzyme that is ribonucleoside or nucleotide binds to the enzyme this cobalt adenosyl part will get influenced get strained and then can give the breakdown of the cobalt and carbon bond to give radical. And this radical now, earlier in the earlier case it was directly transferred to the substrate which is diol, but here it is not transferred to the substrate it is transferred to the enzyme containing dithiol moiety.

So, therefore, you have the cobalt two and the carbon center this radical is transferred to the dithiol of the of the actual protein, and that in turn is transferred to the ribonucle part of the substrate. So, this is not from the adenosyl this is from the substrate. So, in the substrate from 3 prime position the sorry this is the 2 prime position and this the 2 prime position you lose the in presence of proton it will lose the water and then you have a hydride.

And that leads to the kind of a thing that you have enzyme with OH and the S and that results the returning of the cobalt carbon bond in this. And this leads to N oxidized form. And there are other enzymes which will again reduce this then the enzyme is available for catalysis, ok.

So, therefore, in this case there you have the cobalamin adenosylcobalamin coenzyme, and you have an enzyme portion at the interface, you have a dithiol function which is in cool close proximity when the substrate here is there and ribonucleotide. When when it binds then there is a change in the conformation that will make the cobalt carbon bond so what weaker, and they can break down to the cobalt carbon as a radical which is transferred to the dithiol function and to that of the substrate in the substrate in the ribonucle part. And then the ribonucle part accordingly loses its particular this particular the OH with the addition of the H as a water.

And then that that dithiol moiety undergoes oxidation and this oxidation will bring down back to this particular thing; And now if the enzyme has to function this disulphide kind of a formation or oxidized dithiol has to be reduced back to the, back to this then is ready for the next cycle of this one, ok.

So, in effect what we have learned? We have learned unlike iron cobalt is involved in only one the coenzyme that is called vitamin B 12 where the adenosyl is involved. Whereas, iron is involved in 100s of enzymes and variety of reactions and in case of the cobalamin all the reactions are of the hydride shift kind of thing. And in most of these cases is the cobalt and carbon bond is broken in the form of a cobalt 2 and the carbon dot.

For this what is required is the binding of the substrate and the adenosyl moiety itself provides static strain, and you know this vitamin B 12 coordinates or combines or coordinates combines with that of the main enzyme and the substrate binding these will bring a change in the steric factors at the center of the cobalt carbon bond of the adenosyl. And that will weaken the bond and that bond will rupture or break. And that is one which is resulting in the form of a radical.

And then the rest of the story is either the radical is transferred to the substrate or the radical is transferred to the thiol moiety in one case and then transfer to the substrate part of it.

So, we have 3 different classes of classes of reactions class A, class B, class C, but in all the cases it is the hydride shift which is important. So, the class A acyl groups and other one class B of nitrogen containing groups, but in the transformation no breakage or no loss of any moiety occurs. In class C the transformation of the 1 2 hydride shift will transform as a hydroxyl group or a amine group. And in case of amine group it will be ammonia which is eliminated in case of the OH it is the water that is eliminated because jum di hydroxy groups are not stable and they have use.

So, overall what we say is that the cobalt carbon bond is broken in the form of cobalt 2 carbon dot particularly in case of adenosylcobanamin, and then the reactions go through the radical kind of a mechanism. So, this is only one enzyme, so therefore, I will stop at this stage for the cobalt, and in the next class I will take up the nickel containing enzymes which I will continue for 4 5 lectures or so, ok.

Thank you very much.