Biological Inorganic Chemistry
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Lecture 49
Nickel proteins and enzymes

Hello, everybody. So, please welcome to this class lecture number 49 where we will be talking about the nickel ion bearing proteins and enzymes. This is under again module 10.

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So, today we will be talking about something where we see that everything we are talking in that way also that how the metal ions are so important to give you some very useful enzymatic activity. And historically this is also very important to know which is your jack bean urease, JBU, from the plant C. ensiformis, which is Canavalia ensiformis, the scientific name of the plant, so is basically nothing but your food the beans, we take the cater the jack bean urease.

Then what is about the molecular structure starting from the metal ion. Then why this particular thing can be correlated with something which we call as the carbamylation reaction, which is a very important reaction. But when you have the protein structure and when you have the license subunit of that particular protein structure, how the carbamylation reaction is important. And in other two categories we will be talking about the hydrogenase and the dismutase activity bearing the same metal ion.

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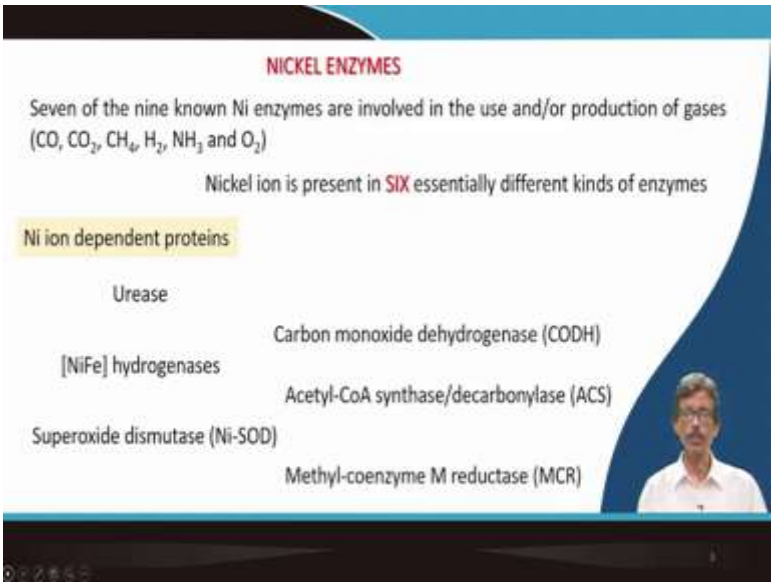

So, what are the different types of nickel enzymes we can think about, because up to this point of time, we do not know much about, we do not teach also the nickel ion bearing, is not the nickel only, is the nickel ion bearing enzymes. Because is the subject is growing a lot. But that is why these are the most recent advances because it is not only recent, but is also very old. The way we can consider it also it is almost about 100 years back people first identified urease.

So, if we have nine no nickel containing enzymes or nickel ion bearing enzymes, but interestingly we will see that we will be talking about something where either they can use or produce some the gaseous molecules only. So, the small molecule activation as well as the assimilation is important. So, the nickel can be a very good catalyst also for our industrially important catalyst also or some biomodified catalyst or some zeolite embedded catalyst that is also important so that we all know that raney nickel from your school days.

So, nickel is a very interesting metal iron and metal itself also. So, all, from all these basically, because seven of them are involving these gases starting from your carbon monoxide to dioxygen. And we will be talking because these classes basically has been divided into two parts. Today we will be talking about out of these six essential enzymes, we will be talking about three. And in next class we will be covering the remaining three.

So, what are the enzymes or the proteins you can have, because there is protein dependent or the nickel ion dependent thing is important. So, these are synergistic in nature. Protein gives certain

structure, certain envelope around the nickel such that you can have the required amount of distortion. And these, that distortion, that amount of distortion plays some significant role when we bring the nickel ions bound to those amino acid residues available from those proteins.

So, first thing what we will discuss today is your ureas. The name itself will tell you that we will be talking about the hydrolysis, the ase activity, the hydrolysis activity of urea molecule. And while we study these, try to remember immediately what is your area molecule, how we can cut it, how we can cleave it or how we can hydrolyze it.

Next we will talk about the nickel iron hydrogenases. This is the most latest one basically if we consider that we can have, the most one we have studied is the copper, zinc, superoxide dismutase type of thing. So, this is also heteronuclear type of thing.

So, hydrogenases iron, iron hydrogenase we can have, but when you have the nickel what are the advantages and what are the species which can use the nickel and how this heteronuclear entity is important, because there will be many such examples where the nickel and iron are living together within this particular protein environment together with that of your sulfur environment so we call it as iron nickel or nickel iron sulfur enzymes.

Then lastly we will talk about the superoxide dismutase, because the basic chemistry related to the superoxide dismutase already we have discussed. Just now I am telling about your copper zinc thing. So, you will require copper as well as zinc. Then the another category we have the corresponding the iron only, SOD, then manganese only SOD.

So, this is the latest discovery during the last 15 years or so. We will identified it and structurally characterized also. In our next class, the final class, we will be talking about the other gas molecules, that means the carbon monoxide dehydrogenase. And to understand nicely we will quickly abbreviate it as CODH.

Then acetyl coenzyme A synthase or decarbonylase reaction, so ACS, acetyl coenzyme A synthase reactions all are on nickel. Then methyl coenzyme M reductase is a very interesting system where we will find that another variety of the macrocyclic ring can come and bind to your nickel ion center.

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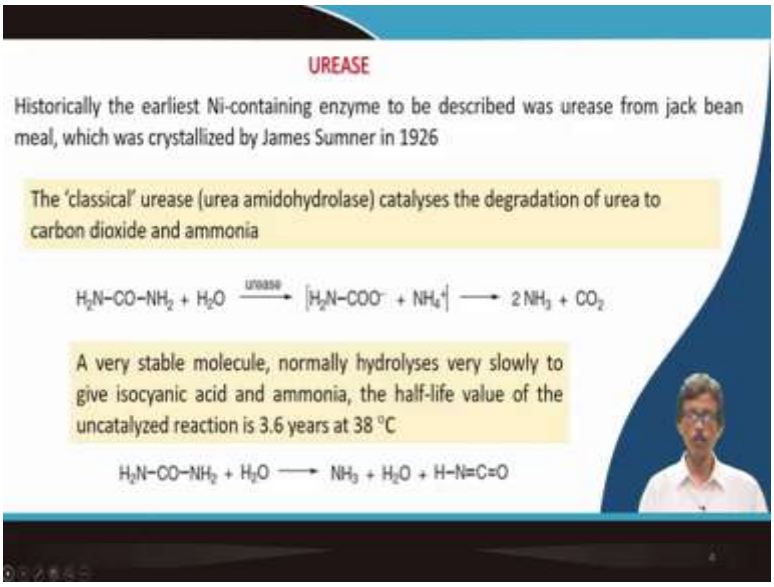

So, what is that urease then? So, we will just talk about the urease. So, how we can utilize this one? So, historically, if you see, you go back to 1926 when the person named as James Sumner is isolated that thing as the nickel containing enzyme, but people were in doubt at that particular time, whether you have the metal ion or not.

They thought that okay the enzyme basically what they have isolated is the, is useful for that particular activity, because at that particular point nothing was known to us. So, it is basically we get from the jack bean meal or jack bean food material what we can have or jack bean urease that is why it is abbreviated as JBU. So, they crystallized it, but the crystallization is not the end thing.

So, they utilize it for the crystallization and once you crystallize it, because for the purification purpose, we know that certain organic compound is in your hand and if you want to purify it, the crystallization is one particular technique such that one form or one polymorph basically can be crystallized nicely such that you can have a single melting point and a very pure compound through crystallization we can get it.

So, crystallization process is basically a purification process. When this person utilize this particular technique, he has that particular idea that we can get the pure form of this particular one, but we do not know about the corresponding structure. It took around another 50 years or so when the x-ray crystallography was established, people can identify the structures of the small

molecules. So, during 1975 basically the people got the structure of the urease thing and then only it has nicely been established.

Other indirect evidences, other spectroscopic evidences there during these 50 years of time, the accumulation of the knowledge which is important, how people can see to on these for so many years to know the identity of that particular molecule or the protein and whether you have any metal ion or not or the metal ion is responsible for this reaction, but the function was known.

But once you are not able to understand the actual form, the molecular structure of the system, it is not possible to identify the exact role of the nickel whether you have a single metal ion center or a double metal ion center or a triple metal ion center. But the function for the degradation is known giving you CO2 and NH3.

So, what is that particular reaction? So, chemical reaction we should know first. They write it. That is NH2-CO-NH2 is not a linear molecule, is angular molecule, always try to understand, but some time to save the space you write in a line. So, is that when you talk about the molecule, you talk in terms of the angular form, because the carbon is a trigonal one C double bond O NH2-NH2. So, when water is attacking that thing, so how that water, water is typically, therefore, a nucleophile. We all know that the water nucleophilicity is important for any hydrolysis reaction.

So, once water is attacking to that particular system is not that you are getting back something immediately, but you are getting ammonium ion and ammonium carbamate type of thing, because the ammonia is there, ammonia is NH3 and if you remove one of the H and put the carboxylate function you will get the carbamate species.

So, the ammonium carbamate is forming through the addition of your water molecules. Then the cleavage is taking place, removing two ammonia molecule and one carbon dioxide. Because we all know that the fixation of dinitrogen is a tedious task. We do not able to do it until and unless the Haber–Bosch process we have from the dinitrogen of the air with hydrogen to produce ammonia.

Then the production of ammonia is also related how we can produce the corresponding urea molecule. So, the reverse thing we are talking about. So, when you have an enzyme which we are hydrolyzing it, but the urea molecule is a very stable mode molecule and its rate of hydrolysis

giving isocyanic acid and ammonia. So, if you get that from the urea this is not that particular carbamate thing is isocyanic acid, in that particular case the process is very slow.

You see the half-life. The 50 percent of the material to be decayed, to be hydrolyzed will be taking 3.6 years at 38 degrees centigrade which is our body temperature, because everything will be trying to correlate from bacteria to the human health, health and diseases and the mammalian system.

So, what we basically get in that particular case that in the first step you get something where you are able to take out one ammonia molecule and the second step you have some other species which is your corresponding hydrocyanic acid and that hydrocyanic acid formation can lead to the corresponding formation of ammonia molecule once again in the second step.

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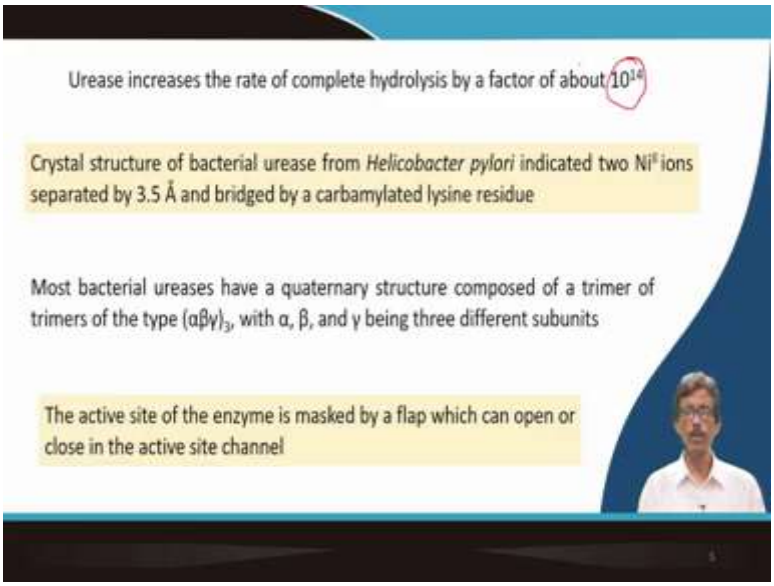

So, why we need the enzyme, we get the enzyme. So, urease basically increases the corresponding rate of that particular hydrolysis reaction by a factor of about say 10 to the power 14. You remember this number, which is very important. Why we need the catalytic path. We know if you keep it for a certain time and we know that it can be hydrolyzed definitely if it is remaining in the soil. As fertilizer we are putting it in the soil.

The bacterias are there. Now that providing urease, but in natural calamities basically can be able to destroy it. But you see we have seen that the characteristic half-life is 3.6. But when we are

doing something that is why we need to have something very fast. So, the rate of the reaction, the kinetics is always important. So, for that kinetic thing to control, we have to bring the enzyme.

So, when you bring this particular enzyme it basically giving a huge amount of acceleration for your rate of the reaction. So, when people got that particular crystal structure during 1975, so is almost another 50 years back also, so people are not stopping on this particular information. They are going further. So, they are not talking to correlation of these urease molecule to the zinc, how the system is getting nickel, how the zinc is responsible for the formation of all these things. So, these are very basic bioinorganic chemistry thing what I am talking to you today.

So, think about the history. History of bioinorganic chemistry basically has started in that particular fashion. So, it is related to the bacteria. It related to some other things, other small organisms also. So, when people identified for H. pylori, H. pulori is the Helicobacter pylori, where you can have, when they find it out, the two thing they identified in the structure that not only one nickel center you have second nickel center, so is a binuclear motif.

And that binuclear motif is basically bridged by a bridging group or the bridging unit or bridging entity, which is nothing but you can have in the protein chain the lysine amino acid residues and that is why from our very beginning we are talking about the carbamylation reaction. So, if you go for some carbamylated form, so you go for the carbamylated form of that lysine and that can be utilized to hold the two metal ions bound by this bridge.

You can have another bridge also from the bottom. So, these bacterial ureases can have a typical quaternary structure like hemoglobin. We all know what is your corresponding quaternary structure, but instead of a typical tetrameric form alpha 2, beta 2 we have for the hemoglobin, here is a trimer of trimer so you can have a trimeric thing alpha beta gamma thing and then is of three time. So, with alpha, beta, gamma being of three different subunits, so you can have three different subunits and they are multiplied.

So, how it looks like because the folding of the protein chain all things are important not only the metal ion, its oxidation states and the coordinates and geometry and the distortion. So, active site of this enzyme is masked, basically covered. We know about mask nowadays. So, masked a flap. So, if you have a flap like your mask for your COVID-19 so you have a flap which can open and close like the putting your mask on your face.

So, it is basically closing and opening of the active side channel. So, depending upon this flap opening and flap coverage, you can have the corresponding entry of the substrate and to the access of that particular active site.

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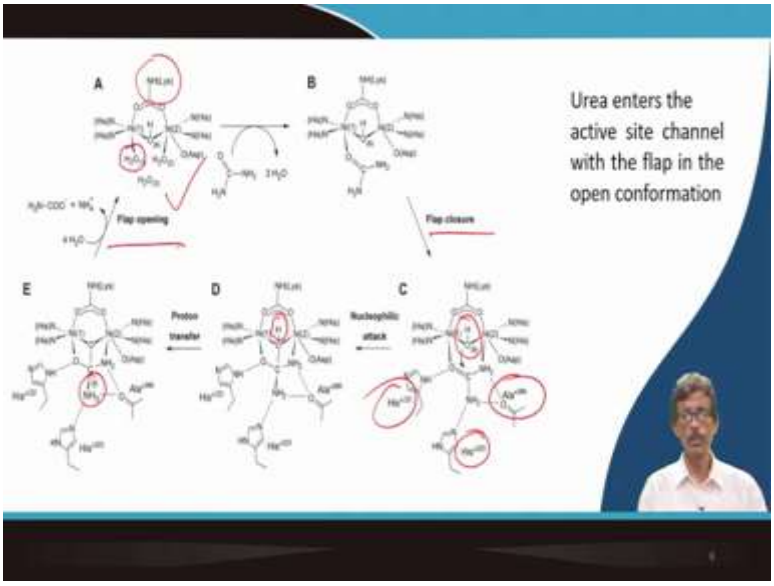

So, when urease basically is entering the active site, channel, so there are some decent channels, so through some channel you have the particular channel site and through that channel site the urea molecules are entering. And with the flap in the open conformation, so when the flap is open, you see the corresponding structure, but it is very fast one. So, within some millisecond time it is happening.

That is why you have a very huge rate of reaction and basically is a very dynamic process. So, do not worry about the different form A, B, C, D, E. So, top left, top from the A. So, you just start from the A. What you can understand from this particular structure A. That is a binuclear system you have. And that binuclear system you will be having two metal ion centers one is nickel 1 and another is nickel 2. Then what will you do.

You just try to have that what are the coordinates sites you can have. So, always we considered that in the biological world these three things, my three fingers are important that you have one coordination site from here, the second, and the third. So, you put three from here and if possible three from the other side. You get a binuclear motive. Is very simple for your di-iron motives also we are discussing from the very beginning also.

Then once you bring, so these two are pushing these two metal ions together and you have the bridges. So, either the hydroxido bridge, Water Bridge or the carbamylated lysine bridge from the top. So, this is your carbamylated lysine unit. So, you have the lysine. We know that the free amino end of the lysine amino acid and which is carbamylated.

So, that carbamylated form is basically you can have the corresponding COH function or you can had sometimes CO-NH2 function also, amide. So, either the carboxy acid function COO minus or CO-NH2 function can sometimes be. So, you have, so urea is entering. What urea will do? Urea will occupy by replacing the water molecule from the active site these water molecules will be replaced and urea will take that particular position through oxygen, because water having the oxygen coordination.

This oxygen will have from the urea also, we have nitrogen as well as oxygen. But when urea is functioning as a ligand, it is the carbonyl oxygen which can have lone pair of electrons on this particular oxygen and that can donate to the metal ion center. So, the nickel 1 site having a vacancy in terms of water. In the right side also we can also have a vacancy in terms of your water molecule.

So, you can have these water molecules. So, when it coming basically you are removing three water molecules. So, interestingly you see when urea is entering why you required the flap opening. When you realize entering, you are removing more number of water molecules. So, is basically going for the removal of more number of water molecules. So, you can have the corresponding entropic contribution for these also that how many number of water molecules you are able to remove when urea is coordinating to your nickel 1 center.

Then the flap is open. Urea has entered. Then the flap will close. So, you have the flap closer step. So, when the flap closer step, so many things are happening, other histidine residues are coming to support the binding of your urea molecule. It is not that area will remain alone. So, all other things, when the flap is closing, so during that closing process, the other amino acid residues will come and they will try to push the urea molecule towards your dinickel site.

So, that push from your histidine residue, so these are the residues, so not only these two histidine residues, but you also the alanine residues. So, these three basically, these three units

basically, three units will try to push that particular system in that particular fashion such that your thing is under strong control of your dinickel site.

Already you have the bound hydroxide ion, that bound hydroxide, nickel ion bound hydroxide it is a strong nucleophile now that will try to attack. So, that is why you have the attack of this hydroxido group to the carbonyl function. So, carbonyl function will suffer the nucleophilic attack from the bound hydroxido molecule between these two nickel centers.

So, you have now the corresponding OH will now be a corresponding one is the tetrahedral O, OH and tetrahedral forming two bonds with the nickel and another bond with the carbon center. Then you have the loss of the water molecule that is called as the proton transfer. So, this proton transfer from here basically the proton will come to this NH2 function.

So, this particular one, then the final step, the flap opening again, one step is that your flap closer and another step is your flap opening at the end when you will come back to the original position A. During that particular process everything has opened, everything has been done. So, you have then again the entry of the four water molecules. So, you can have 1, 2, 3. The water molecules are also leveled nicely. And then you have the first cleavage of that particular bond and you get the corresponding first step of that particular reaction.

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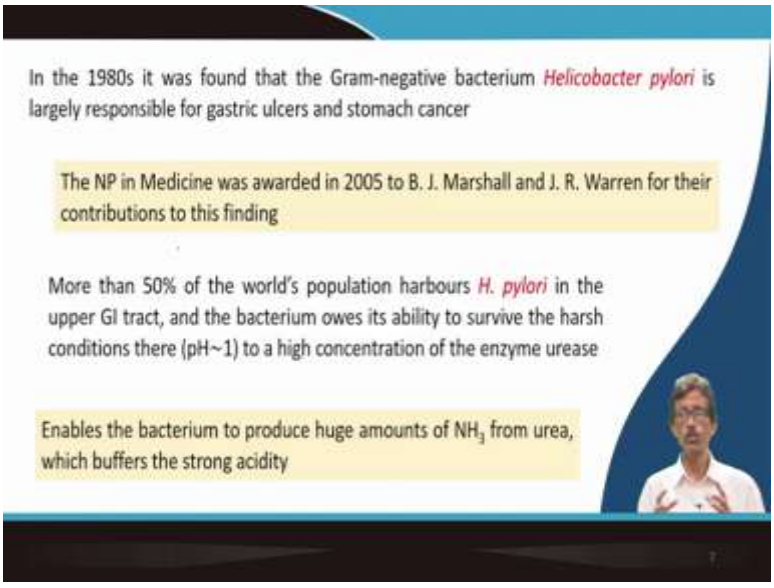

So, during 1980s what happened basically people are started thinking about the other thing also. How we can get, because not only the one particular structure of JBU that is the people are happy, because scientists working on this area they never happy, people always unhappy. So, they try to go on giving different samples from the different sources. So, one such sample is your H. pylori.

So, this H. pylori they found it the connectivity to your disease, to your health, to your well being is related to our stomach cancer and gastric ulcer. So, definitely this bacterium is sitting inside our stomach. That is why this, during this 75 for knowing the urease people got the Nobel Prize for the structure determination. Again another 30 years after JB Marshall and JR Warren also got NP, the Nobel Prize in medicine now for their contribution in understanding of the presence of the bacterium and their connection to different diseases.

So, more than 50% of the world's population try to remember it nicely and you can tell others also in that way, we have, we harbors, we have like the port harbor we call, we harbor basically, we, when we digest the food, it is also deciding our, in our body. But is definitely is not harmful all the time, is the upper GI tract, the gastrointestinal tract it is sitting over there.

And this bacterium owes its ability how it is surviving basically in a very harsh condition of pH 1. We know we suffer, some time we call we are suffering from acidity because our stomach acidity can go down to such a value that it can go down to 2.4, 2.5 and 2.8. But if it is going to pH 1, these are basically surviving.

So, that is the one important thing that even if you have the suffering from the pylori that your pH will not help, your antacid will not help, so because they can survive it. So, when the bacterium is working on it for the urea molecule, they are producing huge amount of ammonia and they basically can give you a buffered condition.

So, when you have acid we know that acetate is there, acetic acid and ammonium acetate or sodium acetate. Similarly, you can have the ammonium carbonate or ammonium bicarbonate and all these bases are there. So, you can have a corresponding the salt and the acid mixture that is known as the buffer. So, it is not possible to change the pH very easily without addition of any other thing or the biochemical thing or biochemical intervention.

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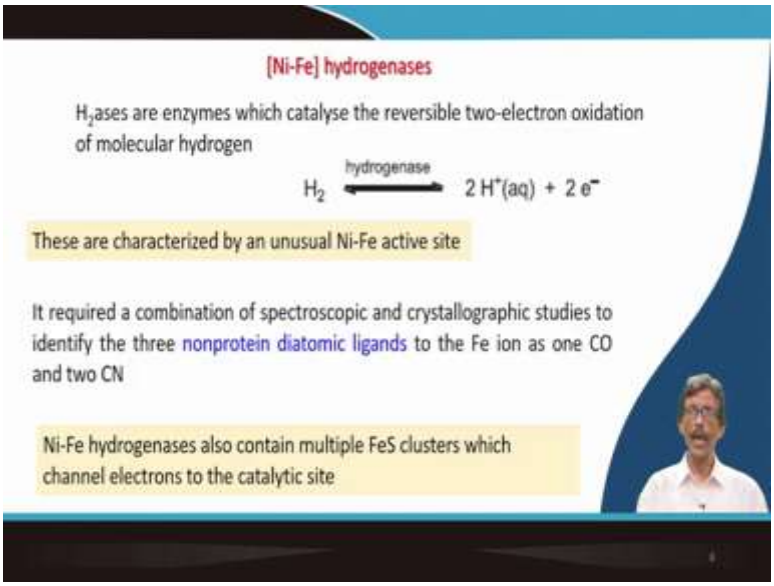

So, next we will go to the nickel iron hydrogenases. Already we know what is known as the hydrogenase, because the assimilation, the reversible assimilation of the H2 molecule, either you go for the cleavage of H2 molecule or you go for the production of the H2 molecule that particular part is very important that whether we will be able to produce hydrogen because we all know that hydrogen is a very good fuel.

So, whether the bacterium can, all these particular species can help us in producing hydrogen. So, this particular one, we are talking about only nickel not iron, so this particular thing has a very good active site, I mean, nickel and iron. But when the crystallographic studies were done, people identified surprisingly that we can have non-protein diatomic ligands and we have studied from our school days that carbon monoxide is a very good ligand and CN, CN minus is not CN, neutral CN is cyanide ion.

So, CN minus is also very good ligand. So, that is why we study the metal nitrosyls or the metal, in the zero oxidation is the metal nitrosyl complexes and metal nitrile complexes. So, these are basically contain, also that is active site is nickel iron, then you can have also other FeS, the iron sulfide rubredoxin, ferredoxin type of clusters which can donate or accept electrons. So, they are very good oxidizing agent or reducing agent to us.

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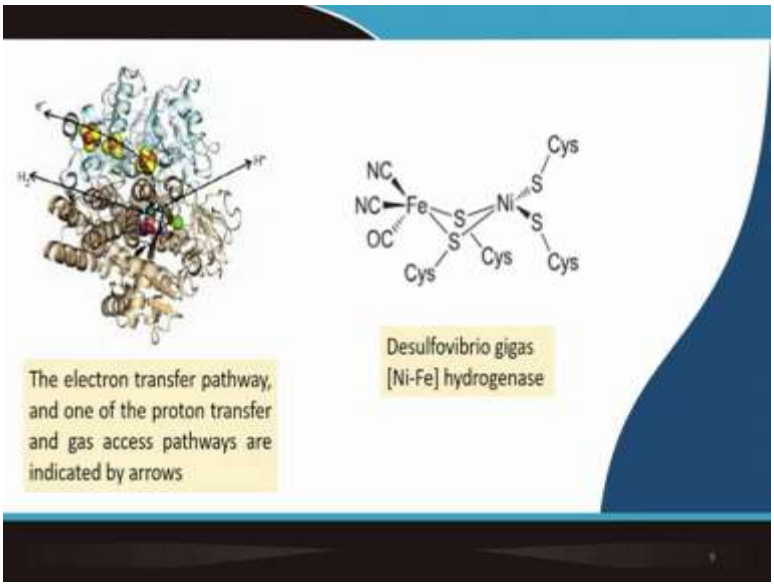

So, this is the structure what you have, the protein structure. And it is showing basically where you can take out the electron or you can put the electron. So, around that active site basically everything is there, but you can have the channel, the electron transfer pathway and one of the proton transfer and gas access pathway. So, gas will be liberated in this site and proton will be liberated on that particular site if it is reversibly consuming your H2 and H plus.

The site, that is why very interesting and very fascinating also. That if you have the nickel site and if you consider I will be able to stabilize that particular nickel site giving you four coordination sites all are sulfur that is fine. So, then if you utilize these two sulfur, the cysteine sulfur residues we know more number of lone pair of electrons are available like your hydroxido group and you attract another metal ions that is your iron.

But interestingly that metal ion, biogenically formed cyanide and biogenically formed carbon monoxide is already attached to it and is coming and binding to that particular site giving you this binuclear site. So, has been identified in desulfovibrio gigas is basically a first example of the nickel when people characterize this nickel center, is roughly around 40 years back during 1980s. But it has developed a lot for knowing all these things, different model compounds and all for all these structures and all these identities.

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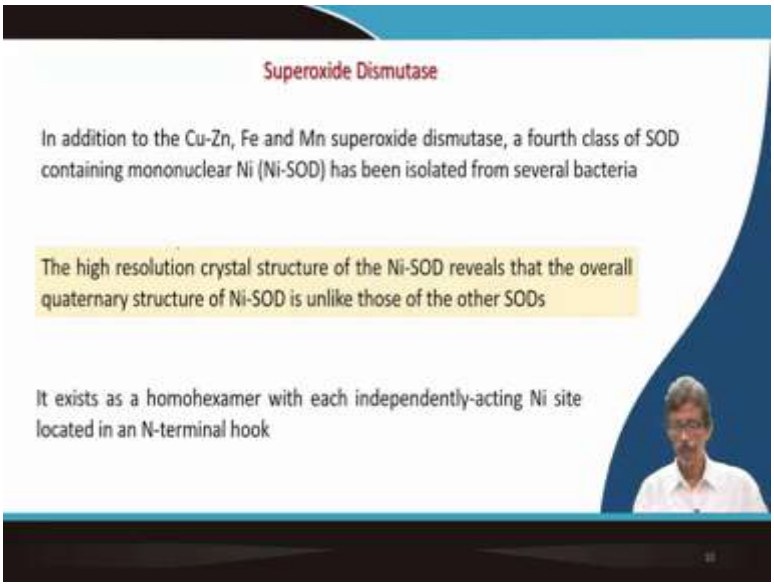

So, during this my last part basically we will talk about now the superoxide dismutase. We already know about the usefulness of your superoxide dismutase system, because our body is basically producing superoxides, peroxides and all these reactive oxygen molecules ROS molecules. We produce this thing for our defense also, because these are very much reactive. We can use those ROS molecules to kill the bacterium also.

But bacterium are smarter than us. They, what they do, they basically can also have some mechanisms such that they itself can produce superoxide dismutases such that they can break the available superoxides or peroxides what our body is producing to kill those bacteria. So, is basically a very good fight between the bacterium and the host like the mammals or the human being. So, how the nickel system is working?

We are very much interested to know about the nickel system. And afterwards definitely, because already you have studied the first example of copper zinc, then we have told you that you can have also the manganese, because you need the redox centers and how you can compare the first category of this particular class of superoxide dismutase or SOD with that of the latest variety that means the nickel SOD, because we are enriching our knowledge.

If something has developed or something has been identified 40 years back and what we are discovering today we always try to correlate that thing that how was your first SOD molecule

and how that SOD molecule you can correlate with that of the latest discovery which is your nickel bearing SOD.

So, x-ray structure definitely and high resolution, because we are struggling also for increasing the resolution, sometime it is 3.4 angstrom resolution, 3.3 angstrom, 3.2, 3.1 we can go down to 2.2 also until and unless we are not getting better structures. So, overall quaternary structure is something different unlike those of the other SODs.

So, is the homohexamer and each independently acting nickel site located in the N-terminal hook. So, you have the hook and end terminal residues are there and that N-terminal residues we try to grab. So, if you have the N-terminal residue and we will try to grab the corresponding metal ion center.

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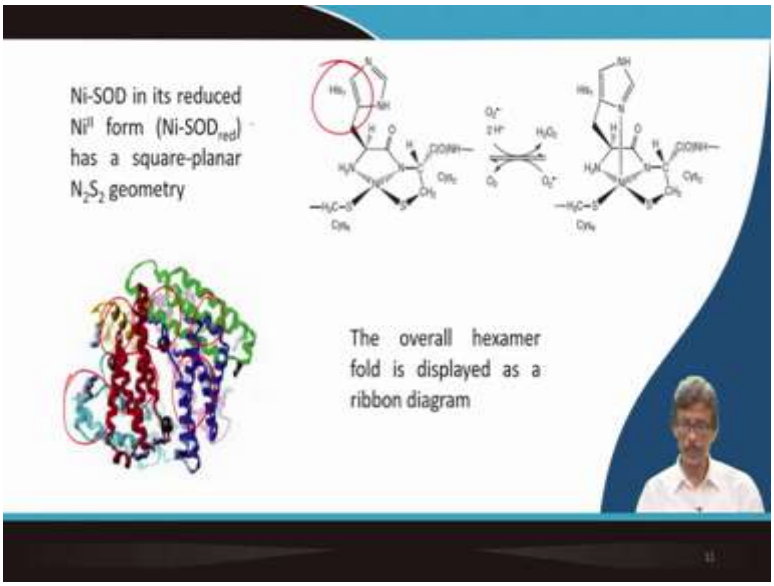

So, this is the corresponding ribbon drawing or the ribbon cartoon structure for your protein envelop where you can have the corresponding nickel centers. So, if it is a thing where we are calling as the homohexamer, so is basically hexameric unit. So, you can have six such units we can have. So, you can have the red and blue you see from this particular part. So, these two color code basically giving one part and the second part.

On the backside you will have the green and the other one and this one other and this is the other one. So, you will have all together six units. So, these six units and arrangement of those six

units gives you the corresponding hexameric arrangement. So, this hexameric arrangement we have, fold is we can have and displayed as a ribbon diagram.

So, what it is can display. But what is the actual environment around the nickel is very much important and very much interesting also. People are excited, particularly the synthetic chemists, the synthetic coordination chemists and the model coordination chemists are excited to know this particular ligand structure.

So, this ligand structure is you can have in the reduced form is the square planar N2S2 environment. So, you can have a bidentate NA ligand. You bring two bidentate NA ligand, you can track a particular site and you can consider this is my nickel SOD site. But interestingly one dangling part at the top basically the histidine 1, you see this histidine 1 residue what is there.

So, this histidine 1 residue is important. When you oxidize it to the trivalent nickel, it can come and bind to that particular nickel center for stabilization, because it is more electron greedy. You have oxidized that particular center. So, it will attract it. So, is oxidative addition case type of thing. So, you will have a penta coordinated situation in the trivalent nickel center.

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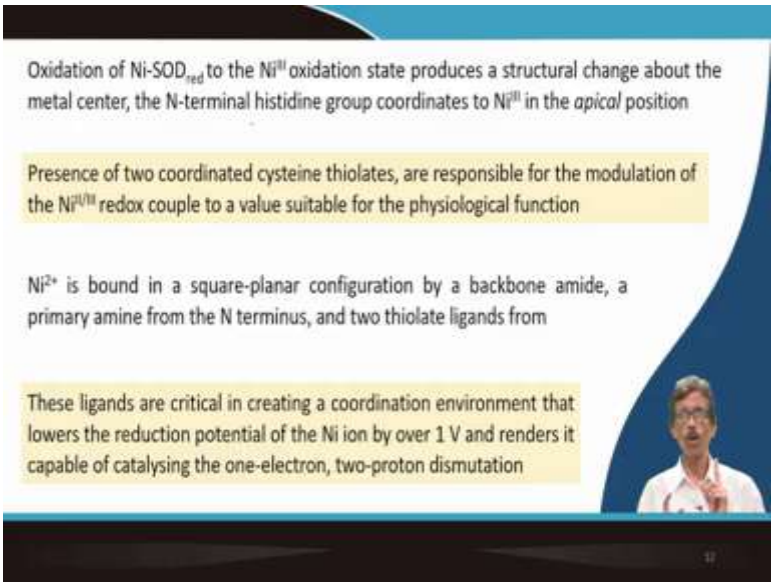

So, oxidation of this reduced form giving you the trivalent nickel and you will have the structural change such that you can have the apical coordination of that histidine molecule. But the coordination of the two cysteine thiolate residues, we modulate the corresponding redox

behavior or the redox values. And when you have the square planar entity, you can have the N-terminus end and the two thiolate ligands from there.

So, these ligands are critical in creating a nickel coordination environment with a huge amount of potential value or the potential window. So, it is capable of catalyzing one electron, two proton dismutation reaction of your superoxide anion.

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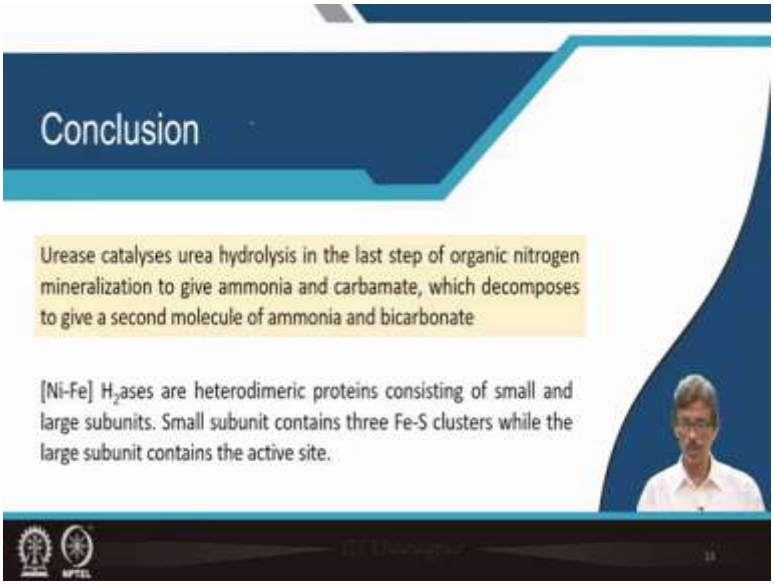

So, that is why we have seen three categories of these examples. The first one we have started with urease, second was in your hydrogenase, and third one is your superoxide dismutase. And there the importance of the ammonia and the carbamate formation and the second step of decomposition gives you another ammonia molecule and the bicarbonate molecule.

Similarly, for your hydrogenase system you can have the iron sulfur cluster and while you can have two units, one is small, another is large, these two are coupled together and they can have the supply of electrons to the active site.

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So, the reference is you can start from urease, then you can go for the hydrogenase and the SOD or the Wikipedia page and the book Crichton. Thank you very much for your kind attention.