Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry,
Indian Institute of Technology Kharagpur
Lecture 48
Cobalt ion dependent proteins and enzymes

Hello, students. So, very good morning to all of you. We have reached to lecture number 48 under module 10. Today we will be discussing about the different other types of proteins and enzymes after say we have discussed already started about cobalt. So, how the cobalt ion play some marvel or some very important role in these, all these proteins and enzymes?

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So, straightaway we will talk about something which is very interesting and first time discovered in biology, the organometallic part. That means when you have the cobalt carbon bond and that has opened up a huge area of understanding and huge area of study even the synthetic organic chemists also take the help of the catalytic activity of cobalt.

So, we first discovered this thing basically the vitamin B12 and we have the discovery of the cobalt carbon bond in biology then we will talk about the advantages of the different oxidation states of cobalt, which can lead us to the isomerases, deaminases and alkyl transferases.

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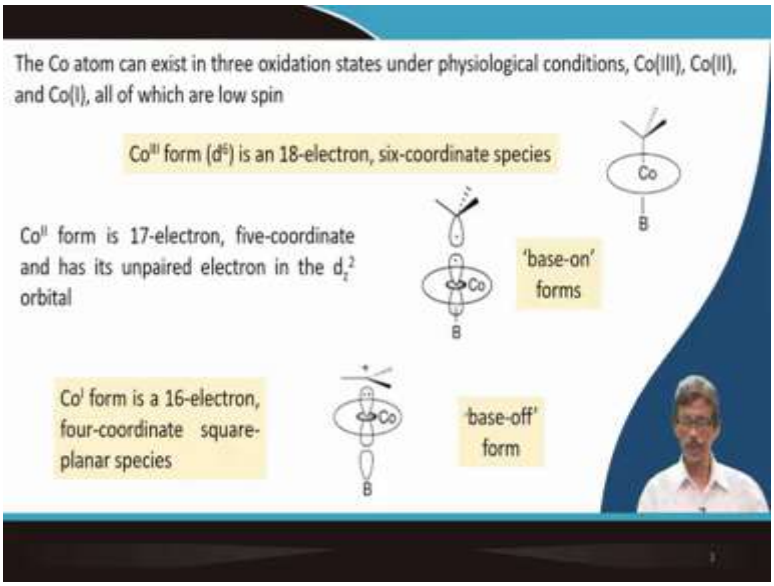

So, basically what you see that if you have a cobalt center, it can exist in three readily available oxidation state. Mostly we know that the bivalent and the trivalent oxidation states are readily available. If you have the simple cobalt II acetate salt in your hand or cobalt nitrate salt in your hand, you always try to understand that which particular oxidation state it has, whether you have a cobalt II center or you have a cobalt III center.

But occasionally we can also go down to cobalt I center and all of which are low spin. We all know that the hexamine cobalt III chloride from the Alfred Werner's time that is a diamagnetic system. So, is a 3 d6 diamagnetic system. So, definitely the corresponding ligand field is such that you will only get a low spin condition.

So, here also we will see there are three different oxidation states from monovalent to the trivalent state all of them are low spin in nature. So, that gives a little advantageous state. And we have the corresponding loop of the corresponding corrin ring, that means we know that you have you have the porphyrin or a corrin ring four in plane donor atoms will be available to the cobalt center and we will talk about the top one and the bottom one.

So, these two are basically the catalytic site and while doing so what is B and what is the other group that is the tripodal one attaching to that particular system. So, if we have a cobalt III form which already I told you that is a 3 d6 system and is the 18 electron system, so six electron of the metal ion and six pairs of electrons from the six donor groups in octahedral geometry.

So, 12 plus 6 is 18 electron system. Definitely it will have a six coordinate species. So, it is typically octahedral one. But if we reduce that particular system by one electron, it will give you a 17 electron system, but at the same time, it will try to lose its one coordination site, because it is not of much electron demand. So, we will have an unpaired electron because all are paired in 3 d6 system, but now once you are putting one extra electron, so that extra electron will go to that particular dz square orbital.

So, this is the situation. You have the cobalt II and cobalt II once it is there you do not have any formal bond between the cobalt and the carbon center considered that particular center which is at the top, say simple methyl or a substituted carbon center derived from a methane type of molecule. So, if it is a methyl one, so we will get a methyl dot center and a cobalt dot center. So, that means you are able to go for a homolytic cleavage of that particular system.

But interestingly, what we try to understand prior is that whether your, that particular fifth coordination site which is your B that the whether your base is attached to that particular cobalt center or not so these two cases that means the trivalent cobalt and the bivalent cobalt system you have that particular coordination of the base with you. So, it is the base on situation.

But if you go down further for one more electron reduction to a 16 electron species, but that will definitely be a four coordinate one. So, you have to remove the base coordination from that particular site and cobalt center in plus 1 oxidation state would be happy, because if you have a 3 d6 system cobalt I is the 3 d8 system which is similar to that of your nickel 2 plus. So, it will be very happy with that of your square planar geometry. So, we will have a base of form.

So, cobalt is sitting over there and you have the species which can be a planar species at the top and the base as the bottom also. These two are available. They are not going away. So, they are close to that, but you do not have the formal bond to that particular cobalt center, making that cobalt center a octahedral one.

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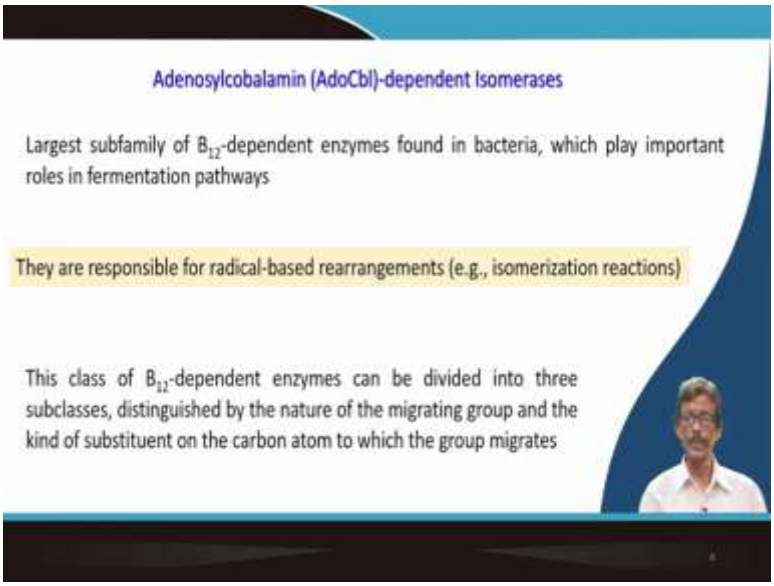

So, first of these cases we will talk about the adenosyl. Adenosyl function is attached to the cobalamin that is why it is abbreviated as AdoCbl. So, AdoCbl dependent isomerases. So, you will talk about the isomerization reactions. They are found in bacteria and many important role they play during the fermentation process.

So, if we go for the homolytic cleavage, so you will have the radical based rearrangement. So, once we get the radical, so you have the bond, you just detach it, you have the two lone pair of electrons or unpaired electron. So, they are sitting on this particular species and the other particular species having one lone pair of electrons.

So, if the lone pair stabilization and lone pair rearrangement can take place then because you do not have the former connection between these two electron pairs giving you the bond. So, that gives us all these informations, all these fantastic level of catalysis for the cobalt 12 dependent enzymes. So, we will have now formed these isomerases you can have three such subclasses and how we can distinguish there depending upon the group which is being produced and the group which is being migrated after these free radical formation on these particular centers.

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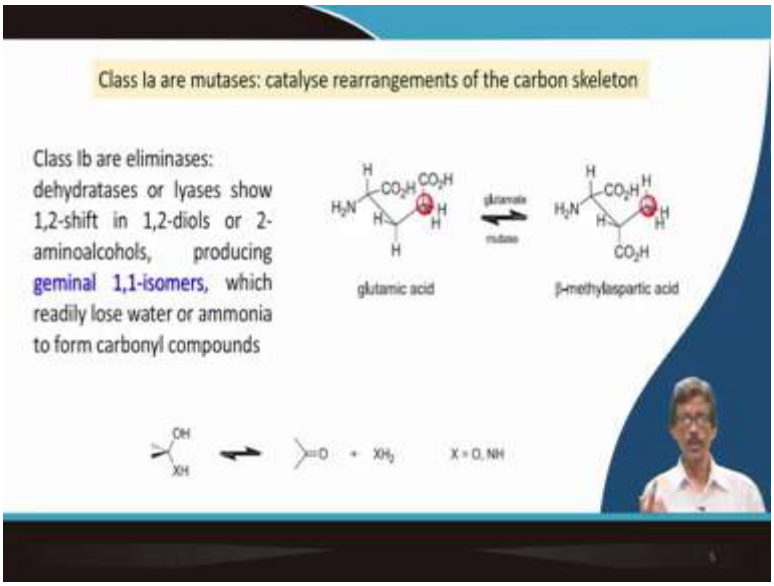

So, the mutases are labeled as the class I category and they are basically available for the rearrangement within the carbon skeleton. So, if you have the mutase, which is applicable on the glutamic acid, we call them as the glutamate mutase. So, the marked carbon center which is CHH and COH will try to rearrange with that of your adjustment center which are having a COH group. So, the COH group from the adjacent center will migrate to that red mark system carbon center such that you get a beta methylaspartic acid.

So, it will be substituted aspartic acid. So, the next category is your class Ib category, where we will talk again which is most important and fundamental for all these vitamin B12 coenzymes giving us the opportunity to study the 1, 2-shift, 1, 2 shift on 1, 2-diols and 1, 2-shift on two amino alcohol which is very well known to us amino ethanol type of thing.

So, basically, that gives us the 1, 1-isomer. So, is 1, 2 is involved then if you get 1, 1-isomer, then finally that 1, 1-isomer can also be unstable, and that stability will basically drive the reaction further to go for the elimination of the water molecule or elimination of the ammonia molecule.

So, if you have like this that means 1, 1-isomer can have both OH and XH. After the movement, after the 1, 2-shift the carbon center we are putting the burden on this particular carbon center having OH and XH on the adjacent sides on the same carbon center which is a very unstable arrangement. That is why the reaction will be driven to the carbonyl formation.

That means that it will be stabilized that the carbonyl function. And when your X is that oxygen you will be leaving behind with the water molecule, when X is NH you will be leaving with ammonia molecule. So, that is why the water as well as the ammonia can go out.

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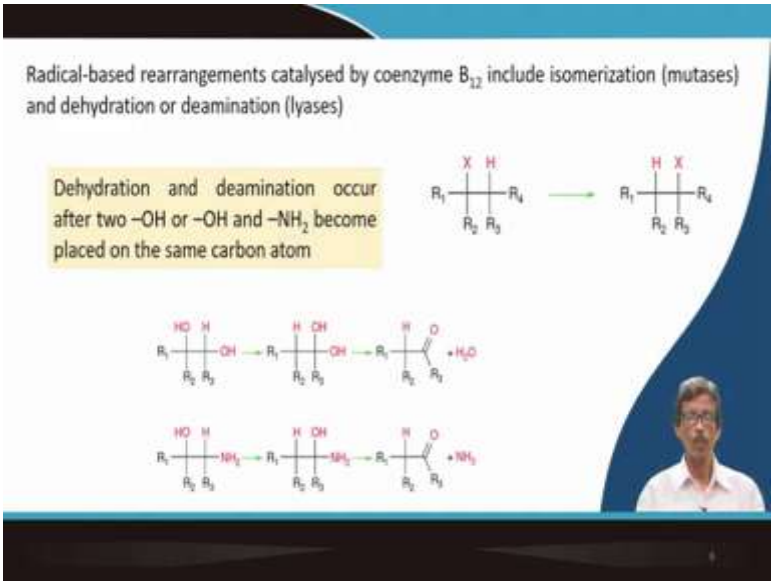

So, this particular case also the isomerization we see for the mutases and followed by dehydration and the deamination. When it is isomerization we call it as the mutases or the action of the mutases and the mutation reaction and the mutase reaction, not mutation, mutase reaction is the hydrolysis.

And when you go for the removal of the water molecule and the removal of the amine molecule, we go for the dehydration and the deamination case which are known as the corresponding lyases from your typical enzyme nomenclature also. So, the red marked X and H, the 1, 2-shift is nothing but is just go for the moment change in the XH to HX.

So, that will give rise to the corresponding processes what we have seen just now that to hydroxido group or OH or NH2 group available for the removal of the water molecule or the ammonia. So, that is why you have these water molecule and the ammonia in your hand and that ammonia is going out from the system in such a fashion that you have the movement and that movement is basically taking care of all this.

So, is a basically a genetically genetic reaction type which can be applicable to any system where you can have the similar thing, that means you have the substitutions R1, R2 and R3, but also you can have HO, H and OH functionality from the two carbon centers.

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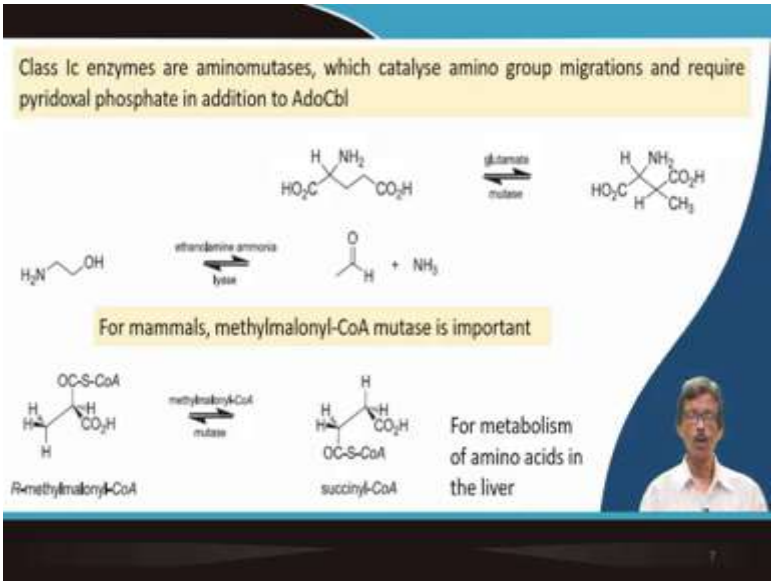

Then what about the class Ic. So, class Ic are, therefore, aminomutases. So, mutase we have seen. Now, you have the corresponding amino function or the amine function which basically go for the migration of the amine function and it also requires the availability of the pyridoxal phosphate in addition to the catalytic center which is your cobalt center, which is your cobalamin center, but is attached to your ado that means adenosyl moiety.

So, already we have seen. Again we are seeing that if you have the glutamate mutase amine function we are talking about that is why it is aminomutase. So, you bring that particular case that means your carboxy group is going away from that particular case and is the movement of that carboxy group is converting one particular system to a methyl function. So, that methyl function was not there.

So, be careful about the species basically try to know that which particular form that means the glutamic acid is going for the corresponding substituted aspartic acid and which is very much important for our system, for the bacterial system and also other biological world because this particular transformation is very much necessary in some particular step.

But if you think about the simple ethanolamine, so when ethanolamine is your substrate and we are talking about AdoCbl, so AdoCbl is working on ethanolamine. What would the product. So that can be a very good question for your exam also that you should be able to know in that way instead of ethanolamine ammonia lyase we write that AdoCbl on the arrow.

The double headed arrow you have over that if I write AdoCbl, you should be able to tell me that the product is your corresponding aldehyde function as the removal of the ammonia from the amine function of any organic moiety. Considered that you have the amine function on the organic backbone, but you are producing ammonia, so you are cleaving the carbon nitrogen bond during that particular radical formation and want to shift, because for us for the human being the methylmalonyl CoA mutase is important.

So, the corresponding all these important molecules, so the corresponding coenzyme A things are there and you can have the corresponding methyl function on the malonyl backbone. So, what are the change basically we are talking about compared to that of your glutamic acid. So, if your, instead of glutamic acid if you have the methylmalonyl function and is the coenzyme, so some part is attached, coenzyme A, some big molecule or big part is attached to it, so is the coenzyme A.

But we are talking about that part which is attached to that particular coenzyme A. So, that methylmalonyl coenzyme A then that particular part is available. And if adenosyl Cbl, AdoCbl is available, you can go for the corresponding CH bond cleavage and that CH bond cleavage go for the 1, 2-shift and that 1, 2-shift can give rise to something where your that corresponding coenzyme A can come from the right hand side to the left hand side and giving you a succinyl coenzyme A.

So, we have read all these things in our biochemical world and we know how these corresponding activities are taking place, but all are triggered, all are catalyzed by your corresponding cobalt based enzyme which is your adenosyl that attached to your cobalamin. And why we are talking these, because we are talking about some amino acids including your ethanolamine. Ethanolamine is not your amino acid.

We require all these steps, very important steps for our metabolism, because we have the amino acid. We can take out amino acid directly. We take amino acid from the food material, but

sometime we also form that particular amine acid from the protein chain or the polypeptide chain. So, this particular metabolism is can, is very much useful for our amino acids in the liver. So, liver is your particular target area or the target organ where these can go or function nicely.

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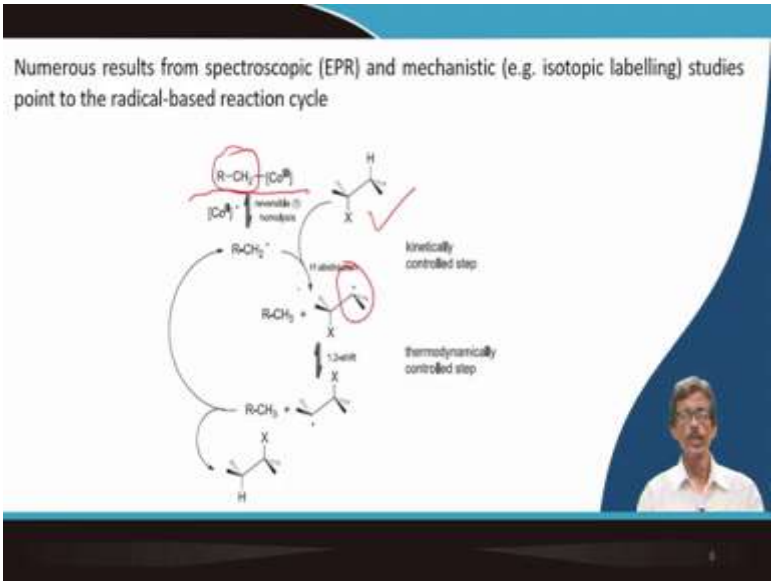

So, how we analyze these, how we go for, how we study these particular reactivity. So, EPR the electron paramagnetic resonance basically can be very much useful from the spectroscopic point of view, because you will be able to track the corresponding formation of the radical species. The way we know that for EPR spectral measurements, your corresponding standard sample is your DPPH which is itself a radical.

So, any radical it forms, so compared to that DPPH signal you can monitor the presence and the type and the nature of that particular signal which is occurring at some other values of that particular magnetic field or other g value. Then further for isotopic labeling studies can point to the radical based reaction cycle, because if you can have the corresponding isotopically labeled amino acid residues.

Instead of H you can have the deuterium labeling and that deuterium labeling can give us some other information, because the removal of that deuterium from the carbon center is also a rate determining step in some way we will talk about. Therefore, if you replace that H by D, you can know that you can see that corresponding rate of the reaction is also changing.

Why, because the abstraction, the formation of the radical species from R-CH2 cobalt III centers, so what you can have that R-CH2 cobalt III center you can have, what we can see is that this particular species is very important. So, R-CH2 cobalt III center and you can go for the corresponding species, the reduced cobalt II radical center if you generate what happens therefore, that immediately this part will go, this R-CH2 part is then go because it will take up that particular one.

So, from that so you will have the corresponding cleavage as well as the production of the cobalt II dot center. So, you have the homolytic cleavage of the cobalt carbon bond that is why we are talking about the corresponding reactivity in terms of your cleavage of the cobalt carbon bond. So, you are producing some species and that species will be responsible for the hydrogen extraction from your this particular substrate molecule.

This is your substrate molecule. So, if you are able to take out, so again we have bought that X and H from the adjacent carbon, but you are taking H not X, you are not removing X, you are removing H only by our R-CH2 dot. So, that is basically a kinetically controlled state where your hydrogen atom abstraction is taking place such that you generate a corresponding radical center at this point and your corresponding alkane is forming by getting H dot to that of your R-CH2 dot. Then this particular case you can have.

So, you have the kinetically control step followed by a thermodynamically controlled step. So, you have a very first kinetic step and which is very difficult to monitor sometime from the thermodynamics or the thermodynamic point of view. The second step can be monitored or can be considered or can be adjusted from the thermodynamic point that you can have some slower rate for that particular 1, 2-shift which can be order determining step also.

So, your dot which was originally there is this moving for the 1, 2-shift then R-CH3 again can supply that particular H what has been abstracted earlier, producing the 1, 2-shift or the corresponding transport molecule in your hand.

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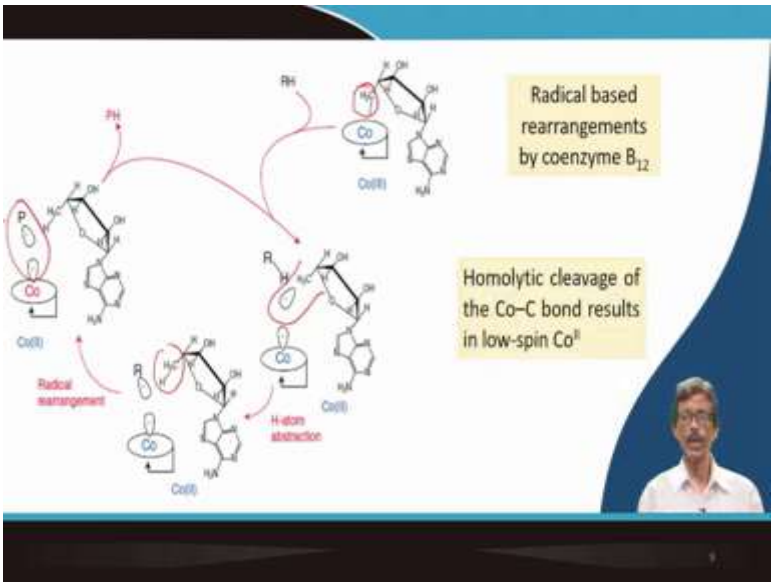

So, that is why this corresponding formation that means the radical formation and its arrangement is important for your coenzyme B12. What we see now that you can have these, you have the RH, cobalt center is there and cobalt center is attached to a very complex molecule basically you see adenosyl.

So, that means your adenine base, then the sugar moiety and the sugar moiety having this instead of that your CH2OH function you have the direct cobalt carbon bond from that particular unit. So, try to remember that in vitamin B12 molecules, vitamin B12 coenzyme which particular carbon center is giving you the cobalt carbon bond.

So, you try to understand it nicely and remember it nicely that this particular thing is there and whatever we have seen just now that you can have the corresponding breakage of the cobalt carbon bond. So, you see now that the dot is forming on this CH2 end of the sugar molecule and your cobalt center is there.

But if your (())(19:35) is not that linear one it can have the angular coordination also such that you can have the corresponding approach of the substrate molecule which is your RH. Because you, initially you have one particular cobalt carbon bond you break the bond, you produce two species, then you allow the third unit to come close by such that your RH can supply the corresponding proton to your CH2 dot function.

So, RH is supplying this that is why your thing is converting to CH2H that means your CH3 function. So, that is basically CH3 function, but is highly active CH3 function once you had the shift R, the reactant is converting after 1, 2-shift the radical rearrangement to product. So, you are forming the P dot, the product dot you are forming and that product dot after abstraction of the proton forming PH.

So, our ultimate goal is the formation of PH from your RH system. So, any kind of substrate can be useful to give that. So, homolytic cleavage of the Co-C bond results in low spin cobalt II center for this particular activity.

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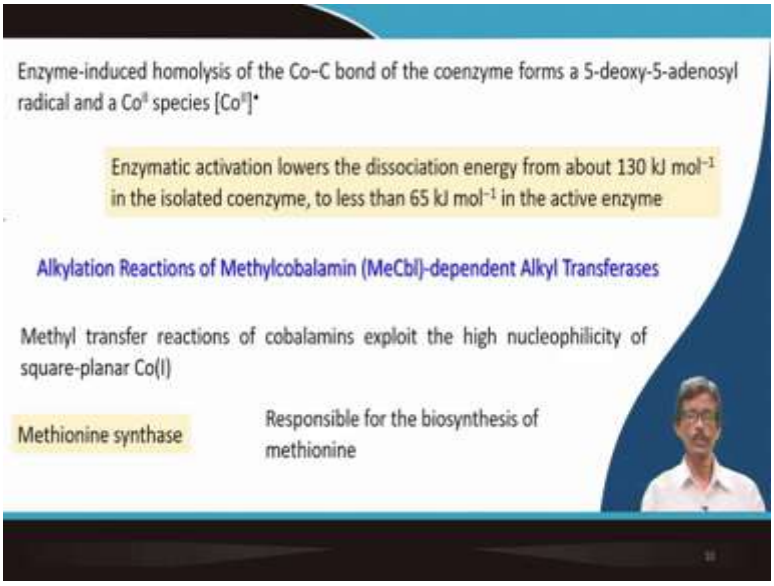

So, in all these cases your enzyme is working and the enzyme induced all these reactions are giving out for the cleavage of the cobalt carbon bond and your cobalt II radical species. In all these cases the enzymatic activation basically why we have the enzymatic, because we have to lower the corresponding activation barrier. For an uncatalyzed step your activation barrier is more. We know the hump, the corresponding hump in the energy profile.

So, you have to decrease that hump such that you decrease the corresponding energy of activation when you are talking about a enzyme, when you are talking about a catalyst. So, binding of that particular enzyme basically lowers the particular energy value from 130 kilojoules per mole to 65 kilojoules per mole in the active enzyme. Is not the inactive form of the

enzyme, but the active form of the enzyme when it is just prone to go for the cobalt carbon bond dissociation. So, you will be able to reduce the dissociation energy for these kinds of reactions.

So, the next we will see how we can transfer or how we can move or how we can shift the alkyl group now is very important now for many other molecules to form. So, different types of alkylation reaction, suppose, you had the simple SH molecule in your hand, 2-aminothiophenol in your hand how to go for the methylation of the SH function to give you S methyl function.

We use in the laboratory we all know we use sodium metal and the dry alcohol go for the corresponding S minus disease and then we put methyl chloride that means, sorry, methyl iodine MeI and MeI is breaking there as Me plus and I minus and go for the corresponding attachment of the methyl function on your S minus.

But whether that particular thing can be followed for different other types of methylation reactions or alkyl transferase reactions that we can see. Basically, it is the methyl transfer reaction for the highly nucleophilic center now is your square planar cobalt I, which is required for methionine synthase.

Synthase we know that a biological synthetic chemist to ask that is why you go for the synthesis of the methionine molecule and how is go for the corresponding methionine synthesis so is the biosynthesis. So, biochemically the catalyst is important and the site of synthesis is important, because you need the RH molecule. RH molecule will be converted to the PH molecule which is your product form. But now you have the corresponding PMe3 system attachment of the methyl function.

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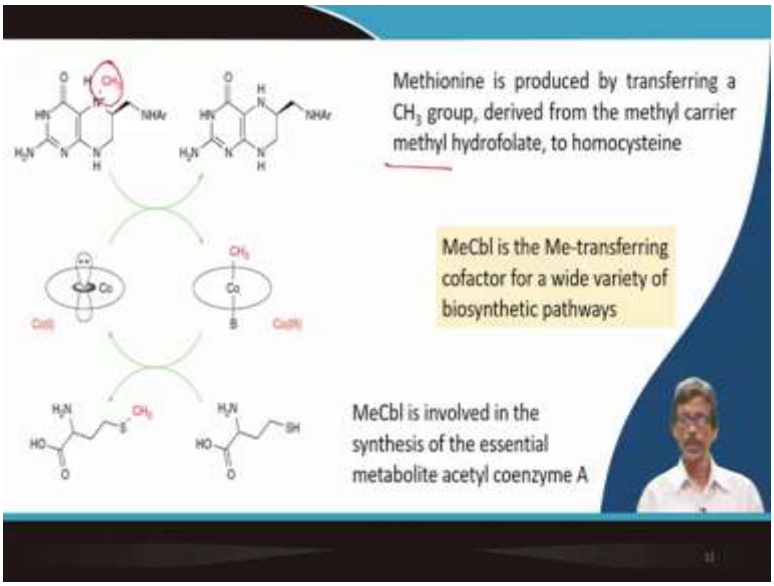

So, how to transfer the methyl group? So, you must have a methyl carrier. So, methylhydrofolate you can have to homocysteine. Homocysteine is the cysteine going for the corresponding methylation or the corresponding previous step or the pro-methionine step. That means you have the methionine in your hand.

So, if you go for these that means you are the corresponding one for you have produced that particular one that means you get it from the corresponding methylhydrofolate. Why it is called methyl, because in the hydrofolate you have attached that red marked extra methyl function taken from the corresponding cobalamin. So, this is your cobalamin center is there. So, cobalamin center is supplying that methyl.

So, you are using methylcobalamin. So, you are using methylcobalamin. So, that methylcobalamin is transferring that group to the hydrofolate. Hydrofolate is that is methylated and that methylation is responsible for the corresponding transfer to that particular cobalt center.

So, if you have that particular hydrofolate function available which can supply the corresponding methyl function to the cobalt center such that you get a corresponding methylcobalamin MeB12 we write, Me hyphen B12. So, methylcobalamin is a very useful reaction for your purpose than for transferring methyl group to your homocysteine to form the methionine.

So, therefore, your methylcobalamin how you can make it, whether you will be able to make methylcobalamin through that methyl iodine pathway that you can also see synthetically in the laboratory whether we will also be able to make it. So, biochemically the biosynthetic route is different.

You required the corresponding intervention of the hydrofolate system for that particular thing that means it take up that methyl function from the methylhydrofolate system and transfer to the cobalt center. So, many other biosynthetic pathways we can use. So, MeCbl like your AdoCbl your MeCbl or sometime we write MeB12. So, synthesis of many essential metabolites like acetyl coenzyme A, where we are writing as CoA coenzyme A, they are very important molecules and we go for the corresponding methylation reactions.

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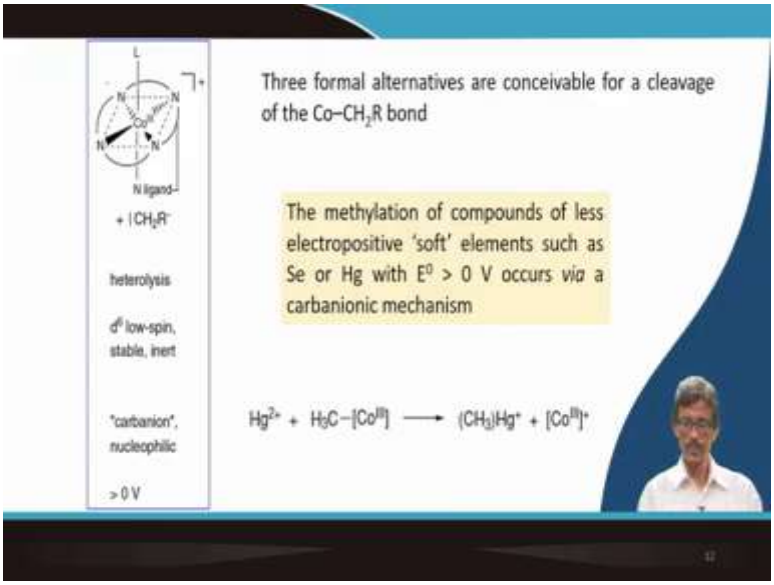

So, we can have the different formal alternatives are configurable for a cleavage of the cobalt CH2R bond. The cobalt CH2R bond what we can have like that of your glucose moiety. Glucose moiety having that CH2OH function and that CH2 is forming the bond to the cobalt center. So, that basically gives us something where we can have.

So, you can have three alternatives. We started our class by talking that also. You can have the cobalt III, the different oxidation states. You can have the cobalt III, you can have the cobalt II and also you can have the cobalt I.

So, in case of one case what is happening now you see that you can have the trivalent cobalt and basically you have the corresponding one that means the corresponding CH2R minus so you can go for the heterolytic cleavage. Heterolytic cleavage is the alkyl function is going as the corresponding carbon ion and that carbon ion is the nucleophilic in character, but the metal ion center is d6 low spin stable and inert we all know.

So, basically, this particular case if we go for this particular reaction, whether you go for the methylcobalamin and that methyl group can be transferred to the mercury also. We know that the methylmercury is a very deadly system, methylmercury chloride also the for the Minamata disaster, we all know, so methylation or we call is the biomethylation, because in seawater it happened. They are basically for the methylation of the mercury chloride.

So, whether this can also be activated by this particular thing? So, you can have the soft elements like selenium, mercury, where your E0 value is greater than 0 volt occurs via a carbanionic mechanism. So, when you can have that thing, you can go for the corresponding carbanionic species that means your CH3 can be is minus form.

So, CH3 minus which is your carbanion and that carbanion can go and correspondingly attached to your metal ion center. So, metal ion methylation is a different thing compared to your sulfur methylation to give you the corresponding s methyl function.

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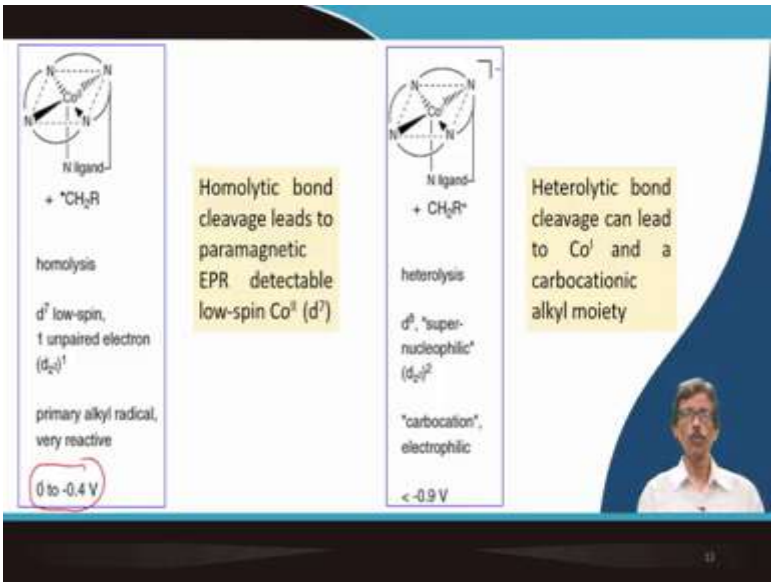

Then if you can have the corresponding homolytic cleavage, homolytic cleavage gives you the cobalt II center. Already we discussed for AdoCbl and the methyl Cbl also. So, is the d7 system. So, you see now what is changing. The changed thing is your corresponding window, electrochemical window is greater than 0, now it is 0 to minus, is ranging towards the minus level, minus 0.4 volts.

So, that thing is important, how we can model at it, how, can electron transfer can take space. So, there will be a thermodynamic control depending upon your E0 value. So, you have the primary alkyl radical which is very reactive then for your reaction. Then finally, you can have another type of heterlytic leverage and lead to a cobalt II center and carbocationic alkyl moiety.

So, you have the carbocationic alkyl moiety what we are discussing about your methyl iodide giving you CH3 plus and this carbocation you now you go farther down to minus 0.9 volts, then only you can have this sort of reactivity.

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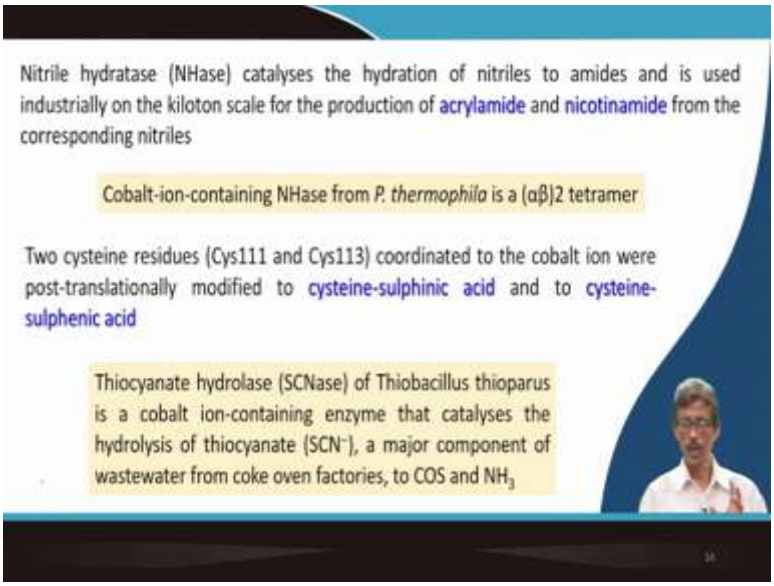

So, lastly we see that how another varieties of molecules like nitrile hydratase can also be cobalt ion catalyzed, which is nothing but the name itself telling you they are looking for hydration reaction and hydratase is nothing but the hydration of the nitrile to amides. C triple bond N giving you C double bond ONH2 that means the entire water molecule attaching to C triple bond N is a very important reaction which is known as the hydratase.

So, water is giving (())(29:13) and hydration is taking place but in a different form giving you amides. And industrial it is also important for the large production of the polymer for the polyacrylamide and the nicotinamide which is the biochemically also important. So, for the production of acrylamide and nicotinamide you go for the corresponding nitrile derivative and immediately go for the hydratase reaction which was originally isolated from P. thermophila.

P. thermophila gave us a alpha beta whole 2 tetramer type of thing which is nothing but your hemoglobin type of thing, where you have the cysteine residues coordinating to your cobalt center but which can be modified post translationally to cysteine-sulphinic acid and sulphenic acid. So, try to remember how you put oxygen atom to the thiol unit or S minus to convert it to sulphinic acid function or sulphenic acid function for these reactions.

Then finally related to that of your C triple bond N we can have also the thiocyanate which is SC double bond N minus, thiocyanate anion also which is Thiobacillus thioparus, like T. thioparus, like your P. Thermophila so T. thioparus, again a cobalt containing enzyme which is basically converting the thiocyanate anion which is a very important anion we know from the inorganic chemistry point of view.

In wastewater it is there, in coke oven factories is basically polluting the environment, the aquatic life or the marine life, but if you are able to convert it to COS and ammonia your environment will be a little bit safer.

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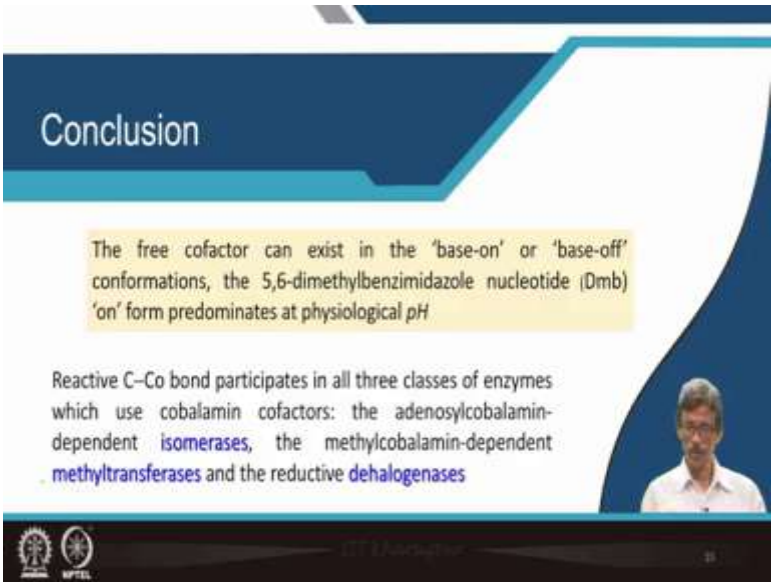

So, altogether what we have seen, we are talking about the cofactor, cobalt based cofactor and this free cofactor can exist in the base on and base off form, where your dimethylbenzimidazole nucleotide is on the base. This is your base B. From the bottom it is connecting. So, you commit from the bottom. So, either it is attached to the cobalt center or it is detached on.

But the on form is physiologically the predominant form, because the physiological pH is basically support that corresponding five coordinated cobalt center where your coordination number is 5 and you have the corresponding radical generated. So, once you generate the radical, you have the cobalt carbon bond which can participate in all three classes of enzymes, not the sub-class.

These are the enzymes which we have already seen for the isomerases, methyl transferases and the reductive dehalogenases. We are not talking here the dehalogenases. But is the newly found area where people can think of the corresponding dehalogenation reaction which is also very much important environmentally.

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So, the references you start with vitamin B12 page from the Wikipedia and also the book of Crichton. So, thank you very much for your kind attention.