Biological Inorganic Chemistry
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Lecture 44
Alcohol Dehydrogenase and Beta-lactamase

Hello students. Good morning, everybody. So, we will just come to the lecture number 44 where today we will discuss basically initially the alcohol dehydrogenase and betalactamase and as your module name is telling you that you have zinc ions and the zinc ion bearing enzymes that means the metal enzymes.

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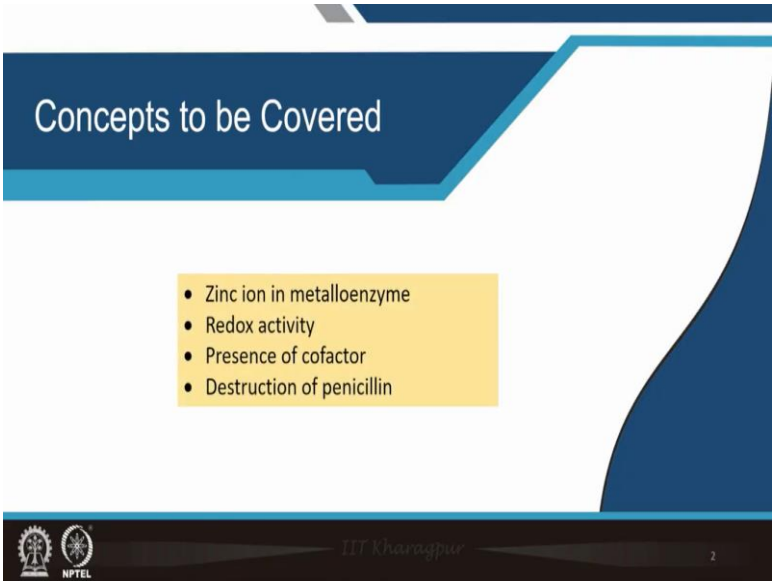

So, what we can see there how important it is in these metal enzymes because there are many, many numbers of these metalloenzymes. The first identification is always a difficult task. But once you identify, you should know the reaction what is going on and how the reaction is taking place.

But another thing now, today we will discuss about we will take the help of zinc ion for its enzymatic activity in the metalloenzyme active site, but it can show that redox activity, because we will be talking about the alcohol dehydrogenase which is a redox reaction. How to find out how to think of that particular redox activity out of the zinc? But zinc ion is required.

Not only one zinc center, but more than one zinc center is required. So, we will bring some redox cofactor. That is why the presence of redox cofactors are important. And finally, we will see how the betalactamase molecules are important for the destruction of penicillin and the penicillin related other molecules.

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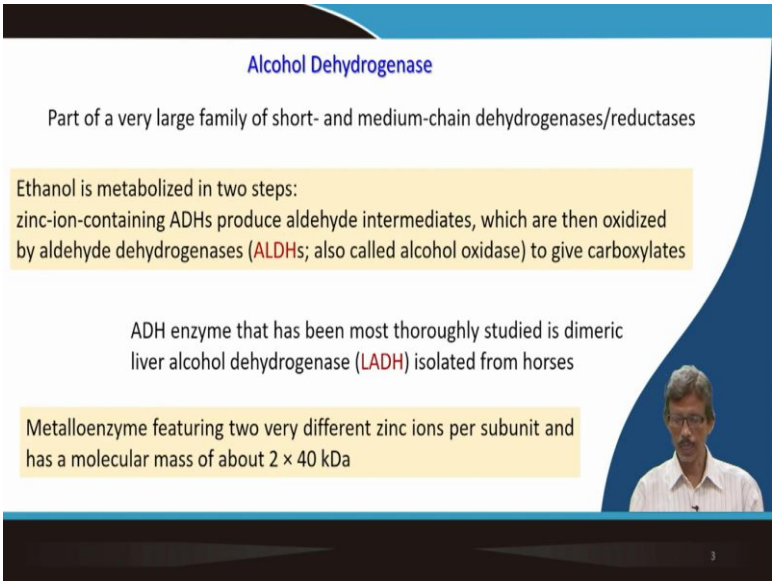

So, first we just see what is that your alcohol dehydrogenase. Your textbook definition always we try to understand. You have the substrate is your alcohol and the reaction is your dehydrogenase reaction. So, by looking at the name, basically, you should always try to understand that what reaction what chemical reaction will take place because, you know, in the biological world, we always have some one specialized area or the specialized subject people study is the molecular biology.

So, the understanding biology at the molecular level and in all these cases in the molecular biology world, we know that the reactions are happening there in C 2 or out of the system that is basically governed by many, many number of chemical reactions. And those chemical reactions are most of the cases are catalyzed by different enzymes.

So, here when we talk in terms of you are the groups of dehydrogenases, always try to remember again, it is the enzyme commission number, the EC number and the 6 or 7 categories of the important enzymes always try to keep in mind, make a full list of that and keep in the site where you study in your study table, adjacent to your study table on your wall always put it.

So, we can have two different types of these hydrogenases or reductases. One is short chain type and another is medium chain type. So, they are also, very important in all these different areas. But briefly, we will just see quickly in this particular class that if we have the alcohol then if we just move to alcohol not as the methanol but you go for the ethanol, ethanol metabolized condition, because we all know the fermentation, the yeast basically, the

brewer's yeast basically, which can format your glucose to alcohol and there is the other pathways also.

So, when that fungus basically can do or produce that particular ethanol from glucose molecule, and if we take externally these ethanol molecules, So, the metabolism of ethanol is always very much important. And whether you require a metal ion or not, that means whether you require or take the help of metalloenzyme or the metal ion free enzyme, or the metal enzyme or the enzyme which is devoid of the metallion.

So, one such category is ADH, is alcohol dehydrogenase, and a group or the class we call ADHS, dehydrogenases. So, when they are going or acting on the alcohol, they basically try to oxidize it, alcohol to aldehyde. And finally, that aldehyde will be oxidized to the corresponding acid.

All we know from our school days, again and again I am telling that whatever we have learned that is the basic and the fundamental knowledge or informations what we can have, So, ethanol to aldehyde or alcohol to aldehyde to acid, these all we know. But how the biological world in the biosynthetic world, how these are happening?

So, you see along with that, ADH will have ALDH, which we terming as your alcohol oxidase, or aldehyde dehydrogenase to give the carboxylates. That means the ionic form of the acids. But when we study these in detail, we will take the source of these alcohol dehydrogenase as the liver source. So, liver source of animals, they were source of some our body and all the time we have the liver.

So, we all know the connectivity between liver and alcohol. We know that people can suffer from the cirrhosis of liver, who are basically drunk, take huge amount of alcohol, but they do not go for the proper assimilation on metabolism of alcohol.

So, you now see that ALDH, which is (red) colored in red at the top, and another is LADH. So, do not confuse these two. Sometimes your question can be set in such a fashion that what is the full form of ALDH and what is the full form of LADH. So, do not confuse why we are going for these sorts of abbreviations because there is no need to write all these things because your focus or attention will be concentrated on the spelling on the big name and all these things.

That is why the bacteria, the fungus, all we abbreviate. Ecoli E stop Coli abbreviated form. So, the liver, which is not the human liver, but the horse liver is also, fine and has been

studied for a long time and is not a monomeric one, is a dimeric one. Dimeric in terms of the protein structure. You have one protein structure here and another protein structure here.

So, you will have a dimeric protein structure, not the dimeric metal ion center. So, do not confuse once again for these understanding. That is not a dimemetallic active site, but is a dimeric protein structure having two parts.

So, have two different zinc ions there So, one protein part will be taking care of one zinc center, is the mononuclear zinc center or mononuclear zinc protein or enzymes that per subunit you have one zinc ion and a very small molecular weight of 40 kilo dalton each. So, they are basically of that particular category of short chain dehydrogenase or reductase molecules.

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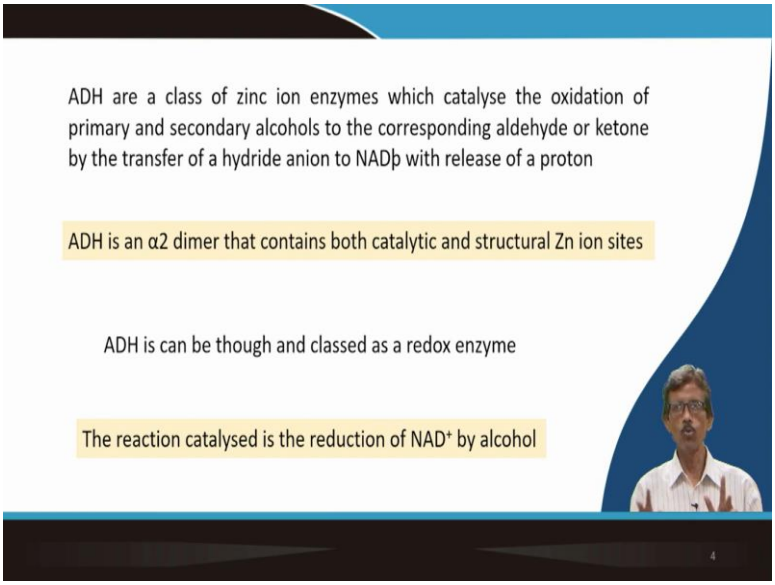

So, they are the enzymes which can catalyze the oxidation of primary as well as secondary alcohols to the corresponding aldehydes and ketones, as we all know what is the oxidizing product of primary alcohol and what is the oxidation product of your secondary alcohol. But along with that, we need to transfer the hydride ion to NAD is positively charged one, So, it should be superscript positive. So, NAD plus with release of a proton. So, we are talking about hydride and then we are talking about proton So, what does actually mean?

Hydride is nothing but one proton plus two electron, is not also, a hydrogen atom transfer, it is hydride transfer. So, that's why in the indirect way, we are bringing the electron from this NAD plus and NADH between these two spieces and we will take some redox activity.

So, this will play some important role apart from your zinc ion, which is not providing any electron or which is not accepting any electron. So, is alpha two diamond type of thing and contains both catalytic as well as structural zinc ion sites that we all know earlier. In case of superoxide dismutase, we have seen that zinc is, one of the zinc center is playing the function or role of a structural part. And another one is your copper ion which is your catalytic site, but here both of them are zinc sites in the polypeptide chain of O2 isolated parts.

But one of the zinc site is basically playing the structural role dissolve that is the most essential part of this particular metalloenzyme, but that particular zinc can play only the structural modification or the conformation of the protein structure during your catalytic activity.

So, it can be thought, thought is thought, the thought and classed, that means the classification you can do as a electron transfer enzyme that means the redox enzyme, but is not derived from the metallion because you do not have iron or you do not have the copper metallion which are redox active. But zinc is present for substrate location, for the substrate abstraction, and subject attachment, and electron transfer is taking place from that your redox cofactor.

There are a large number of redox cofactors are there. Sometime we can find a particular metal enzyme can depend on three, four cofactors of different types NAD, FAD, and all these. So, these cofactors are that is why very important and they are basically the helping the enzyme activity. So, is basically what the reaction is being catalyzed is the reduction of NAD plus by alcohol. So, what is happening in a sense?

We are going for the oxidation of the alcohol, alcohol dehydrogenase is doing that particular job, but at the same time the redox active cofactor, NAD plus, will be reduced by the alcohol. So, these two things will be going parallely and we will find all these things and what is the basic reaction what can take place.

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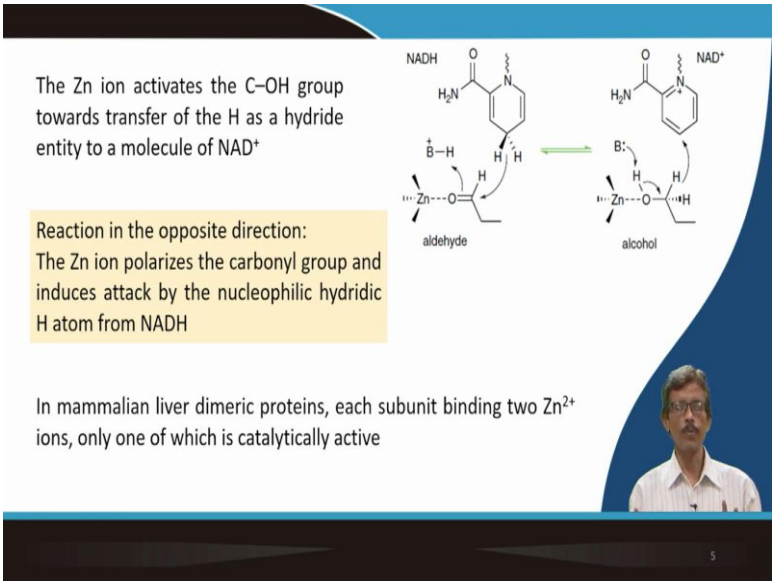

So, you should not only know the active site structure of the zinc center or zinc bearing metalloenzyme, but you should also, know the structure of the cofactor that means NADH or NAD plus. So, if you straight away go to the right-hand side, we will find that one part of the nicotinamide adenine dinucleotide, it is a dinucleotide. You should know about the structure and the essence, the function of that.

So, it is the nicotinamide part. So, is a pyridine ring attached to CO NH 2 function is the nicotinic acid amide. So, it is nicotinamide adenine dinucleotide. So, only that particular part, the functional part, that is why upper part is shown as the curly line. Its not a straight line, its a curly line.

That is, it is the other part of the NAD plus. So, what is happening there? The pyridine ring is there, is charge is on the pyridine nitrogen, is the quaternary nitrogen and is positively charged. As we all know, when pyridine ring is attaching one proton, you will have the pyridinium ion. If you add hydrochloric acid to a pyridine solution which is a liquid one, we get pyridinium hydrochloride which is a white solid like any other salt, like sodium chloride or any other. So, it is a very useful organic salt pyridinium chloride.

Similarly, here, we can find something as NAD plus which is n plus, the positively charged pyridium ion and you have the support from your amide function at the ortho position. Then (your) the ring is getting activated for the hydride transfer from your substrate alcohol. But while doing So, your zinc is required, zinc is coordinating from the alcohol, alcohol is functioning as a typical water molecule. The lone pair on the alcohol oxygen is being donated to the zinc center.

That is why you have the coordinate bond or coordination control on that particular alcohol OH. While doing So, another external base like some other site chain of the amino acid, maybe one other histidine residue with the nitrogen available that can be protonated. So, that will come but on the coordination position, you are modulating the corresponding pKa value of this alcohol.

This bound alcohol OH pKa we can modulate such that your bass is effective, the B lone pair. B lone pair is nothing but your base, your lewis base. So, the B with lone pair, the Lewis base, can now abstract that particular proton. So, proton is getting abstracted, you are leading towards alkoxide formation and that alkoxide formation can go for your hydride transfer and that hydride transfer is basically leading towards the formation of your aldehyde.

But the reaction is irreversible in nature. It can go from aldehyde to alcohol also, that is a reduction step. Oxidation is the formation of the aldehyde but reduction is the corresponding formation of your alcohol. But in that particular case, your cofactor is the reduced form of NAD plus, which is your NADH.

So, that is why in language what we can write, what we have seen now that the zinc ion can activate the COH group of the alcohol and transfer the hydrogen as, not as H plus, or not as H dot is the hydride, that means H minus and DD to NAD plus. And we also, discussed the opposite thing is also, happening that induces basically the attack by the nucleophilic hydridic hydrogen atom from the NADH because that hydride ion which is already attached to your NAD plus is NADH.

So, that hydride can attack. So, nucleophilic attack from that NADH is basically taking place again through the activation of the carbonyl function of the aldehyde molecule by the zinc center. So, zinc is playing, say, dual role. It can not only activate the corresponding carbonyl function, but it can also, activate the alcohol function from the alcohol.

So, the human liver protein, what is given to us, ultimately it is giving the corresponding enzyme, the metalloenzyme. You have two subunits and one already I told you that one is the structural part and another is the catalytic part and the catalytic zinc is showing all these actions or the activities. So, what that cofactor is already I told you.

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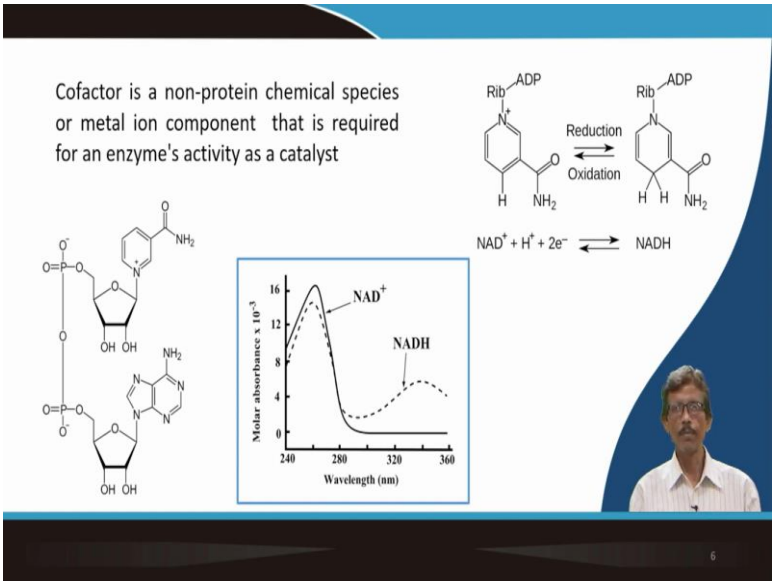

So, quickly now see the language and what is the structure of all these. So, the cofactor is nothing but a non protein chemical species. It is not of the protein origin, but it can help the species which is of protein origin that means your metalloenzyme or sometimes simple metallion for increasing the corresponding activity of any catalyst.

So, if you have NAD plus, you should nicely know the structure where you have nicotinamide function or nicotinic amide function, nicotinic acid amide function is there, adenine where is adenine, and dinucleotide. So, you will have two sugar units and two phosphate units. So, that is your NAD plus.

And when you have the ribose sugar, rib is given. So, you have the ribose sugar attached to the remaining part of that. So, the ribose sugar what is attached to your nicotineamide function is shown here for the rib, and then you have the ADP molecule. So, adenosine diphosphate. So, that part is nothing but your adenosine diphosphate.

Also, you can have this transaction between these two forms, this NAD plus which is basically accepts hydride and that hydride is nothing but H plus plus two electron, producing your NADA. So, it is basic and fundamental reaction. Everybody learns this thing, a biochemist learned that thing, a clinical biochemist also, have to know that thing, and also if we want to know a little bit about our liver functioning of the liver, and particularly when you take alcohol and all these things, how these things are operating because people will have less amount of this enzyme in their body. So, how to detect that?

So, a clinical biochemist only will take the help of the visible spectra, and we will also, can take the help of the visible spectra because these two species are different in terms of their electronic transition properties. So, you see the band in one case your energy band at the position of say around to 268 50 is a little bit diminished, but characteristically if you see the band at around 330 nanometer, it is only NADH which can show that band having some molar extinction value or the molar absorbance values, but your NAD plus will not.

So, if you are able to produce NADH in some reaction, we will be able to monitor in the reaction medium by monitoring simple visible spectrum. That is why if you have some metalloenzyme and if you want to monitor the binding of the substrate, you can go for the corresponding enzyme kinetics. So, enzyme kinetics is very important thing such that you can monitor the corresponding mechanism and all these things apart from your structural identification in terms of excess structure determination.

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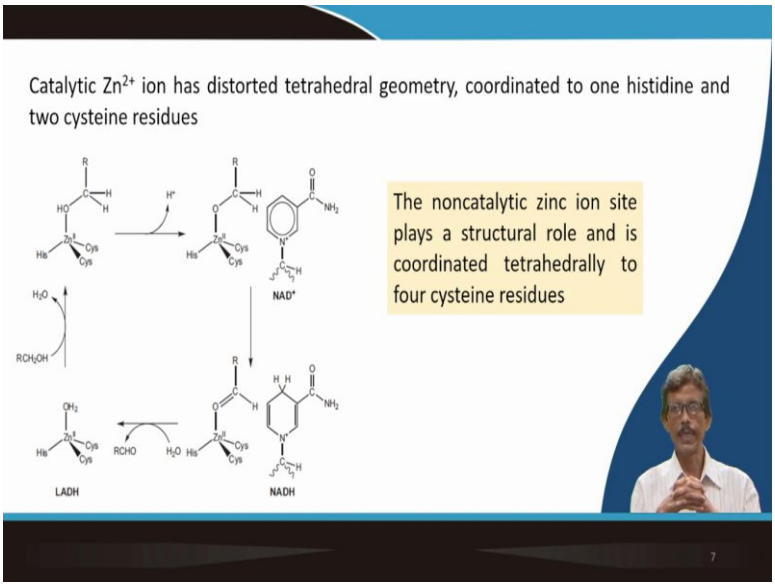

So, the catalytic site already we know that you have the facial thing, the facial triad of these three donor groups and you bring the zinc over it and you get that thing. So, now if you bring the NADH and all these things around that tetrahedral geometry part, this tetrahedral geometry earlier I discussed it, we have a table. Go back to that particular table and find out what are the groups when you have the attachment to the zinc center such that you can get a carbonic anhydrase, you can play the carboxypeptidase, or you can get a alcohol dehydrogenase.

Here, now you have not three histadine residues but you have now cysteine residues. So, two cysteine, one histadine residues, again in a tripod. So, on that particular tripod, So, tripod, if it

reverse direction, its zinc is sitting on the top. And when it is binding basically, the LADH in the lower bottom part is the your LADH, water is bound. So, water is the catalytic site.

Alcohol will come, bind that alcohol and then that transformation already I showed you. But you see that two things are coming and binding to that particular active site, your substrate is coming and your cofactor is also, coming. So, we have to identify, we have to establish which one is coming fast and which is giving all these reactions such that you can have an arrangement which is not at all a binary arrangement, but a ternary arrangement.

Your E is there, your capital S is there, the substrate is there, and to activate your that E or E plus A, you have to bring the corresponding coenzyme NAD plus. So, NAD plus will show the usual action what we have seen just now and then alcohol will be formed bound again on the zinc site because this thing is reversible. So, we are showing only one arrow because it is alcohol dehydrogenase, but if we are looking for some the reverse reaction, we have to go for the reverse reaction.

So, the reaction wise, these are all same. If it is a reversible reaction what we are talking about that alcohol to aldehyde and aldehyde to alcohol is a reversible reaction but you require two different enzymes. One enzyme for one conversion and one enzyme for the other conversion. So, the reaction is so, selective. So, we are focusing our attention there only that you must know the corresponding reaction and how these reactions are happening and why those reactions are so selective that is why the enzymatic activities are so, selective in nature for a particular type of reaction.

So, this is your catalytic site, but you have the non-catalytic site playing the structural role which is also, coordinated giving you a coordination number of four, but there you have all our sulfur environment. So, that is like your celulo plasmine or something like that. The zinc is binding towards the sulfur and the sulfur cysteine residues from the protein chain is basically giving a tight structure for Jaden S four type of coordination around your four cysteine residues around that particular zinc site.

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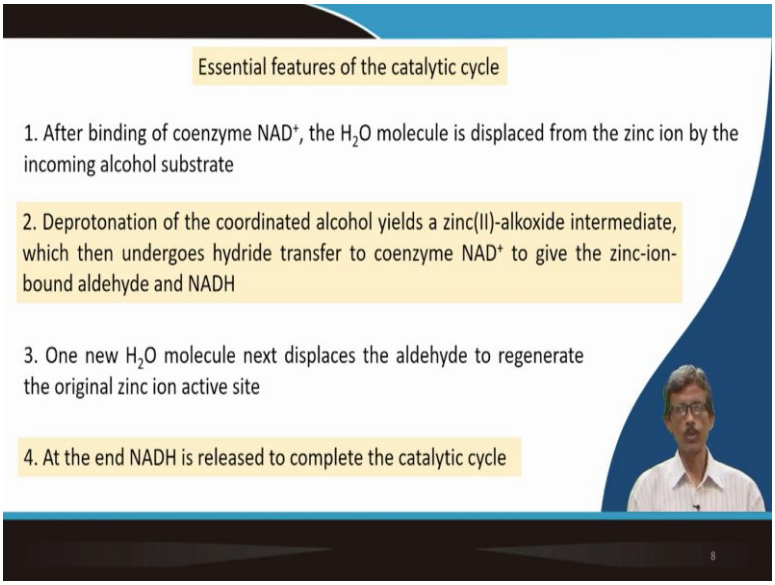

So, what are the steps and what are the features quickly we will see we have already discussed just now for your record, for your writing you have to write it and assimilate in your own language, but I am giving you all this material is also, important not only your PowerPoint slides are giving all these things, how you can explain about the structure and all this but also, the material what you can have. You only just go through these, you read all these, you will be able to understand what is happening there within the entire catalytic cycle.

So, the binding, the removal of water molecule and the incoming alcohol occupying the position then the deprotonation of the alcohol base is coming into the picture. Zinc is giving you the alkoxide intermediate and once it is alkoxide intermediate is forming you can have the hydride transfer from the alcohol to the coenzyme NAD plus. So, that you can have the corresponding bound aldehyde and NADH. Everything is in close space.

So, new water molecule will come now and will kick out the product that is why you have the cycle and you have more and more alcohol will be entered into the catalytic loop and you will more and more aldehyde will be formed from there from that particular active site. What is happening then at the end?

So, when we have one cycle, So, that is why we know that the catalytic turnover, all these numbers are how much you can produce in a particular time or per second, the rate of the molecules the rate of the reaction is important, is 10 to the power some big number. So, at the end basically, we consider that the cycle is closed, and now you complete the catalytic cycle such that you can again come back to that particular molecule where the zinc center is bound to that fourth coordination site, the water molecule.

So, at the end, the NADH is released. So, the release of the NADH is important. So, its not going far away because it is again come. So, the movement of that NADH is also, important not only the coordination and the coordination of the zinc site, but the movement of the NADH within that particular pocket is alSo, important to complete your catalytic cycle.

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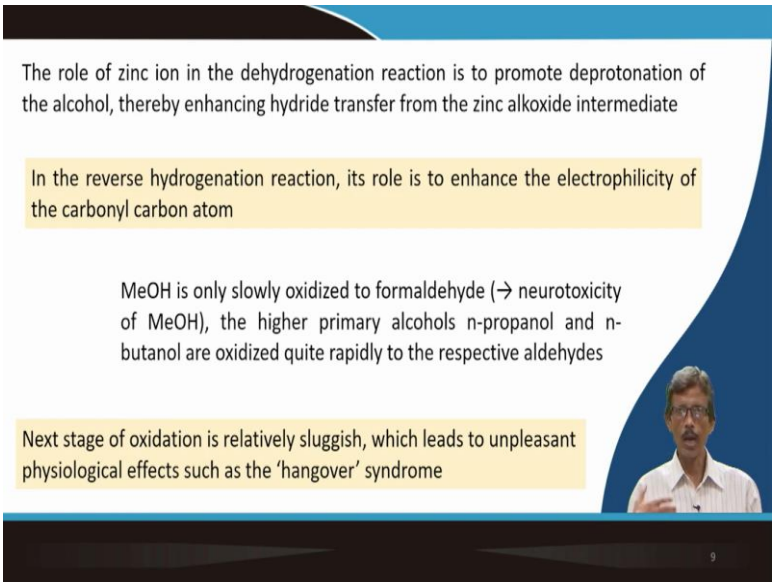

So, is that the zinc is playing a very useful role for hydrogenation reaction basically by promoting the deprotonation and just directing us for your hydride transfer and zinc oxide intermediate formation. So, if you are asked to explain all these things, what is the zinc is doing or the zinc ion is doing. You should be able to tell us all these things, but you should always remember, these zinc ion dependent hydrogenation reaction is not only dependent on the presence of the zinc ion, but you require the corresponding coenzyme.

That nicely explains the importance, the function, and the role of the coenzyme when we are talking about some enzyme, which is in our case, these are metalloenzymes. And also, we discussed the condition for the reverse hydrogenation, the role, and to enhance the electrofelicity of the carbonyl carbon atom such that you can go for the corresponding attack on the carbonyl carbon.

So, why this thing is not happening on methanol because is a corresponding one if we can have the formaldehyde will be reproduced, which is neurotoxic, and the neurotoxicity of the methanol that is why we know. But for the higher primary alcohols n propanol, and n butanol are oxidized quite rapidly to their aldehyde congeners. So, the rate of the reaction that is why I am telling you that the rate of the reaction, the enzyme kinetics, or you can go for the

Michaelis Menten plot and the Lineweaver-Burk plot also, and all these you can extract out the key values, the turnover number, the turn number and all these things are important.

So, you can have if you have huge amount of these oxidized form of the aldehyde in your body, you will be definitely in trouble, people who are consuming alcohol regularly. That is why you will see the physiological effect we define these as the corresponding hangover syndrome, if you have huge amount of aldehyde in your body and via through your liver.

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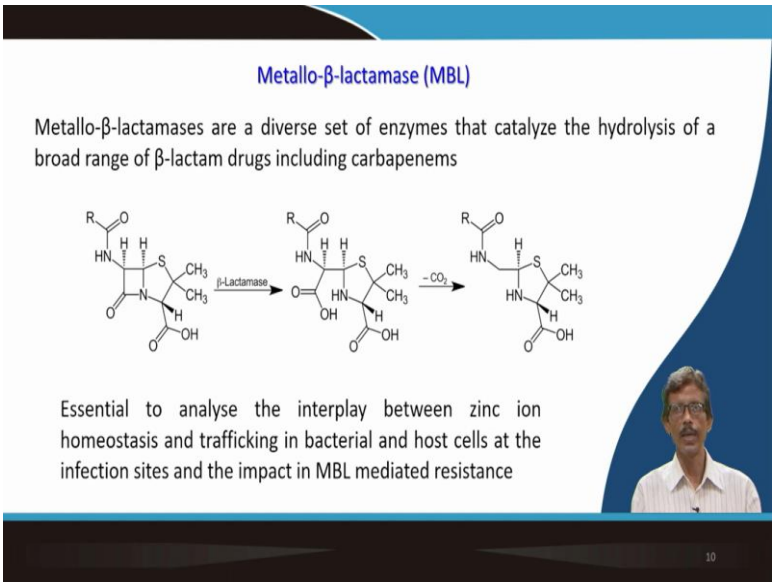

So, last to this class is that your another important molecule is not a some sort of single zinc type but some are double zinc types MBL molecules. So, MBL molecules or metallo betalactamase. So, once you know that what is beta lactam ring and what are the betalactamase, they can hydrolysis of the beta lactam ring and the requirement of the metal ion.

So, basically the broad spectrum beta lactam antibiotics we can talk about, and including you have the I mean the penicillin we have, the ampicillin we have. So, the simple reaction is very easy to understand because in our body if you have betalactamase and if you are consuming at the time, the penicillin, doctor has prescribed penicillin to your body or betalactamase will come and break the ring.

Once the link is broken, its antibiotic efficacy will be lost. So, you have to put something which is your secondary part. Nowadays all these drugs are coming in that way is basically a hybrid form of this and another acid is given, clavalunic acid is given to inactivate the

betalactamase, such that your ring is not broken. And the second step is also, followed from the first step is the decarboxylation reaction of that particular molecule.

So, we have to analyze the between the reactions of the zinc homeostasis and the trafficking in bacterial and host cells. The bacteria has attacked me and I am the host cell, I have the host cells. So, where I have some infection, So, how the zinc ion availability is trafficking and movement and the availability to the MBL mediated resistance is also, important.

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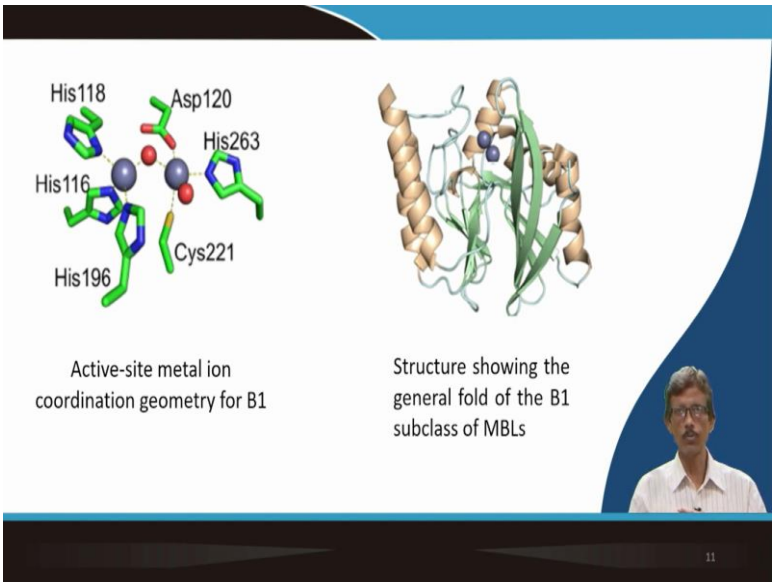

So, if the zinc is not getting by all these betalactamase, you are happy and you are safe. So, all always we try to look at it, you try to love it, seeing all these things, the cartoon drawing trying to master yourself in the drawing of all these things where you can have two zinc sites. So, we are talking about only one type, B 1 type of this particular metallobetalactamases. We can have B 1 type, we can have B 2 type and we can have also, B 3 type.

So, it is basically showing the folding of the protein chain and where your zinc ions are sitting over there. But what is your real active site structure? So, real active site structure where you have the metal ion coordination first and you know then you put in entire thing within the protein envelope, we will get the whole structure and whole geometry in your hand.

So, this is the structure. It is not a mononuclear zinc metalloenzyme but that alSo, gives us the corresponding example that you can have a binuclear zinc site which can be effective one. So, I have taken that example that is your B 1, MBL. So, that is a binuclear type and one water now, a single water is bleaced between these two zinc sites and your coordination from

the three sides, the cystedine, histidine, the cystenite ion, the histinite ion. And now you have the aspartate and in one case you have three histidine residues.

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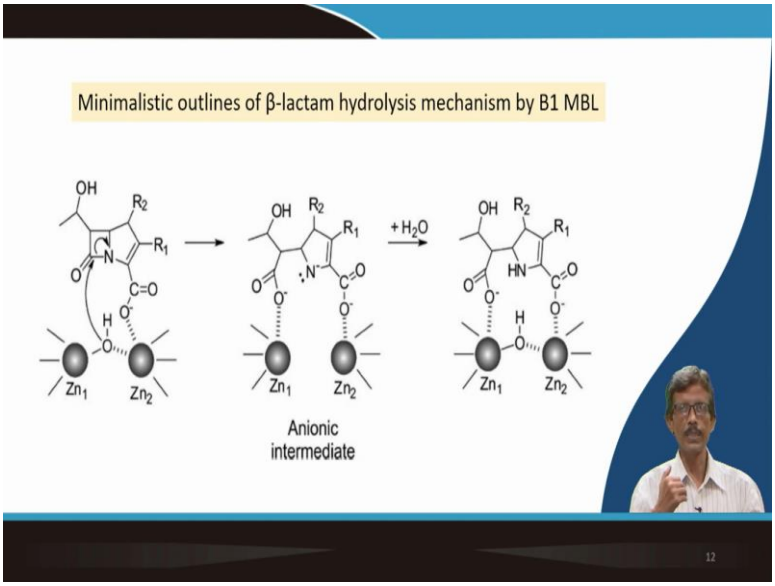

So, the coordination environment is completely different from your alcohol hydrogenase of the mononucleotide and that is basically now effective for all these the reactions. We only look at the minimalistic outline of the enzymatic action. What is the minimum thing what you can understand, what you can think of that what we can have is your intermediate, So, your whole substrate will come and you now require two metal ions, not one metal ion since its a big substrate.

So, if you have a big substrate which is coming on the top of the two metal ions, So, these are the two metal ions sites and will come and try to be fixed or we always say the enzymatic activity is nothing but your lock and key. So, you have the corresponding lock. So, lock you can consider as your site, active site, and your key is coming and the key is now tried to open the lock such that you can have the reaction, you can have the cleavage, and you can have the breakdown of your penicillin molecule.

So, we can have the anionic intermediate once the ring is broken, the betalactam four membered amide ring is nothing but the CON amide ring, four membered that amide ring is the beta lactam ring we call it. So, that is open up. Your N is forming and that N will have a charge. So, you have the anionic intermediate and that is getting protonated and during that particular process, what we see that two metal activated hydroxide ion is your corresponding nucleophilic agent for your hydrolysis.

So, we have seen the nucleophilic attack on carbon dioxide molecule. We have seen the nucleophilic attack of hydroxide ion activated by bound zinc to that of your corresponding carboxypeptidase activity. Now, a very simple reaction what we see now that once we do these because the intermediate is very simple one but it is bound by two carboxy ends on these two zinc sites.

So, the binding of the carboxylate groups also, can have that particular affinity on the fourth ordination site around these zincs. But ultimately when you have this particular one that you have the protonation and it goes back and you once it is protonation formed, the water molecule, your thing will come again back to that particular bridging, and we will have this particular formation of the open form.

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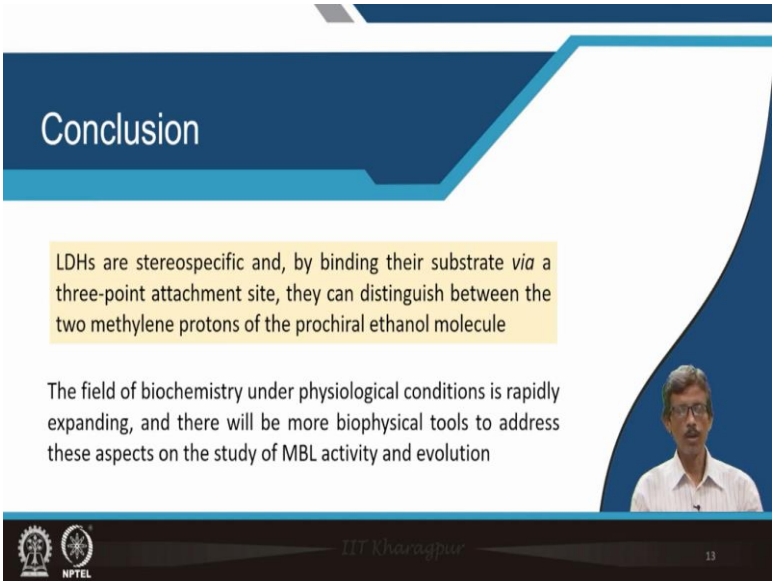

So, in conclusion what we have seen that your alcohol dehydrogenase are not only functioning a good way but from the organic chemistry point of view, the stereochemistry point of view, is stereospecific in nature because the binding of the substrate you have the three point attachment. So, you can have the three point. So, the recognition is also, important, like that of your facial coordination to your zinc site and distinguish between two methlyn proton and the prochiral ethanol molecules.

So, the chirality you can bring the prochiral molecules can sit on these enzymatic site. And for MBL basically is the field of biochemistry and we study the biochemistry that we will call as the clinical chemistry then physiological conditions, physiological pH environment and all these and we can use different biophysical tools like a simple spectrophotometer to many

other things to address these aspects while we are studying the activity and evolution of these MBLs.

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So, the references, you start with alcohol dehydrogenase. You can go for the betalactamase alo, and the book also. Okay, thank you very much for your attention.