Bioinorganic Chemistry
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Lecture - 18
Nickel Enzymes - II

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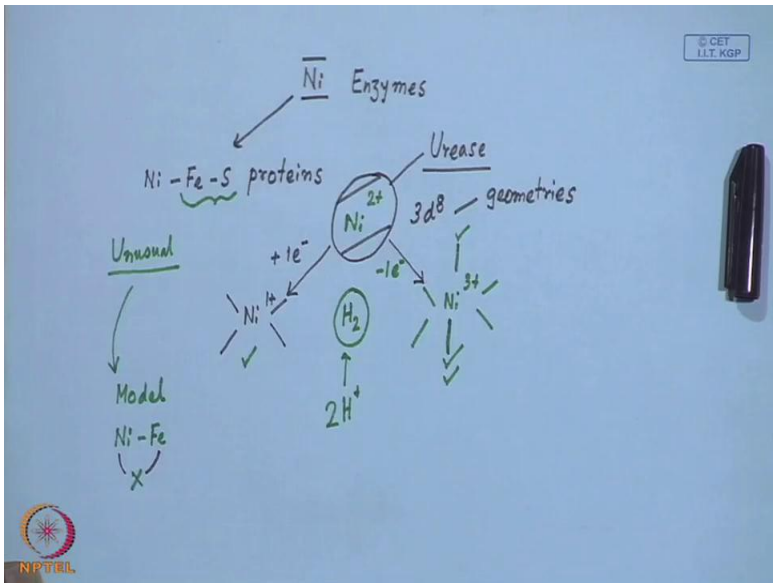

Hello, we will just continue the same nickel enzymes, what we are discussing last time. So, in these nickel enzymes, so we are focusing our attention mostly on nickel, and its coordination chemistry, and slowly we can move to some complex species, which is based on nickel iron and sulfur, so this can also be classified as nickel iron sulfur proteins. So, which will be definitely a different one, if we just compare with that, with what we know as the corresponding irons sulfur proteins.

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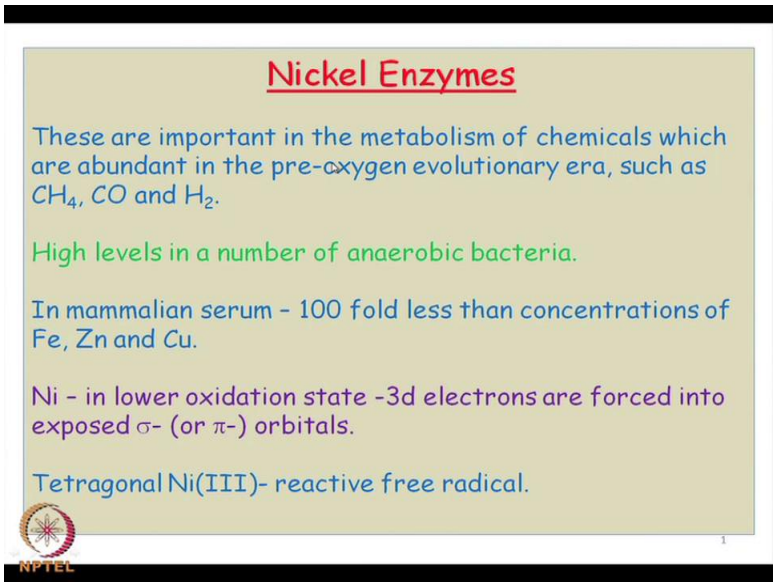

So, these we know that, there are some nickel enzymes, which are very much useful in metabolism of chemicals, which are abundant in the pre-oxygen evolutionary era. So, we want to assimilate some of this species like methane, weather we can produce it or we can get some species, where the methyl group is attached to the nickel centre, then carbon monoxide and hydrogen. So, these has some similarity with that of our organ metallic chemistry, where we can have direct nickel carbon bond, and you can something, where we can make some nickel methyl bond, or nickel carbonyl bond, or nickel hydride or some other species related to hydrogen.

And all these species are mostly, they present in very high level in anaerobic bacteria; that means, when we do not have in the environment dioxygen. So environment is fully populated with the gases like methane, carbon monoxide, and hydrogen. And if we just compare the amount of that nickel what we find in our system; that means, the mammalian serum, which is simply hundred fold excess, then the concentration what we require for iron in our blood, and any other important species like ferritin or transferring, zinc in the different proteins and the enzymes, and the cooper.

So these are the three most abundant, which are 100 fold excess the nickel, so nickel concentration is very less, but still nickel can function very easily, due to its characteristic property, related to its coordination chemistry or bi-coordination chemistry, which we cannot get for iron centre, or zinc centre, and cooper centre. So,

what is that, so the stabilization of nickel in different oxidation states. We all know that, the nickel is mostly stabilized in last two oxidation states. So most of the compounds where he get in the laboratory also, the coordination chemistry of nickel is mostly dominated by nickel 2 plus, and what we have seen earlier also in case of areas; that is the hydrolytic species, which is responsible for hydrolysis of the urea molecule, and the coordination chemistry is mostly based on a species, which is 3d 8 in different coordination geometries. So, first time we will be focusing our attention, on nickel in lower oxidation state. So, you have, large number of 3 d electrons. Already you have a 3 d 8 system in plus 2, and if we go to nickel one.

So, there will be more then that species; that means, 3 d 9 system and electrons are forced into exposed sigma or pie orbitals. So, those electron, the available electron from those sigma and pie electron would be responsible for, very different or characteristic property what you can get, based on nickel. So, not only the lower oxidation state; that means, nickel in plus one oxidation state, but also the tetragonal oxidation state; that means, the nickel 3, which can give rise some reactive free radical, and which is also very much useful, like the if you can recall the corresponding organometallic chemistry based on palladium.

So, palladium we all know we start from palladium 2, then if we are able to reduce it palladium 1, then immediately 2 electron transfer can transfer that particular oxidation state from plus 1 to plus 3. And not only this one, but also something else; that means, when we have a nickel in plus 2, and if we can reduce it by one electron. So, you get a nickel 1 plus species, so this particular species. So, in this particular case, you have a coordination geometry which will be very much similar to that of a square planar geometry, but if you go for a corresponding oxidized form; that means, nickel in 3 plus.

So, it will have a general tendency to form a corresponding octahedral species. So, not only the electron transfers, because all these cases what we will find for hydrogen gases. So, you will be getting something, where you have the corresponding assimilation of H 2 molecule, or you are producing that H 2 from proton. So, you can sometime you accommodate; that means, the expansion in coordination number, as well as transfer of electron.

So, if you have 2 such H plus. So, you require 2 electrons for that transformation of H plus 2 H 2. So, nickel is ideally suited for that, if you have some stable oxidation state. So, this is the most stable oxidation state, and this is the catalytically active one, and this also is the catalytically active oxidation state, and it can change the corresponding coordination number also, like that of our organometallic transformations based on palladium, that when you go to plus three oxidation state, it can attract two more coordination site, such that if you have H based some species; that means, a hydroid or H plus or other species something like that of your solvents.

So, it can occupy this site, as well as it can occupy this site. So, this tetragonal nickel 3, which can also give rise to some reactive free radical is also important to know, when we talk about this thing. So, if you go for some nickel iron sulfur species, this is very much unusual, which is very difficult to make also. So, if we want to make it in as a model compound in the laboratory, which is also very difficult to make; that means, what we are talking about something, where you have a nickel iron binuclear compound, because they have some different preference for oxidation state, and some time when we go for this nickel and iron species, there must be something; that means, there must be some bridging units, and that bridging units should have some reference to bind these both nickel and iron.

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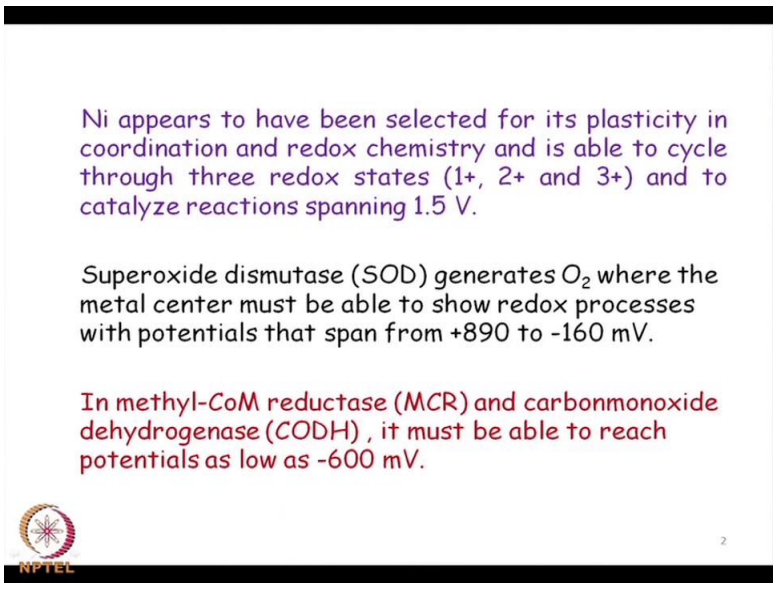

So, it is definitely a very unusual site, and that site what we get, in case of this particular reactivity pattern. So, initially we can have some of these enzymes, where nickel appears to be selected, in its plasticity of coordination in redox chemistry, that just now I am talking about, that you can move from one coordination site to another coordination sites; that means, you can move from a square planar geometry to the octahedral geometry. So, that is why we are talking it as a plasticity in the coordination geometry, as well as the redox chemistry; that means, how much your oxidation states are accessible to you, to a biologically visible oxidizing agent, or reducing agent.

You cannot use some other strong chemical reagent, chemical oxidizing agent, or reducing agent, to get some reduce form or the oxidized form, and when it can cycle through the three different oxidation state; that means, when you start from the most stable oxidation state, which may not be catalytically active, but you can go down to plus one oxidation state, and you go up to plus three oxidation state, and it can catalyze large number of reactions, within a potential window of 1.5 volts. So, we are not going much, for this oxidation and reduction potential. So, you have a window, for plus 1 oxidation state to plus 3 oxidation state, which lies between 1.5 volt.

And one such example, though we will not discuss in details all these, this is superoxide dismutase family of molecules, which we know that generates O2, where the metal center can be able to show redox processes, where you have the potential, you see the window the very large window, so plus 890 to minus 160 milli volt. So, between this window and between 1.5 volt window, we see some other examples for one such example we will find is the carbon monoxide dehydrogenase, and they are inter related.

So, one is that carbon monoxide dehydrogenation reaction, is nothing, but we abbreviate it as C O D H, so it is nothing, but its corresponding transformation form carbon monoxide to carbon dioxide and vice versa. And another is methyl-coenzymes M reductase, which is also possible for the reductions reaction, and again if we talk in terms of the corresponding potential values like this, and the superoxide dismutase is, and for this it, is a quite negative potential, which is minus six hundred milivolt. So, you required some strong reducing agent, to generate a species which can be nickel in plus 1 oxidation state, and that plus 1 oxidation state, if you are able to generate, that can show some catalytic activity.

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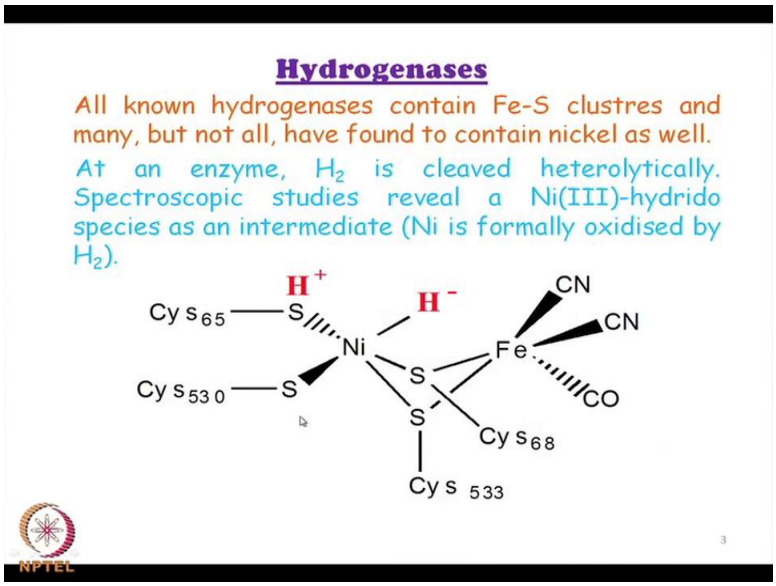

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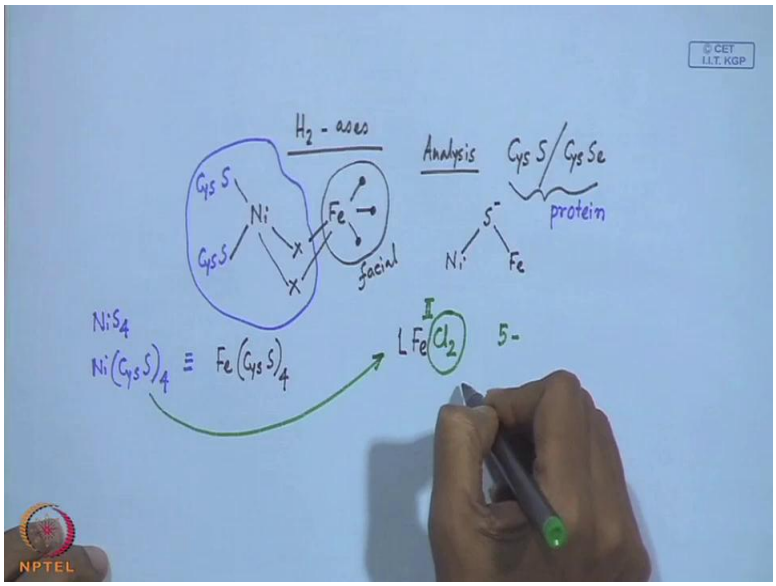

So, the potential is important, as well as your corresponding coordination geometry. So, when you go for these hydrogenases, and this hydrogenases one important thing will be, you get the binuclear specie, based on nickel and iron, and some bridging group is there, and another bridging group is there, and we have two other coordination sites, and three more coordination sites. So, the analysis, initially establish that, you have cysteine sulfur residues, or selenocysteine residues sis selenium based system, sulfur is replaced by selenium. So, if we can have all these groups and these, we know that cysteine residues are based on s minus. So, this can very well used as a bridging unit between nickel and

iron, and these are coming from protein chains. So, these are protein ligands. So, if you have all four; that means, if these are also cysteine sulfur, and this is also cysteine sulfur. So, what you need, you need four cystein sulfur groups, around a legal centre.

So, that is also very important, if you are able to make in the laboratory, that some compound, where you have the environment is simply Ni S4, and that S4 at one side if they are all thiolate groups; that means, the thiol ligands are very good ligand, for bridging the second group. So, already you have the mono nuclear nickel centre, and two of these; that means, if it is in the different geometries not a square planar one, this can be a typical square planar geometry, but if you have available thiolate units from these two sides, and if they are used to bridge the second metal, you get something, where you can gave a hydroid nuclear system.

So, this is one such strategy, that you do not use any thing exogenous; that means, which is not present within the protein chain, or within the biological system, but it is already bound to the nickel center. So, this nickel is nothing, but your nickel cysteine sulfur four, which is equivalent to that of our well known iron site rubidouxine, this we all know. So, rubidouxine is also a mononuclear site, where four cysteine sulfur residues are attached to (( )) site.

But in this particular case, if something happens like these, that if you bring the second center; that means, the iron center, with some of the ligands, like this ligand, this ligand, and this ligand, say there are in a facial orientation. So already we know that, anything in a face, basically some well known ligand, we know that the triazacyclononane type of ligand.

So, one face is occupied. So, basically if you have some species like that, in the laboratory also you can make ,one ligand l, which is like a tridented one, and that tridented one is capping face of the iron, and you can get a corresponding salt which is chloride salt. So, this iron side this iron salt you can make in the laboratory, a synthetic molecule of this type, which is five coordinated. So, now, if you put something; that means, if you are able to make some of this type of compound, that how you attached this particular units, where you have 4 cysteine sulfur are already attached to the nickel site, and you have this particular unit 5 coordinated side.

So, what we can do you just if you are able to remove these chlorides from iron site, and if this cysteine sulfur can bridge your iron site. So, you get some assembly which will be very much similar to this, and in this hydrogenase this particular motive is present; that means, you have already you can make these in a 5 coordinative species based on iron, which is allowed to react in the protein chain with the nickel site, which is already occupied by four cysteine sulfur residues, and you get some interaction, such that you bring the iron site to the nickel, it is not that these hydrogenase can react in a different fashion, when both of them are iron, or both of them are nickel, but you need iron, because the coordination preference for this iron, is completely different compared to your nickel site. So, if you have a hetro binuclear system, that two catalytic sites, the metal based catalytic sites, one is based on iron, and another is based on nickel, would be completely different. So, that we get for the hydrogenases, and all known hydrogenases contain this iron sulfur clusters, and many, but not all have found to contain nickel and as well.

So, basic thing is that, you have most of these hydrogenases, it should have these iron sulfur clusters, and these iron sulfur cluster is already present there, and these iron sulfur cluster we can have. And in this particular case, you are considering these as you have; this is also cysteine sulfur, this cysteine sulfur, and these two cysteine sulfurs are the bridging cysteine sulfur residues, and which is attaching to another site which is iron, but you can now have instead of a facially capping trident triazacyclononane type of ligand, you have three inorganic ligands two cyno and one carbonyl.

So, these three groups basically can stabilize, this particular iron site in low oxidation state, and that was the strategy for the biological system, that you should stabilize this particular iron site in a low oxidation state; that means, the plus 2 oxidation state, and in electron transfer reaction, because these hydrogenases are all electron transfer proteins. So, they are basically electron transfer enzymes, but electron transfer will take place based on iron, and these corresponding proton or the hydride units, which is coming from the cleavage of the hydrolytic cleavage of the H 2 molecule, but your iron site is not participating in any such electron transfer reaction.

So, this H 2 molecule can be cleaved hydrolytically, and spectroscopic studies reveal a nickel 3 hydroid species. So, that people have identified, that once you have and we all know that the most important spectroscopic technique for identifying a nickel site is, the

EPR, electron paramagnetic resonance. So, electron paramagnetic resonance once you have, which cannot identified very easily the nickel in plus 2 oxidation state, but it can easily identified nickel in plus 1 oxidation state, and nickel in plus 3 oxidation state.

So, these two means, weather your nickel centre is settling between plus 1 or plus 3 oxidation state, that can be very easily identified by EPS spectroscopic measurements, because these two species, these two oxidation states, can have characteristic spectral feature; that means, it has the corresponding and or whether it can be a rhombic one, or a monoclinic one, so axial one or a rhombic one, the spectral features for the EPR, and when nickel three is there, you can find that in the catalytic cycle when we find that, the hydrido species; that means, when we you have this nickel, we will find in the entire catalytically cycle also, that you have this particular.

So, nickel centre oxidation state of the nickel you can identify; that means, the nickel center is in plus 3, but the nature of these species; that means, weather is a hydrogen atom or H plus or H minus; that means, it is stabilized by H minus. So, this particular assembly; that means, is Ni H to nickel 3 hydrido species, which is the most characteristic species which is present in hydrogenase.

So, you have H 2 which is attach to this nickel, and it cleave between H minus and H plus, and one particular centre; that means, the metal centre is utilized for taking up this H minus, and when H minus is taking up, it can also if your these two distance, because already you have the bridging sulfur groups. So, we all know that if you have a single bridging units between two metal centre, you have a corresponding distance of nickel iron. If you go for a second bridging your distance is less. If you go for a thought bridging, your distance is further less, because when we find that if your two sides are octahedral in geometry, you can have a corresponding face. So, one face of one metal centre, and another face of the second metal center are bridged, so when it is triply bridged bi nuclear units.

So, these hydroid is bridged, the sulfur is bridged, and second sulfur is also bridged, you get the shortest possible nickel iron distance. So, that distance is also very important for its catalytic activity, and how you modulate these two distances, and interestingly when the cysteine sulfur is attached to nickel side, you can have something; that means, this sulfur when it is attached to the nickel side, it can bridge the second metal center.

Similarly when this sulfur, is already coordinated to your nickel site, it can bind a proton like these; that means, already if you have s minus, which can be bond to your nickel site, or S H in the neutral form S H in neutral form can also loosely bond to the nickel side.

So, either the protonated form of cysteine sulfur, or the deprotonated form of the cysteine sulfur can bind to the nickel site, and that basically can give some interesting feature that then when it is protonated what happens, your nickel sulfur distance will increase. It will show some loose coordination to your nickel side, like binding of water molecule to any metal centre, compare to your hydroxide binding. We know that the hydroxide binding is stronger to any metals centre, compared to your neutral water molecule, so these bindings. So, binding interaction will still be there, but you have a longer distance and which is weekly bond. So, what we are basically generating, we basically generating two sites, and for hydrolytic cleavage of the H2 molecule, giving H minus and H plus. So, nickel site will be available for binding H minus, and your ligand site; that means, the sulfur site from the cysteine sulfur will be available for binding your proton.

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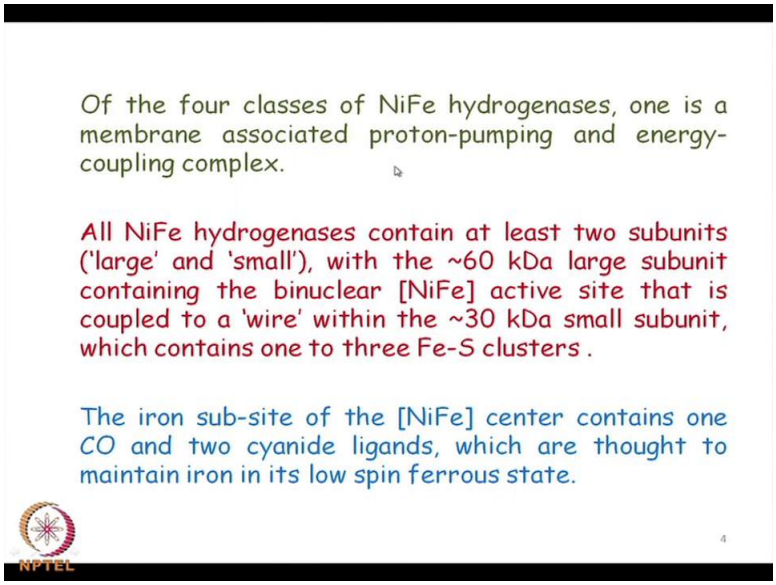

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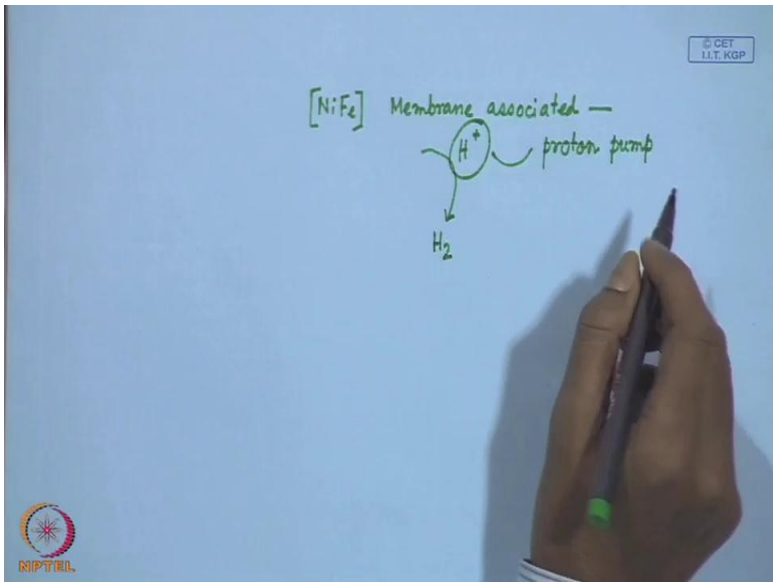

So, that basically gives some important reactions, based on all these nickel iron hydrogenases. So, this class of molecules basically we will call them, as because there are several only iron hydrogenases, but these we will call as nickel iron hydrogenases, and they are associated to the membrane. So, they are membrane associated protein molecules, and that basically handling, or taking care of proton. So, it has something related to that, we will later on we will see something related to what we basically considered as a proton pump.

So, if membrane is associated, and that membrane is there, which can reversible bind the proton into the system. So, because we are talking something related to the assimilation of the proton, and the production of H2 for these hydrogenise reactions. So, there are basically four such classes of nickel iron hydrogenases, and one of them is membrane associated. So, when it is membrane associated, we call them as it is related to something, which is considered as a proton pumping mechanism, and energy coupling complex, because when you know that there is some proton gradient is formed from one site to the membrane to other site, we can have some good free energy change same and which will be utilized for A T P synthesis. So, in all these cases, these are little bit complicated.

So, we get some spared one such part, or one such unit, which we will consider as sub unit. So, one sub unit we considered it as a large sub unit, and another sub units as a

small sub unit, and the large sub unit has a molecular weight of 60 kilo delton, and small is for the small, and already we have seen how a bi-nuclear nickel iron system is important. So, you have, you have identified it that from the analysis also, that identification of the metal sites; that means, you have iron and the nickel sites, so active sites.

So, that is coupled to a wire within 30 kilo Delton small sub unit. So, if you have one such unit and another one such units, and in between you have, you see the total thing; that means, only one part we are discussing which is based on your nickel irons. So, one is large now, and another is small, and these two units are connected by further are your iron sulfur clusters, because all we are know that the use of these iron sulfur clusters, because they can supply electron, they can donate electron to the system, or they can take off electron from the system. So, if you have. So, all this hydrogenases are your furridoxinen or rubidouxine dependent; that means, the iron sulfur protein depend.

So, number of electrons, supplied to the proton, will be dependent on the number of these irons sulfur clusters, which are present, and sometime they are in the oxidized form, they take of electron from other biological reducing agent; like NADP and others, and they will be in the reduced form, and those reduced form will be in the near vicinity of the nickel iron based hydrogenases, and those electrons will be supplied to proton for the production of hydrogen.

So, if one such these thing; that means, the sub unit of these nickel iron center contains carbon monoxide ligand, that we have identified, which is very important and that will find how nature has divide something; that means, not only we are identifying the presence of nickel, the presence of iron, but also we are identifying the two different inorganic ligands; that means, the carbon monoxide and cyanides ligands attach to the iron site, but this nickel and iron based hydrogenise, can also tell that something, that though this carbon monoxide is attached to the iron site, but we can have something; that means, this biological site, or any other biological site, you can have some mechanism where the nickel center, the nickel can function as an active site, which can go for assimilation of carbon monoxide.

So, that gives as some clue that for carbon monoxide hydrogenases also, not only hydrogenases, but carbon monoxide dehydrogenases, which we are considering at

CODH. So, those CODH can also have something to play in this system that, it can go for binding carbon monoxide in a transit form; that means, it can transfer this carbon monoxide to some others species, and it can activate this carbon monoxide, either in the form of transferring it from carbon dioxide to carbon monoxide or carbon monoxide to carbon dioxide, and when it is attached to the iron site, already I told you that it is stabilizing the low oxidation state that means the ferrous state.

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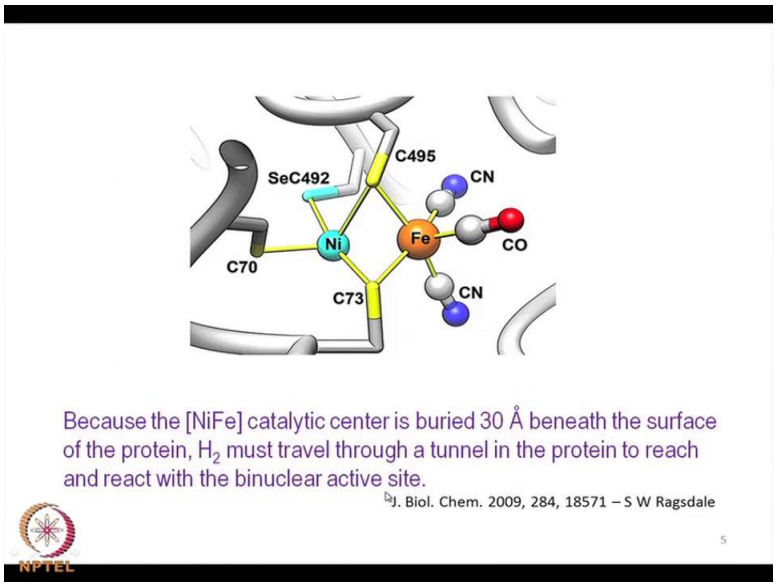

So, this geometry already, the diagram I showed you. So, this thing is that how this the large sub unit and the small sub unit. So, this is the thing; that means, you have coverage of these; that means, it is under a 30 Armstrong surface of the protein, and under that it is there. So, you have to cross this particular distance; that means, hydrogen is a very small molecule. And that molecule basically tunneling and going through this particular part, and approaching this particular by nuclear active site. So, if it is fully covered, and if say something is membrane bond thing, and if you considered the membrane is not allowing for the gas permission.

So, permeability of the hydrogen is very important there, and that permeation is allowing that particular membrane, that hydrogen is entering there, and it is passing or crossing that proteins SIDH basically or protein coverage, and then ultimately approaching your catalytically active site; that means, it is approaching basically to your binuclear active site. So, this already I told you that you have one iron site; the carbon monoxide and

cyanides groups are attached, and these two cysteine sulfur are the bridging units, and these two are can be cysteine and sometimes it can be also selenocysteine.

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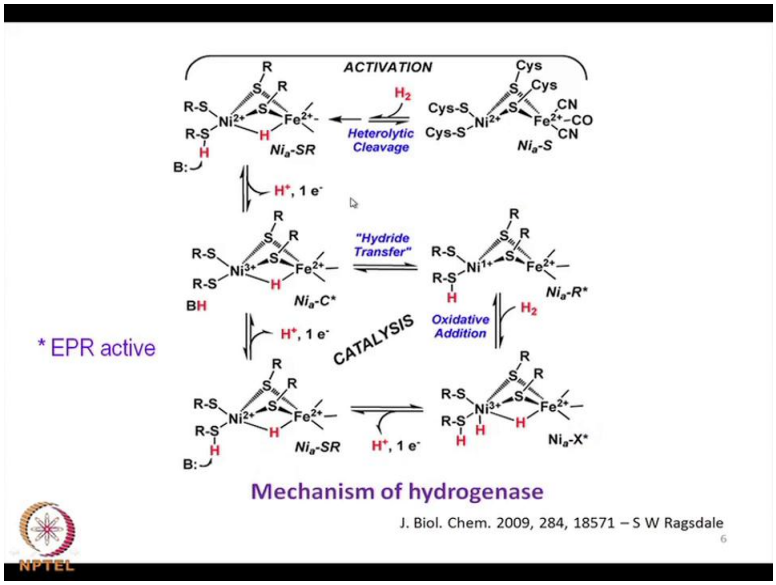

So, this active site you can have now, so how you go for this mechanism. So, mechanism is a little bit complicated one, and you have this unit, because you are talking here. Your sub unit is like these, on the iron on one side you have these groups, and on the left you have the nickel, and attach to your cysteine sulfur. So, what basically we are getting there, that you have of some amount of vacancy here, is not triply bridged by nuclear unit.

So, if you just able to considered it, that this is not our system where you have two metal centers which are triply bridged. So, it is not a triply bridged system. So, if you get the introduction of hydrogen, as for the hydrolytic cleavage already I showed you, that you have the hydride function the red hydride group is bridging these two metal centers, and the second hydrogen, which is coming out as proton, which is attaching to your cysteine sulfur. So, this is cysteine sulfur is getting protonated. So, this cysteine sulfur is protein protonated, and when you have. So, what is the driving force.

So, you have these one, then H2 is attached to this particular form, and if you take out these particular age, and this particular one is the environment is such that; that means, the. When people discovered say 20 or 25 years back this particular system, initially there were puzzled to know, that how in this particular environment; that means, the

nickel is basically in S4 environment four sulfur environment. So, these four sulfur nickel environment, how immediately from this particular state; that means, your proton is moving from this cysteine sulfur to some nearby base, this is a base basically.

So, nearby base in the protein envelope, so that base is taking up this proton, and your nickel centre is responsible for oxidation. So, basically what we are talking about, we are talking something related to your proton coupled electron transfer. So, this has the uniqueness for these things; that means, sometime we consider only electron transfer; that means, those metalloenzymes or the protein cysteine you can have, only electron transfer, and some other time we will find that you can have the proton transfer from one side to the other.

Sometime you can find it for different hydrolysis, but in this particular case, you have the proton transfer as well as your electron transfer. So, once it is going there, so your nickel centre is basically oxidized, and this particular active state; that means, is a start from. So, all these start form are EPR active. So, this would be EPR active. So, people have identified initially that the spectroscopic signature what you can have, that the catalytically active site is EPR active. So, this particular state you go for the activation of the active site, activation of the binuclear site, but the real catalytic cycle is this four, so this is the catalytic site.

So, you get the nickel is in plus three oxidation state, and when it moves; that means, if these hydride function; that means, the hydride group is moving, and your nickel center is now getting reduced; that means, the hydride is transferring electron. So, hydride is transferring electron. So, hydride was H minus it is going to H plus immediately. So, it is a two electron step. So, those two electron are donated to the nickel three plus oxidation state, to reduce it to nickel one. So, these two are basically people initially were puzzled, people could not identify for several years, that which particular site is responsible for the catalysis of hydrogenases, weather it is a trivalent nickel state or the mono valiant nickel state.

But both of them are EPR active, but the nature of the EPR spectrum are different. So, that we should know, that the EPR spectrum for the mono violent nickel is completely different, to that of the trivalent nickel. So, trivalent nickel is simply most of the cases your typical axial and rhombic signal what we find for the copper, which is a 3d 9

system. So, this is your 3d 7 system and this is 3d 9 system. So, this can be very much similar to that of your copper system.

So, if these two are different, then people can propose that these two thing; that means, the hydride transfer is taking place, and your site is moving from a trivalent site to a monovalent site, and this particular site again if you go for this; that means, the oxidative addition, which we all know from the from the organometalic chemistry or anything, what we know that nickel can settle between the two oxidation state, what we all know for the palladium chemistry, that if you can have a trivalent nickel and the monovalent nickel, and the palladium chemistry is well known to you, that when we go from a trivalent state to a monovalent state, not only you are transferring electron, but also you are you have to move the ligands from the nickel side, because the nickel will have the preference for low coordination number.

So, it cannot accommodate a coordination number of 5 or 6. It will always try to prefer a corresponding coordination number of 4. So, when you go for oxidative addition on this monovalent nickel immediately it can go again to a trivalent one, but this particular function is a different one. Now you have three, it's you already you have the protonated thiolate function, plus you can have this type of arrangement; that means, the hydride as well as these two groups already attached to the nickel. So, this is one such proposal for that is already in your trivalent state, but this is completely different from N i a C star N i a R star this is N i a X star. So, these two are different C star and X star are different. So, you will have again some signature for the trivalent nickel, but your attachment for the hydrogen's are different, and this particular case you have single electron transfer over here, you can have one more single electron transfer like these; that means, you have the proton transfer as well as electron transfer from these.

Again you take out this particular form of this hydrogen, so all involvement for these; that means, you are basically assimilating hydrogen, involving proton and the two oxidation state; that means, the plus one oxidation state of nickel and plus three oxidation state. So, like your catalysis based on palladium, what we find occasionally in organometalic chemistry, that we go for some catalysis based on palladium. similarly in hydrogenise, which is the simplest possible catalysis for hydrogen that you involve these two oxidation state; that means, plus one and plus three, but now it is based on nickel plus one, and this particular one is giving you some attachment to your iron site.

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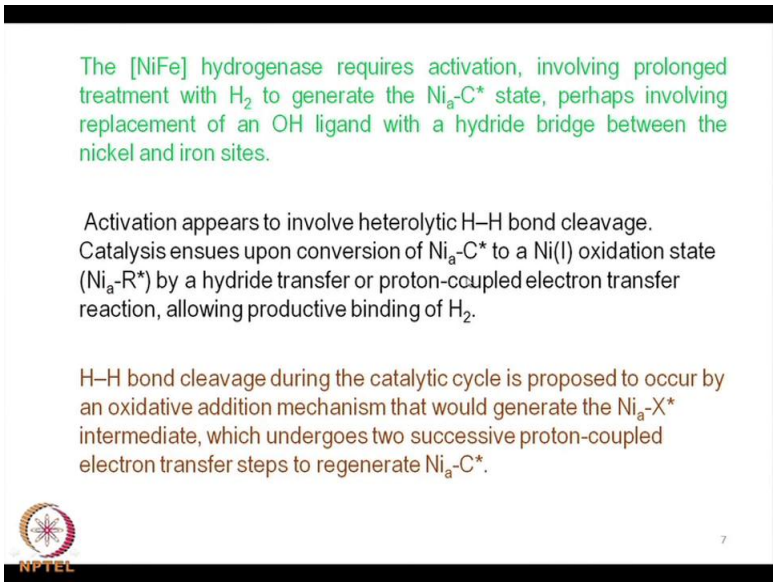

So, what we basically finds, is just you have written in the language that, required activation initially involving prolong treatment of H2 to generate Ni a C star state, and replacement of some of the oh ligand if it is not at all vacant; that means, if you have a vacancy what is attached to hydride bridge; that means, if you go for these, that the corresponding water molecule is present, and some time depending upon the p H of the medium you can have some loosely bond hydroxide function over there, and that hydroxide through ligands can be replace by hydride bridge between nickel and iron. So, you need this hydride bridge, between nickel and iron site.

So, then you need the activation; that means, already you have seen that how you can cleave the hydrolytic cleavage of H-H bond, and the catalysis is started over there, and which is responsible for the conversion of C star in one plus one oxidations to clusters, for hydroid transfer and proton coupled electron transfer, so this statement is very important. So, most of this cases, but we find reaction is nothing, but you have a proton coupled electron transfer reaction, allowing productive binding of H2. So, how initially if we start from proton, how you get some species, where your hydrogen is loosely bond to the system; that means, if it is loosely bond to the system, you can immediately get hydrogen from the system, so that particular case.

So, this H-H bond cleavage, when it is forming, so when it is involving your nickel plus one oxidation state, so oxidative addition mechanism that will generate, the third one;

that means, not C and R X star. So, in another case it is also possible to have the corresponding intermediate, and it undergoes. In this particular case two successive proton coupled electron transfer species, to generate back the Ni a C star. So, involving three such species, we can assimilate hydrogen from the proton. So, that is the thing, that how loosely you can get. So, when your hydrogen is living from the system, which is eliminating from the system; that means, you have loosely bond hydrogen to the system. Already the electron transfer as taken place; that means, already you have transformed this from the proton, and you have the loosely bond hydrogen to the system and that hydrogen can be removed from the system.

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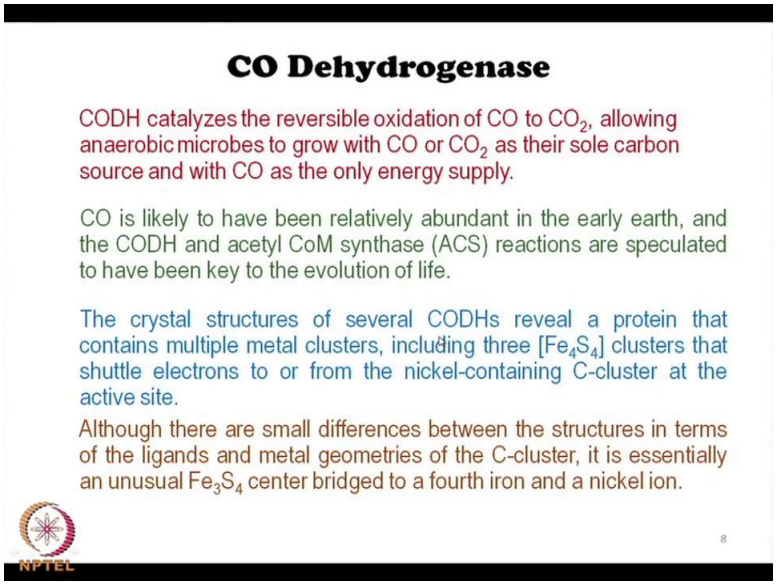

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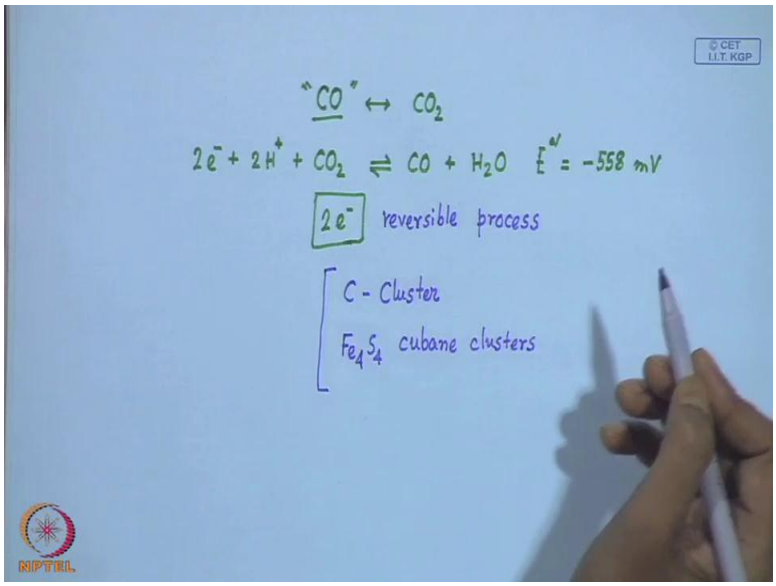

So, once we go for these binding of iron on the other side. So, now we can little bit talk about the corresponding carbon monoxide dehydrogenase. So, which is a very useful reaction, where we can talk about like that of our assimilation of hydrogen, which is nothing, but your corresponding reversible assimilation, where we can inter converged this carbon monoxide, between CO and CO2. So, this is basically inter converted reaction what we have seen in case of H plus and H 2 this is CO and CO2. So, this reaction you should remember little bit is a very simple reaction, but we cannot reproduce it nicely in the laboratory, for the assimilation of carbon monoxide, and carbon dioxide. So, this giving us CO plus H 2 O, and it has a potential. So, of minus 558 milivolt is a negative potential we can have. So, this particular reaction, also like that of our hydrogenase reaction, is a two electron transfer process. So, we have a reversible process, which should be two electron transferring process.

So, we have a reversible two electron transfer process, and now the system is little bit complicated compared to your hydrogenises. Now will bring some clusters, we will call them as c-cluster, and also we can have, what is already present there in hydrogenase also, because we are talking about large number of electron. So, iron sulfurpheridoxin like cubing clusters will also be there. So, involving these and if there are some other clusters also present we will find that.

So, we find that the CODH which is nothing, but your carbon monoxide dehydrogenase the reaction is therefore, your reversible oxidation of CO to CO2 allowing anaerobic microbes to growth with co and co two at their sole carbon source. So, these anaerobic microbes can take up this carbon monoxide and carbon dioxide, and your carbon monoxide, is your energy supply for all these microbes. So, basically when this carbon monoxide and carbon dioxide assimilation, we are talking about, we are taking something, you can have the microbial environment which is anaerobic in nature, so this particular case.

So, in the earlier, when you do not have oxygen in the atmosphere, your CODH was active, along with CODH we can have another species which we will considered as acetyl co-enzymes M-synthase. So, what we are making this. So, here the nickel site can function with that of your carbon monoxide. Now we are talking about something which is your acetyl function. So, your acetyl function is attached to your nickel site. So, your iron site in hydrogenise was attached to carbon monoxide. If that carbon monoxide is now transferred to the nickel site, you have the CO group attach to the nickel site, and if we can have something which is the methyl function, like your cynic coalmine or methyl coalmine. So, methyl coalmine we know that you have the methyl group attach to the cobalt center. So, you will have the corresponding acetyl function, attached to the corresponding nickel site, because these reactions are very useful reactions of some of these nickel dependent enzymes.

So, these nickel dependent enzymes are therefore, very much important when we find the corresponding crystal structures of several of such carbon monoxide dehydrogenases, and the we are we are saying that you have the c-clusters you have some other cluster. So, you have multiple metal clusters. So, one is your active site, or one or more active site you can have including three iron sulfur clusters, which is providing you electron, to form a nickel containing c-cluster at the active site, but at the active site you get the corresponding electron pool from the iron sulfur sites, and those electrons are consumed at the nickel site, and you get the corresponding catalytic transformations.

So, the differences in all these cases are the ligand differences, and the metal geometries of the c-clusters it is basically a different type of iron clusters. If you have Fe4 S4 clusters and if we can have some other clusters, where one of the iron sites is removed;

that means, you have generated a vacancy within the iron sulfur Cuban, and that Cuban can be bridged to some other metal site that the fourth iron site and a nickel site.

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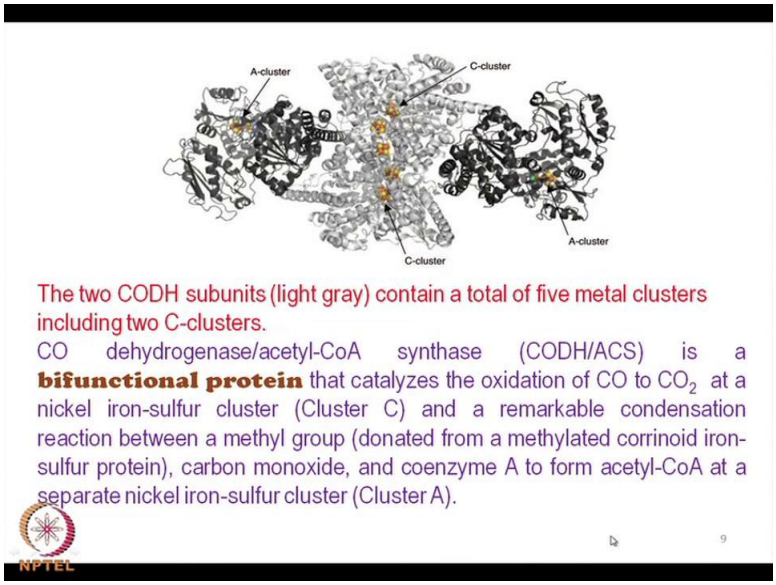

So, that is basically a typical example for the different positioning for these clusters. So, you can have A cluster, and the C clusters, and again one more A cluster. So, all together here within this C clusters you can have one two three four five, these five, means this five. So, five clusters are there, including some C clusters. So, in these cases; that means, while we are talking these two together that CODH, as well as ACS which is acetyl coenzymes synthase; that means, we are able to produce some acetyl function. So, acetyl function can be produced from the assimilation of the methyl group as well as carbon monoxide.

So, these are also very useful reactions where we go for some different acetyl transfer reactions. So, these two basically go for two different type of transformations. So, this is the thing that we considered them as bi functional proteins. So, in one closed related structure, it can function for the catalysis of corresponding conversion of carbon monoxide into carbon dioxide; that means, CODH activity at one nickel iron sulfur cluster, and in another case it can function on coenzymes a to form acetyl coenzymes a, at a separate nickel iron sulfur clusters which is known as clusters A. So, you have apart from your iron sulfur cluster, you have cluster C and cluster A nothing else. So, you have two clusters one is your cluster C and another is your cluster A. So, the C cluster when

people have identified that like that of your hydrogenases, you have iron is present nickel is present, plus you have sulfur.

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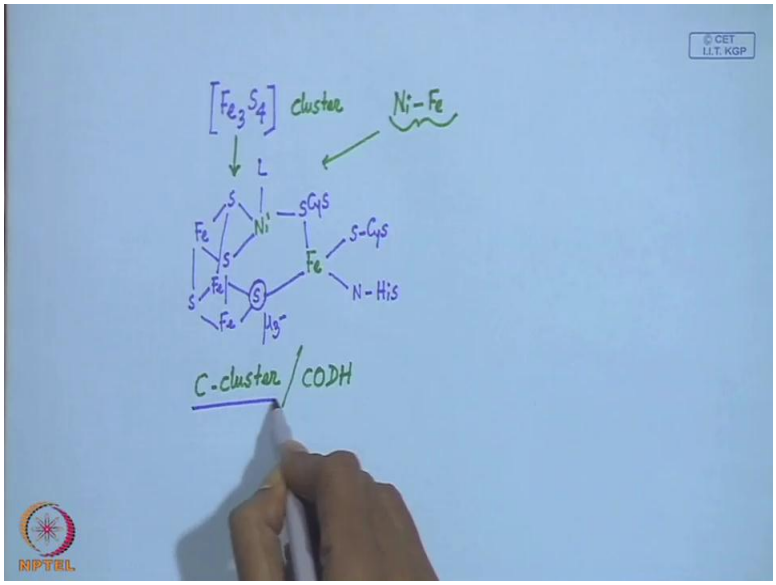

So, this is basically, just now I told you that if you have some vacancy from the Fe 4 S4 cluster. So, if you remove your one such iron center from there; that means, you have created one vacancy on Fe 4 S4. So, simply you can draw, is how nicely you can draw that thing, where is your vacancy. So, this is your sulfur. So, if you have sulfur over here it is basically Fe 4 S4. So, now you have generated this one; that means, you have Fe 3 S4 unit and these Fe 4 S4 cluster.

So, since you have generated some vacancy. So, it is still a cluster and now within that cluster we will be putting a bi nuclear motive, which already we have seen in case of hydrogenases. So, in the previous case, where we have seen that you have separate Fe 4 S4 cluster, but now here you have some degradation is taking place and you have generated some vacancy, and these vacancy is attracting something, and will responsible for producing your C cluster of, where the C cluster is present, we have seen just now that how much complicated your thing is that, when you see the protein structure.

But the c cluster, but we are all the time we are focusing your attention on the metal center, do not worry for that. So, we are focusing on the metal based cluster. So, you have c cluster which is present in CODH, responsible for the reduction of CO to 2 CO and now you bring, like your this particular unit; that means, nickel iron site, and you

have iron. So, already we know the mechanism form, that nickel iron hydrogenases. So, you know that this particular motive can be stabilizing very nicely by protein cysteine sulfur.

So, you put cysteine sulfur, you get the bridging unit. So, once you know that is we starting our discussion form the hydrogenases is that this part if you know, that this particular ligand is a very useful ligands cysteine sulfur any thiol ligand is very much useful not for giving you by some binuclear entity, but also a hetro bi nuclear entity. So, your cysteine sulfur will be available there, which can be utilized for binding your iron site and this nickel site, and this site if you bring this; that means, this particular unit is there if they are close enough; that means, they are at the binding distance, close to 2 Armstrong.

So, if this distance is closed to 2 Armstrong distance, this particular sulfur can function as a mu 3 bridge, which is well known. So, in this particular case you already you can recall little bit about your iron sulfur ferridoxine molecule, that what type of cuban you can have, and in that case when you have this sulfur attached to this; that means, all these sulfur that means which are occupying the corners of the cubes of the clusters, all the sulfurs are mu 3, but when you create some vacancy, your mu 3 sulfur is becoming mu 2.

And again when the iron is available, it is approaching to make some new bond, and that approach basically giving you some new binding; that means, this sulfur already attaching to this iron for the Fe 4 S4 unit, is showing some other binding so; that means, the entire cluster, not only accommodating a new hetero metal; that means, the nickel, but it is functioning as a bridge for iron. So, this cysteine sulfur is there, and you have some other ligand, some loosely occupying sulfur, or from the protein chain, and this sulfur is again your cysteine sulfur, and this is your nitrogen from the hysterine.

So, that basically completes the entire system, this is a very useful catalytic site where you get the corresponding C cluster within the system. So, we see that you have nickel, iron and sulfur in sulfur plus, in this particular cluster C. So, now we know the corresponding structure; that means, you have the nickel iron sulfur, how the cluster C looks like. Then you have the another cluster, so this particular part; that means, the C cluster and this C cluster and the C cluster the structure is well known to us now.

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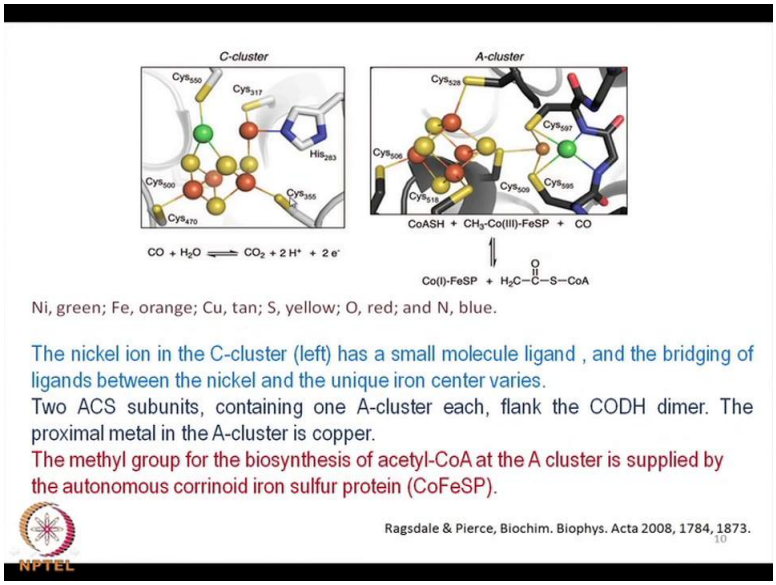

And now you have one more cluster which is your iron cluster. So, this we will just, do not worry for that, this will continue to the next class, that this particular entity what just now I have drawn in the piece of paper. So, this is your structure, basic structure. So, this people have identified from the determination of the protein crystal structure, and you can find this particular reference initially appeared in biochimic or biophysica acta, so this, when people have identified that, people are puzzled initially that how you have this particular.

So, this is the cluster, you have this Fe 4 S3 cluster, it is attracting your nickel. So, not only that nickel it is attracting, but it is bringing that corresponding cysteine sulfur and these cysteine sulfur, can establish some amount of bridging interaction, with this particular this cysteine sulfur, with that type of our iron; that means, you have both these nickel as well as iron in close proximately, because this particularly C cluster will be responsible for these very simple reaction; that means, the transformation of carbon monoxide to carbon dioxide.

And next day what we will see that this a little bit complicated reaction, for the transfer of this particular one. So, this is some square planar nickel based another one molecule we will considered, then again and that particular center which is nothing, but is attached to the methyl function like your methyl co vitamin. So, if you have some biological fault, or biological system, and methyl function is attached to that particular unit, and another

coenzymes is coming. So, this basically the reaction between two coenzymes, which is supporting these two enzymes function.

So, these coenzymes are reacting in a fashion; that it is basically trapping your carbon monoxide. So, in the C cluster we are getting some reaction, where carbon monoxide is transferring to carbon dioxide, but in case of a cluster, we basically require this particular carbon monoxide, in presence of this, this is CH3. So, this CH3 CO; that means, you use this carbon monoxide for the generation of acetyl functions. So, you get the corresponding acetyl coenzyme function. So, acetyl coenzyme function, again will be dependent on your nickel center. So, how nickel is useful, or how nickel is so useful as a catalytic site that we will see, in our next class also.

Thank you.