Inorganic Chemistry of Life Principles & Properties
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Lecture - 44
Role of Molybdenum in life - Introductory aspects

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the previous few classes, we have looked at enzymes pertinent to zinc; you know that zinc does not undergo redox, but there are reactions which undergo redox. And a lot of reactions are hydrolytic type, like the peptide bond hydrolysis ester bond hydrolysis and addition of water to carbon dioxide, etcetera. So, variety of things, we have looked at. We have also looked at the group transfers, we have also look at the ligands properties where the 2 broken pieces are joined together. So, all of these were pertinent to the zinc.

Now, in this class, let us begin with the biological inorganic chemistry of molybdenum. So, when it comes to the word molybdenum, I am sure every one of us is reminded of one particular thing that is what, that is a nitrogen cycle and because nitrogen fixation is involved in this ok. So, this is the famous nitrogen cycle that you see, which we will study in a high school, you know general biology course basically we see at.

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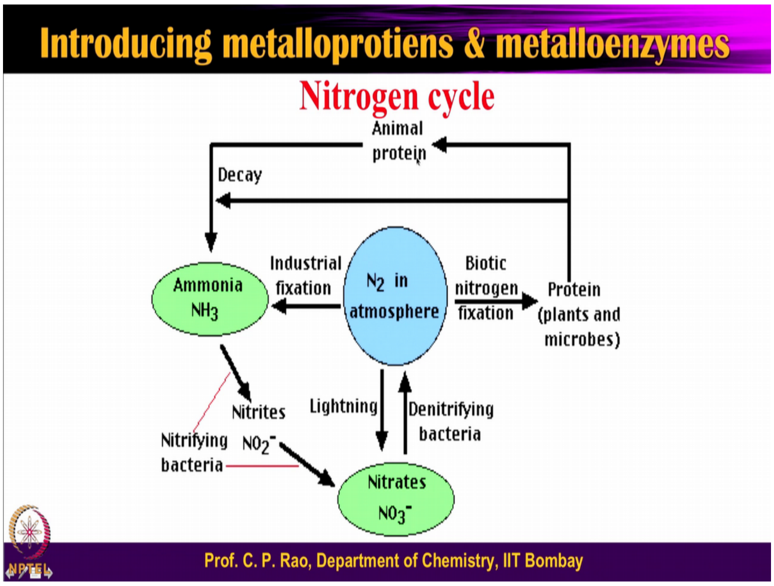

So, nitrogen present in the atmosphere on one side, you have the industrial way conversion to ammonia and ammonia converting to nitrates nitrites and to the nitrates and these are you have a lot of enzymes nitrifying bacteria; when we say bacteria is working its basically it is the enzymes of the bacteria that works. So, we need to as chemists, we need to look at the bacteria as an enzyme, a biologist will look at as a bacteria as a creature which does it.

Now, so, the nitrate ammonia to nitrate is what process oxidation. So, this is an bacteria, then NO 2 nitrite to nitrate is what process is again oxidation. So, you have an oxidative kind of a reactions on this side and from N 2 to ammonia what do you have; N 2 to ammonia is a reduction. So, you have a reducing type of an enzymes oxidative type of enzymes to in the biological system what you would see there is a nitrogen fixation, you call it as because nitrogen source is required for synthesizing protein, nucleic acids many other thing even modified carbohydrates, etcetera, etcetera lot of cases.

So, in other words, entire biological mantle requires a input of nitrogen which comes from the metabolizing the or by converting the nitrogen or fixing the nitrogen into the form of for example, ammonia. This part is not very important for us and this is. So, this particular thing is done by an enzyme called nitrogenase. So, that is what actually happens when we say molybdenum immediately what comes to our mind is nitrogen cycle and what comes to our mind is nitrogenase enzyme nitrogen fixing.

So, we will see very soon that the enzyme some molybdenum not only involved in the reduction of nitrogen to ammonia. They are also oxidation oxidized kind of enzymes are there too, we will come to all that as we keep moving across this molybdenum story ok.

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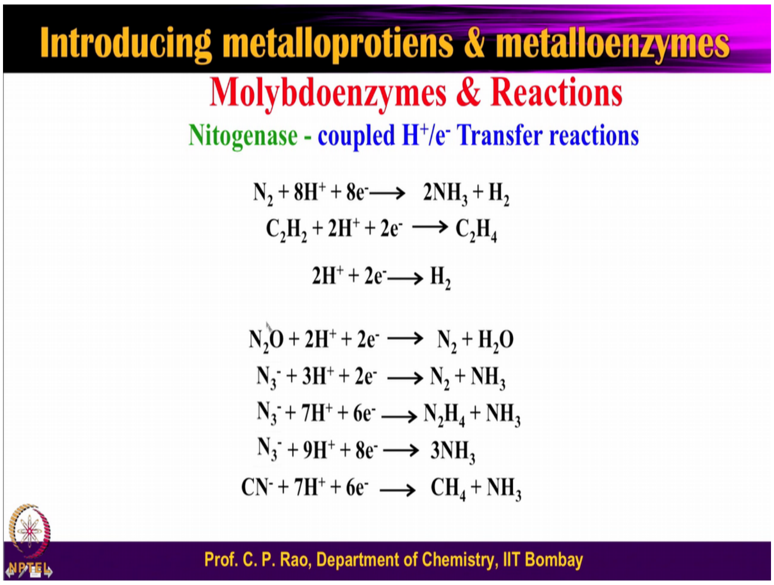

Let us look at the kind the enzyme that we were talking about the nitrogenase, nitrogenase based on the molybdenum, I have mentioned the term nitrogenase, earlier stage can anybody recollect; that was in the time when we were talking about the vanadium. And I mentioned that the some of the organisms acquire nitrogenase even in the absence of molybdenum, but for them vanadium is required.

Let that I have a promise that the molybdenum enzyme nitrogenase will be fully detailly studied and that is what we are going to take up now in this particular class and maybe this class perhaps, even the next class also. So, we will try to look at those things.

So, nitrogenase nitrogenase is primarily a reducing agent. So, for a reducing agent what do you require electrons ok. So, to remove some oxygen when you reduce, you do not need protons also. So, basically you require for a reduce reducing substrate you require electrons some cases you require both electrons and protons as well. So, basically it is a proton electron coupled transfer reactions are carried out by the enzyme nitrogenase ok.

If you give what all the nitrogenase enzyme, when you take in a test tube, what it can do in view; what it can do the first reaction. the N 2 plus 8 H plus plus 8 electron is 2 NH 3 plus H 2. This is the first reaction, this is the enzymatic reaction though the required, a you know equivalence is different from this.

Let me show you the required equilment equivalence or the actually what you need is N 2 plus H 2.

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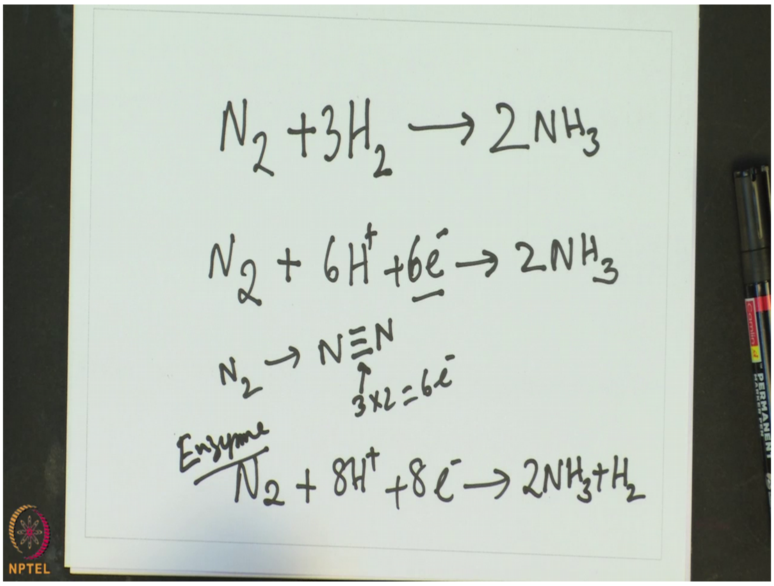

This is the 3 H 2, then this is nothing, but 2 NH 3 ok. So, generally the hydrogen is not supplied in the biological system. So, as far as a biological system is concerned; that is you require 6 H plus plus 6 electrons which is giving 2 NH 3 why do we need 6 electrons the NN N 2 has NN triple bond. So, the triple bond requires 3 into 2; 6 electrons.

So, therefore, if we put 6 electrons into the anti bonding orbitals on the nitrogen molecule, you start breaking the bonding between the nitrogen and nitrogen. So, if you put 2 electrons; 1 bond is reduced if you put 4 electrons; 2 bonds are reduced; if you put 3 electrons; 6 bond 6 electrons 3 bonds are reduced. So, that is how it is.

So, the enzyme does by electrons and protons does not give directly the hydrogen. So, as so, this is the reaction, but the reaction that we saw in the case of enzyme is N 2 plus 8 H plus plus 8 electrons giving rise to 2 NH 3 plus H 2 this is in the enzyme in the enzyme. So, this is basically because the initial protons are reduced to hydrogen gas in this case as you can see 2 H plus plus 2 electrons hydrogen gas and once the hydrogen gas leaves the active site, then the nitrogen gets activated.

So, therefore, if we add this reaction of the N 2 reaction of the hydrogen nitrogen plus hydrogen reaction; let us take as I have written already in the previous sheet.

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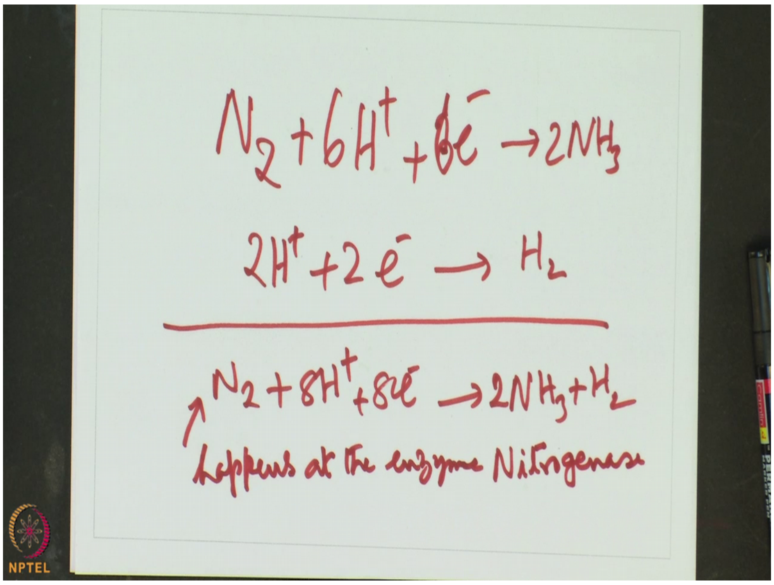

I will rewrite here plus 6 H plus plus 2 6 electrons giving 2 NH 3 and the enzyme also can do 2 H plus plus 2 electrons giving H 2; the total reaction is N 2 plus 8 H plus plus 8 electrons giving rise to 2 NH 3 plus H 2. So, this is happens at the enzyme happens at the enzyme nitrogenase.

So, this is what happened that is why you see the reaction as the N 2 plus 8 plus plus plus 8 electrons giving to H 3. The nitrogenase is also capable of reducing acetylene to ethylene acetylene is 2 C 2 H 2 ethylene C 2 H 4. Again, you require 2 protons plus 2 electrons N 2 O can be reduced to N 2 and water because this oxygen will be pulled out by 2 electrons as the O 2 minus plus 2 protons and that will become the H 2 O and azide can be reduced in different levels. At a lower level of reduction, it will give N 2 plus ammonia, a little more number of electrons 6 electrons, it will give hydrazine plus ammonia 8 electrons, it will give ammonia fully ok.

So, even azide ion. So, what is happening azide initially goes for nitrogen and ammonia and the nitrogen in turn goes to the hydrogen and the hydrogen in turn goes to the ammonia and that is what the total reaction is so; that means, this enzyme is capable of supplying 2 electrons, 4 electrons, 6 electrons, 8 electrons, all of these that you can see ok.

Let us look at another reaction that is the cyanide reduction cyanide reduction; there is a carbon based reduction nitrogen based reduction. So, one can easily expect chordal carbon will give carbon hydride which is methane nitrogen will give nitrogen hydride which is nothing, but the ammonia. So, nitrogen ok.

So, therefore, you get the carbon and is in the form of a methane nitrogen in the form of ammonia. So, you get this methane plus ammonia ok. So, what are the things that we can notice from this all the reactions have got electrons taking in; that means, they are all reduction; All the process has got protons and the proton plus electron can be 1 proton plus 1 electron is 1 H dot 2 protons plus 2 electrons is H 2.

So, therefore, you have a incipient hydrogen therefore, this hydrogen can react and therefore, you have a reduction reactions and other thing that you see all the products are the gaseous in nature mostly. So, therefore, you have a mostly gaseous kind of a reactions coming in to all of these

So, therefore, nitrogenase is an enzyme which is capable of providing electrons and of course, proton chain connects it. Therefore, it is a reducing nature enzyme where the proton electron coupled reactions take place ok, let us look at this is only the reduction.

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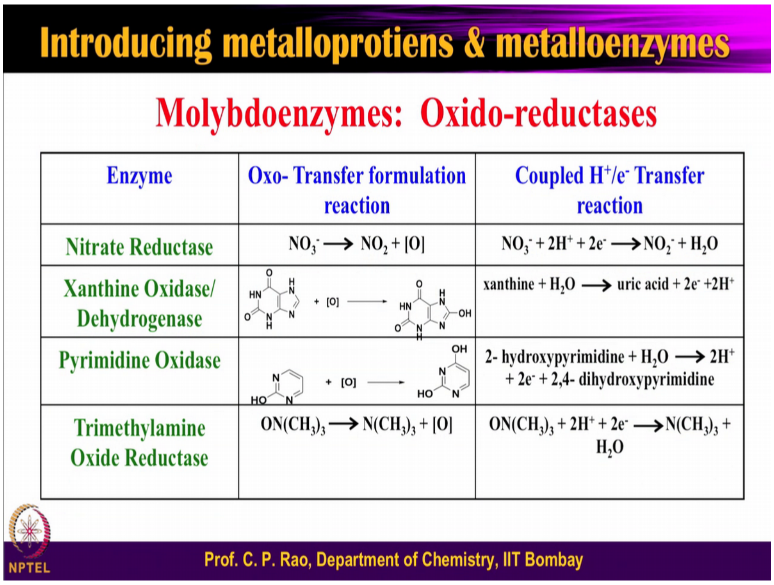

But in the beginning I mentioned that the reactions go even for oxidative direction ok.

So, what kind of things of the oxidative reductions, what kind of things or reductive kind of things both oxidative and reductive oxido reductases, you see that molybdenum enzymes, molybdo enzymes, oxido reductases, nitrate reductase going from nitrate to nitrite. In fact, you can take the reverse also nitrite to nitrate. So, this is this is one is in one direction, you call it as a nitrate reductase in the other direction, you can call it as a nitrite oxidase. So, it is the 2 forms of the same enzyme 2 oxidative states of the same enzyme.

So, in one enzyme in the reduced form the molybdenum will be in the molybdenum 4 state and the oxidized form the molybdenum will be in the molybdenum 6 state that is all, the difference enzyme is the same. So, you can have a nitrate reductase nitrite oxidase reverse. So, this reaction is NO 3 minus plus 2 H plus plus 2 electrons giving the NO 2 minus plus water. So, basically pulling out one of the oxygen to give water in the presence in the in the in the process you consume 2 electrons and 2 protons.

So, let us see a different one which is xanthine oxidase or oxidase can also be told as I mentioned earlier dehydrogenase. So, removing the hydrogen is the oxidation adding oxygen is also oxidation adding electrons is reduction removing electrons is oxidation. So, keeping all those things in mind you see that this is the moiety of the xanthine and oxidation giving rise to uric acid; So, this is the; so, what is the number of electrons protons 2 electrons plus 2 protons and water.

Pyrimidine oxidase; so, it is basically 2 hydroxy pyrimidine going to dihydroxy 2 4 dihydroxy pyrimidine. So, it is oxidation. So, the first reaction is kind of a reduction then this is an oxidation, this is an oxidation. So, if the enzyme is in reduced form, it will act like a reductase and the enzyme is in the oxidized form it will act like in oxidase.

But these are reactions are not done by the nitrogenase, I will be coming to that in a while; they are nitrate reductase xanthine oxidase pyrimidine oxidase, this is another example here shown trimethylamine oxide reductase. So, this is reductase, the first one and the last one are reductase the second one and third one are oxidase.

So, here trimethylamine N oxide giving raise to trimethylamine plus O again you require, this O will go as water ah; that means, you require 2 protons and 2 electrons. So, that is where we have in the trimethylamine. So, you saw 2 reactions are they are being reduced 2 reactions are being oxidized, these are basically oxido reductases as I said these are not carried out by nitrogenase.

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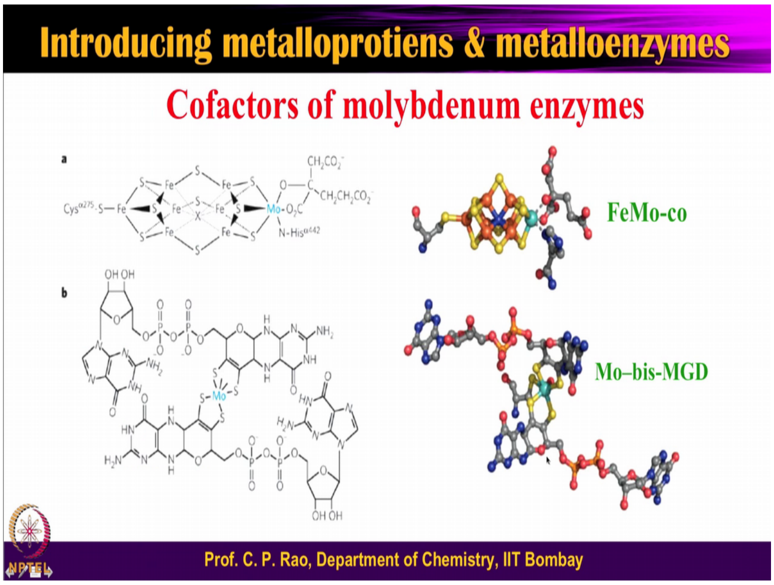

So, what are the carried out by just before coming to that in the nitrogenase actual species is called cofactor; where the reaction occurs, where the nitrogen becomes ammonia. This particular cofactor have you come with the more details later on in the kind of a iron sulfur, there is a kind of a iron molybdenum sulfur coupled together or joined together or bridge together by these sulfides and at the molybdenum. You have certain species bound and this is called iron molybdenum cofactor, I will explain you fully in a detailed fashion after a few slides from now ok.

So, this is a cofactor which functions in the nitrogenase. Now, look at another enzyme which we were talking about oxido reductase enzyme, this enzyme has got a molybdenum here and this are the 2 thiols and these are the 2 thiols and this is the eine bond eine thiol moiety.

Now, little bit examine, this whole moiety here there is one molecule here there is another molecule exactly inverse in structural relation; this part is here this part is here. So, let us look at this part is basically called as a terine. So, this is the terine moiety which is involved in the redox kind of a process which is involved in the redox systems this is I will write down here so that you will P P silent terine.

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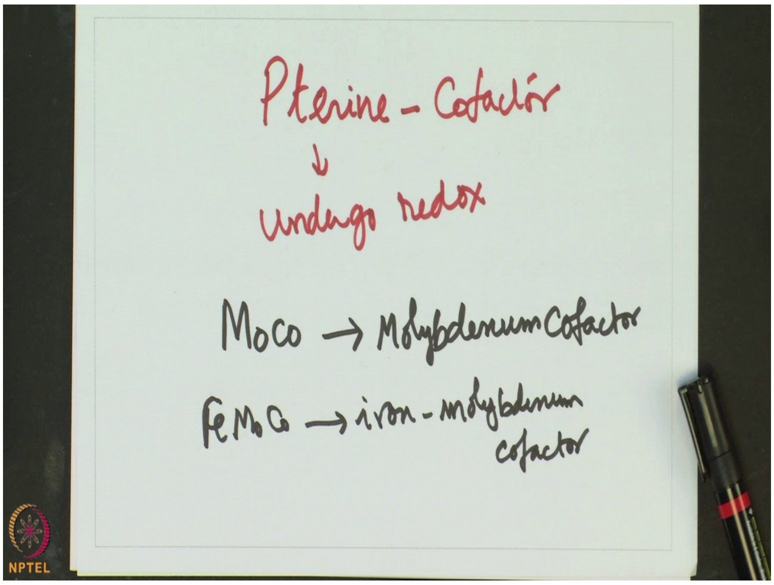

So, this is a cofactor and this can undergo redox this can undergo redox. So, this is a redox type of a cofactor. So, these are called terines.

So, you can see, so, there is one terine moiety connected to 2 phosphate the bridge phosphate with a sugar and with a nucleic base ok. So, this whole part is nucleotide this whole part is terine terine base nucleotide nucleotide base terine, this will add as a electron transfer system.

So, it is a basically organic electron transfer then further you can have the molybdenum 2 and same thing is 2. So, it is a 1 is to 2 complex where the molybdenum center is bound by the enine thiols conform both sides.

So, this is again an enzyme for the oxido reductases not for nitrooxidase; this is for the nitrooxidase, this is for oxido reductases.

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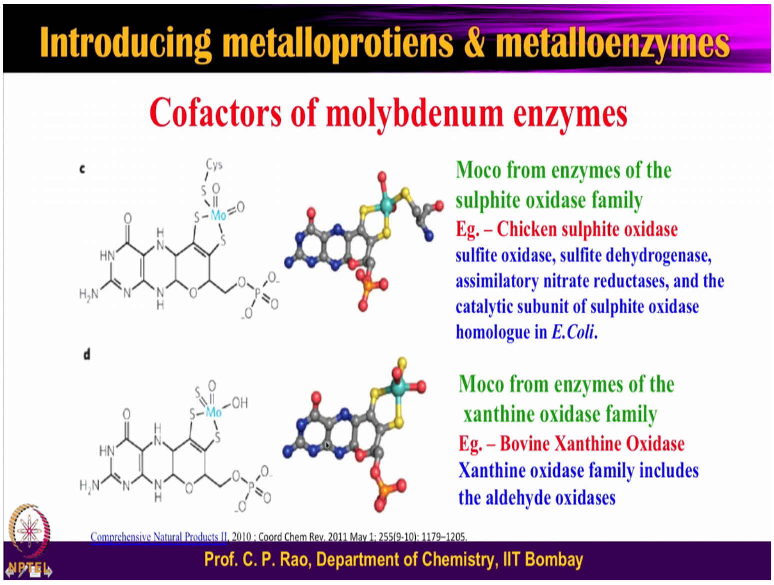

And also some more oxido reductases are there where you have only one terrine only one terrine. So, one molybdate center with one terrine one molybdate center with one terine and this terine is modified by phosphate terine is modified by a phosphate both are same except for this at the molybdenum center, you have S cysteine here and you have double bond S double bond O and OH. So, here you have dioxygen.

So, this is a basically a dioxo center generally to keep in mind whenever you have a dioxo double bond oxo center, this is molybdenum 6 when you have one single molybdenums oxo then it is 4 suppose one double bond oxo one double bond sulfur again 6. So, molybdenum has 2 favorite oxidation states this is a structural form of the same as you can see that these are called molybdenum cofactors. So, molybdenum cofactor a small in the short form is written as moco moco. So, that is moco is molybdenum cofactor.

So, these are moco is molybdenum cofactor and in the earlier case femoco iron molybdenum cofactor iron molybdenum cofactor. So, I hope you understand this. So, there are quite simple to keep in mind and now this one example this particular thing is a cofactor which is a part of the sulfite oxidase sulfite oxidase family. We will come to this later in explaining their mechanistic aspects some more examples example chicken sulphite oxidase, sulfite oxidase, sulfide dehydrogenase, dehydrogenase and oxidase as I said they are similar assimilatory nitrate reductase and that is what is one of the nitrogen cycle reaction and the catalytic subunit of the sulfite oxidase in the homologue in E coli. So, this is present this cofactor is present in all of these systems all of these examples ok.

Now, let us come to the other part of it there is one more enzyme where the molybdenum center just shifts by instead of molybdenum double bond as you have molybdenum S cysteine or in other words, molybdenum S cysteine is replaced by one molybdenum double bond sulfur and that is the difference between the C structure and d structure and this is again a molybdenum cofactor which is a part of another family the family called xanthine oxidase family. So, bovine xanthine oxidase bovine, you know that cow. So, xanthine oxidase; xanthine oxidase family includes the aldehyde oxidases too.

So, we will come to all these examples in this particular CO, in this particular topic, maybe in next one to half an hours of the classes that when we take. So, now, what we have seen in particular. So, we have looked at the two aspects, one is the nitrogenase nitrogenase is a reductive kind of an enzyme and then we have looked at some oxido reductases other than these nitrogenase kind of thing and these have got the nitrogenase has got iron molybdenum cofactor.

And whereas, the molybdenum enzyme 3 oxido reductases have got the carine based cofactor as you can see over there and this particular case is a 1 is to 2 and the ones here sulfite oxidase and xanthene oxidase family have got 1 is to 1 with a slight difference at the molybdenum center in one case molybdenum with a bonded to cysteine in the other case, molybdenum bonded to sulfur with a double bond. So, these are the kinds of things.

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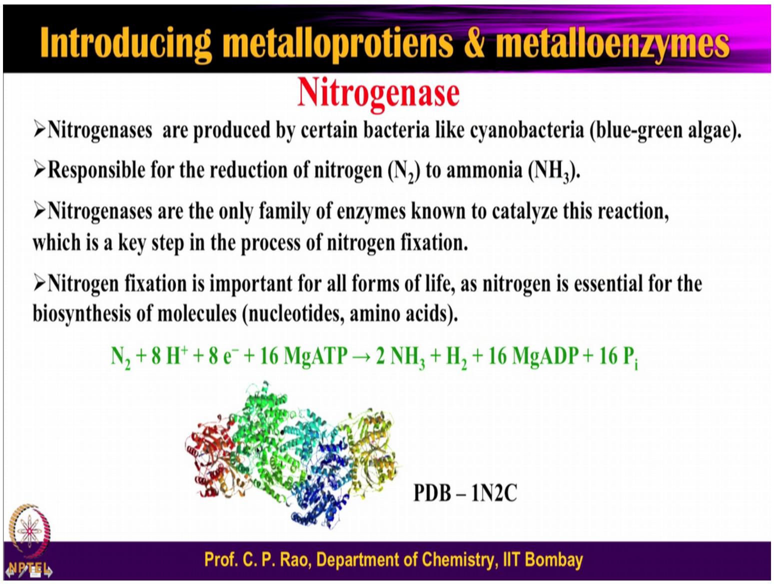

So, essentially we have seen two categories of enzymes nitrogenase enzyme and the oxido reductase enzymes.

First, let us start with the enzyme nitrogenase. So, enzyme nitrogenase is basically, this is produced in a variety of bacteria for example, cyanobacteria blue green only and also this is present in a large number of plants because there is those bacteria is present in their roots of those bacteria those plants and there, again nitrogen is converted or nitrogen fixed.

So, this particular enzyme is responsible as I have been telling on the time reduction of nitrogen or dinitrogen to ammonia. So, this is the reducing type of an enzyme. So, this is the only kind of a category of enzymes nitrogenase are the only category of enzymes which are known to catalyze the nitrogen to ammonia reaction and there is no other enzyme known till now which will fix the nitrogen.

So, to fix the nitrogen, it is only the nitrogenase, either the nitrogenase can be either a molybdenum based on the nitrogenase can be by based on the vanadium vanadium is less prevalent molybdenum is completely present everywhere. So, most common thing is nitrogenase with molybdenum.

So, therefore, we study more details of nitrogenase as we keep going, we will understand the complexities of this enzyme, please keep your focus on these nitrogen fixation is an important form of all life as nitrogen is an essential component for the biosynthesis of molecules like nucleotides amino acids modified molecules, etcetera, etcetera.

So, therefore, the the nitrogenase is only the enzyme which reduces the nitrogen to ammonia and this is a very important enzyme because this is the one which gives the usable form of nitrogen species by the plants to convert into amino acids, etcetera.

So, the total reaction is as I mentioned earlier, I had written much better way here much more extended way I do not mean the better way extended way that is I have what I have written earlier was N 2 plus 8 electrons plus 8 protons giving rise to 2 ammonia molecules and it does not happen in the enzyme as it is earlier I said that this reaction goes from N 2 plus 8 H plus plus 8 electrons giving2 NH 3 plus H 2; this is what I said enzymatic reaction

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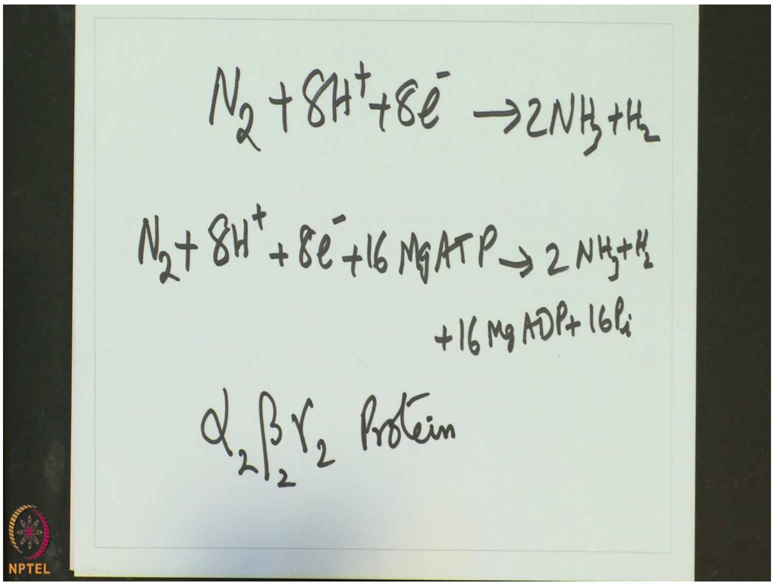

Now, I am saying this is not the only the enzyme reaction; this is a part of the enzyme reaction, but require something more what is that something more to do this reaction electron transfer should take place in order to do the electron transfer to take place it requires the energy, therefore, the reaction is using the ATP which consumes ATP plus 16 Mg ATP. So, gives rise to2 NH 3 plus H 2 plus 6 Mg ADP plus 6 P i.

So, what does this tell this tells this reaction is not a free flowing reaction, it is not a spontaneous reaction, it requires a lot of energy to be involved it requires a large number of electrons to be involved it requires a large number of protons to be involved.

So, to bring proton to bring electron to hydrolyze being issue an ATP so; that means, enzyme requires a huge number of different components that is why this enzyme is a complex enzyme. As you can see I will explain the later slides more this is one color, another color, another color, another color, another color etcetera; you had see so many different colors to show different kind of a subunits.

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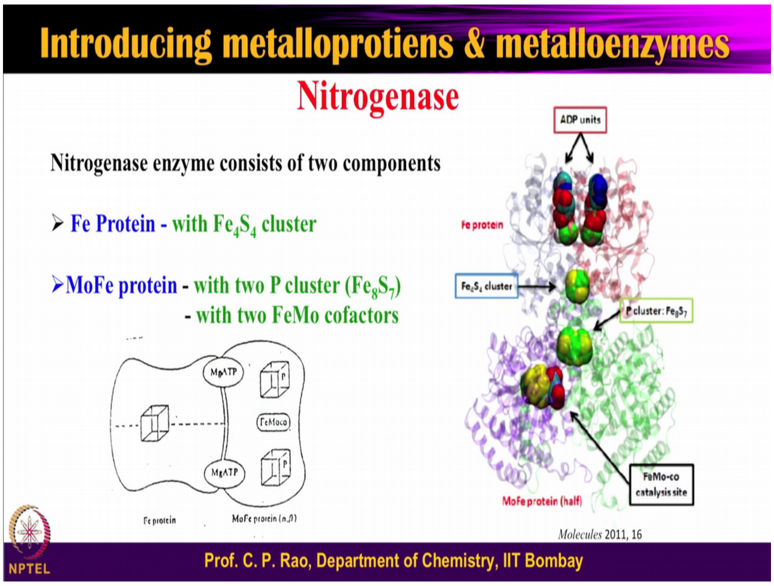

So, the nitrogenase nitrogenase enzyme basically at the first stage let us learn that it has two components, one of the component is called the iron protein other component is called molybdenum iron protein. So, there are two systems. Now, how you focus on this particular figure this smaller portion of this; where I am drawing this is the basically the iron protein and this huge one both the violet and the green one together is the molybdenum iron protein

So, this entire complex protein having 6 kinds of a subunits 1, 2, 3 and back side 4, 5 and 6; totally 6 subunits. These 6 subunits thing is basically nothing, but it is known as alpha 2 beta 2 gamma 2 protein. So, you will understand more when I keep moving across this. So, at the right now, you take it is understanding the 2 portions; 1 portion is called the iron protein portion; other big portion is called the iron molybdenum protein, iron, molybdenum, protein has got 4 subunits iron protein has got 2 subunits, I will show you that also.

Now, the in the iron protein you have two things one is the iron sulfur cluster and the magnesium binding site for ATP for the at ADP initially ATP when it is hydrolyzed it is ADP and as you can see that this is the iron protein part, this is the iron molybdenum protein part. So, or molybdenum iron protein part this is the iron sulfur cluster and this is where magnesium ATP complex and this is the molybdenum iron protein ok.

So, as you can say in the molybdenum iron protein there is the Fe moco; this is the molybdenum iron protein has got Fe moco, it is called iron molybdenum a cofactor Fe moco and these are the P clusters; I will explain. So, this is a basically a P cluster and this is a molybdenum cluster the molybdenum iron molybdenum cluster iron molybdenum cofactor cluster, this is now we understand 2 parts; 1 is this one, one is this one ok.

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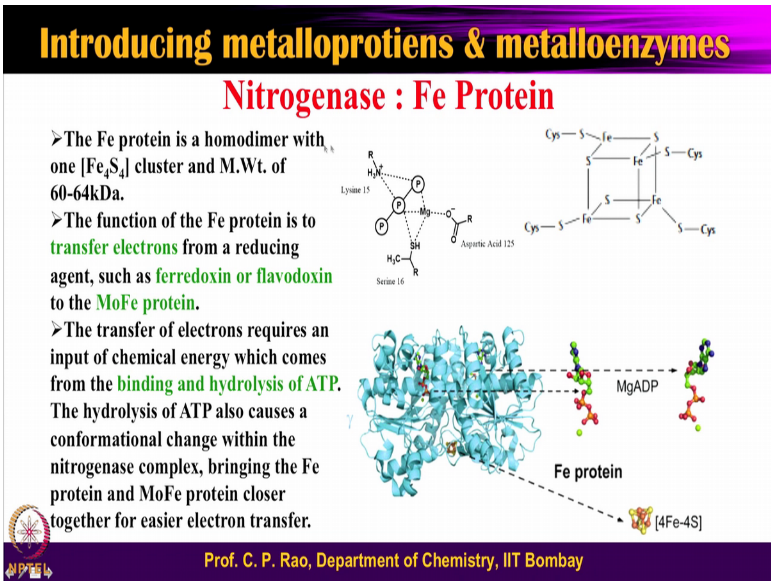

Now, as I said the first part is iron protein iron protein has got the iron sulfur cluster and the magnesium phosphate ATP bound structure it can also bind to magnesium ADP as well and this is a dimer which is called gamma 2 dimer. So, the function of this particular protein is what it is a iron sulfur protein? So, it will be involved in the electron transfer reaction.

And then the reducing agent and it will pick up from ferredoxin or flavodoxin, etcetera. So, it will pick up from there and give it to this protein. So, it will transfer the electrons required by taking from outside by using energy the energy is ATP hydrolysis ok.

So, therefore, the important components of the iron protein is the iron sulfur cluster and the magnesium binding ATP ADP side and this particular thing this is the top portion.

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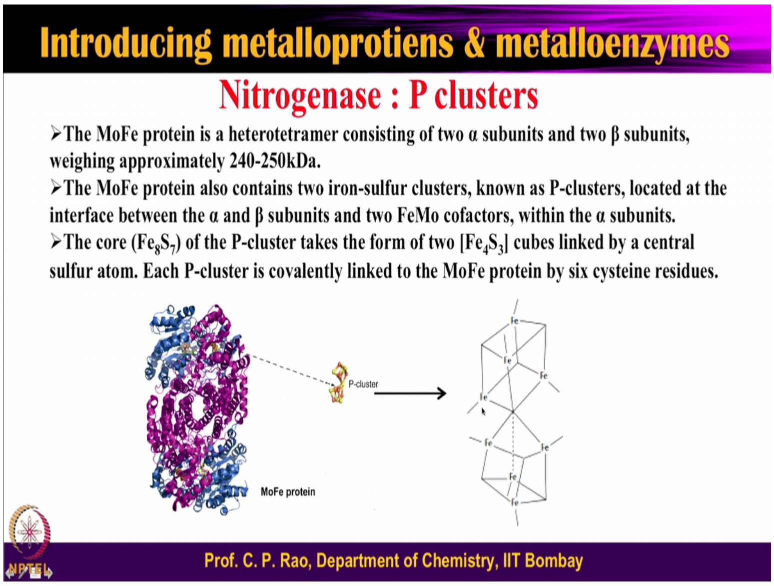

In the previous one, sorry in this previous one, this is this top portion and that is what we are looking at. So, we will continue with this particular protein nitrogenase in the next class.

Thank you very much.