Inorganic Chemistry of Life Principles & Properties

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Lecture – 22
Role of vanadium in life – Haloperoxidases

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the previous class we have started looking at the Role of vanadium in biological systems, we have try to look at the among the various oxidation states of plus and minus of the vanadium that is possible in chemical species, the most you know prominent and prevalent oxidation states in the biological context or plus 5 plus 4 and plus 3; we have looked for the reasons for these ones because the potential reduction potential from plus 5 to plus 4 is quite large positive and the plus 4 to plus 3 is smaller, but positive and beyond that when you go to the plus 3 to plus 2 becomes a negative potential and the vanadium 2 plus is strongly reducing agent.

So, therefore, vanadium 2 plus is being not identified in the biological systems at all. So, that is what we have noticed. We have also try to understood the vanadium presence in the form of a vanadates multiple kinds of vanadates mono, di, tri, tetra etcetera, which is dependent on both the concentration as well as the p H because the p H will change the protonation, deprotonation states of these species. Then we have looked at the vanadium 51 NMR, how that is relevant to the coordination sphere and the basically dependent on the summation of the electron negatives of the a primary coordination species to the metal center and its very nice that when such a such a vanadium 5 is present in the proteins or biological enzymes, one can find the coordination sphere quite nicely by using that.

On the other hand if you have a vanadium in the form of plus 4, you can use EPR as the useful tool because the vanadium 4 plus is d 1 system. And so in fact, also shown in the in the previous class that the vanadium 5 that is entered into the biological cells can get reduced to 4 and in some cases even 3 therefore, in biology would see as a plus 5, plus 4 and plus 3. And you have seen the special case of vanadate which is acting as a inhibitor for the the ATPase. You know the ATPase goes through the 2 types of a 2 parts of the cycles, the first cycle where the sodium binding followed by a releases this then the

second cycle potassium binding followed by the release of this and the first one is driven by the phosphorylation and second part is driven by the dephosphorylation, and the second stage where the dephosphorylation takes place if the vanadate is present vanadate binds to these protein much more strongly than that of the phosphate and it is irreversible hence the vanadate acts as a inhibitor for the ATPase cycle. Now let us in this class, let us look at the a positive role of the vanadium that is the vanadium enzyme.

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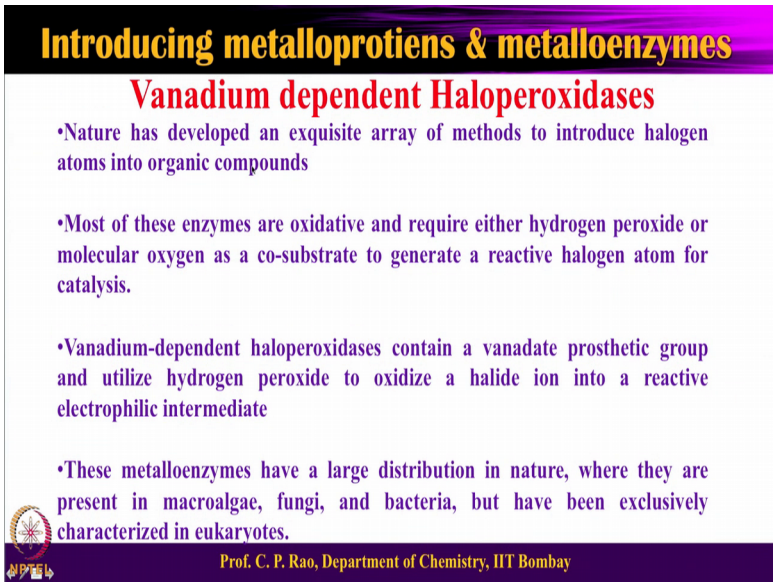

So, one famous example is the haloperoxidases so vanadium dependent haloperoxidases. So, haloperoxidases are quite popularly known particularly from the their presence in a sea algae, where a lot of organic moieties in the ocean or converted to the halogenated ones. This could be a chloro, this could be a bromo, this could be a ido. So, therefore, chloro peroxidase, bromo peroxidase and a ido peroxidase. So, these are general class of the haloperoxidases and these haloperoxidases work in the presence of the halogen of course, is required and what is required in the hydrogen peroxide.

So, so one can read this particular slide to get the most background information about this, let us look at one example of this vanadium bromo peroxidase we will come to the reaction etcetera bit later.

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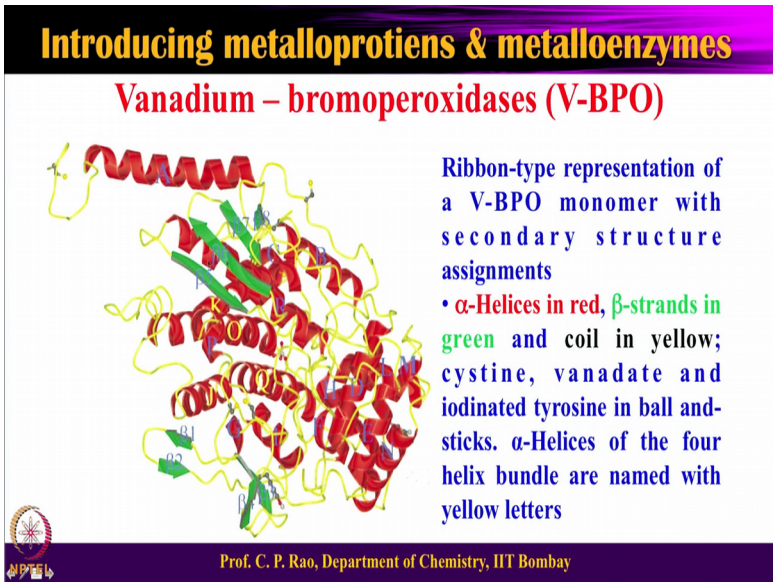

And you see a huge protein of course, this is a one monomer or the entire structure and you can see the structure is having the alpha helices, which are mostly in the red in color and the beta sheets, which are in the you know a green in color and then you have the random coil which is which are in the yellow color.

So, this whole thing together you have a 3 dimensional structure and such a 3 dimensional structure would basically you know is responsible for the activity of the enzyme. Now how is the vanadium is placed;

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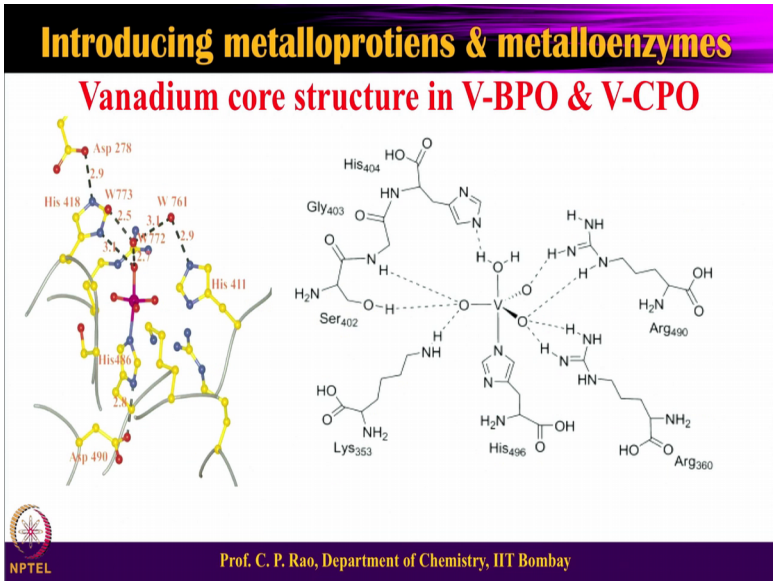

So, let us look at to understand this, let us look at the this slide and in this slide there are 2 structures one on the left side, one on the right side ok. So, the left side one is taken from the vanadium bromo peroxidase; V dash BPO means vanadium bromo peroxidase V dash CPO means vanadium chloro peroxidase.

So, bromo peroxidase and chloro peroxidase that you have and this is the enzyme structure not the entire enzyme structure is shown only the structure very close to the vanadium part. So, how is the vanadium is placed? Vanadium is here in the centre and the 4 oxo groups are there. So, which is basically vanadate is suspended in the protein, where the only one direct covalent connection between the vanadium and the protein is this particular thing which is a histidine.

So, vanadium 2 yet on the histidine is only one single connection that you have between the vanadate and the enzyme and as the oxygens present on the vanadate or indeed involved in the in the hydrogen bonding, and this is from boro bromo peroxidase and the complete hydrogen bonding scheme is not shown here.

However if you look at the another case chloro peroxidase, we can just see only the vanadate you see that 1 2 3 and 4 kind of a oxygens being present here and these are connected by the a lot of hydrogen bonding situation. So, it is looks like as if a vanadate species is suspended into a protein and wherein it is attached through a one covalent connectivity with the nitrogen or the if it is one of the histidine moiety.

So, you can see that all these arginines this arginine and so other serined and of course, the histidine this histidine is a covalent bond. So, this is how roughly the a active center or the vanadium looks like in haloperoxidases. And as you see that this whole species is stabilized by all this therefore, these you called as a secondary interactions and these hydrogen bonds with the side chains in the protein is called basically the secondary interactions of whereas, this is the primary coordination sphere.

I think this much of information about, how the vanadium center is present is more than the sufficient enough to understand of course, the rest of the protein is always there which is not shown over here ok.

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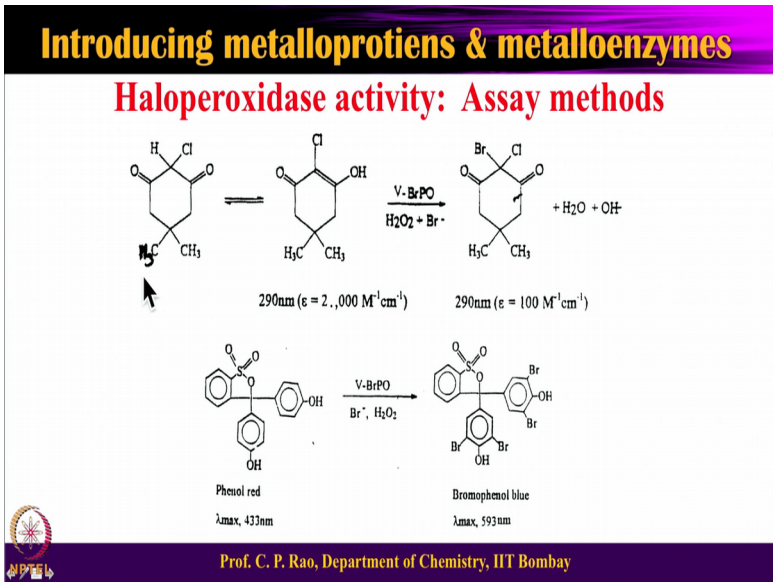

And let us see what kind of a reactivity and how do one measure such a kind of a reactivity. Haloperoxidase activity is basically it does halogenation of the substrate. We have given here case for how to as say in the such a kind of a the halogenation. So, this is a substrate we have and the substrate has 2 forms which are shown and this has an absorption spectrum around 290 nanometers, 20,000 mole inverse this is 20,000 mole inverse E is the epsilon value.

Now, this when you add the vanadium bromoperoxidase V B r PO in presence of the H 2 O 2 and bromide is the of course, inorganic bromide like sodium bromide, ammonium tetra alkali ammonium bromide any of these things can be taken, and that bromo will may added here and then what will happen is, the absorption effects will not shift, but the absorption epsilon that is the extent to which it absorbs is diminished its going from 20,000 to 100. So, a lot decrease in this ones you can also use other kinds of trans substrates like a phenol red you have and the phenol red will become the phenol blue here, this goes from 433 nanometers to 593 nanometer which is almost 163 nanometer shift.

So, you can find either by the intensity variation or by the epsilon maximum position, the rather the enzyme activity and this is what is referred to as a Assay method. So, you want to identify the enzyme activity always you require a assay method and this is one of the

assay methods are actually 2 different assay methods for the haloperoxidase system that you have ok.

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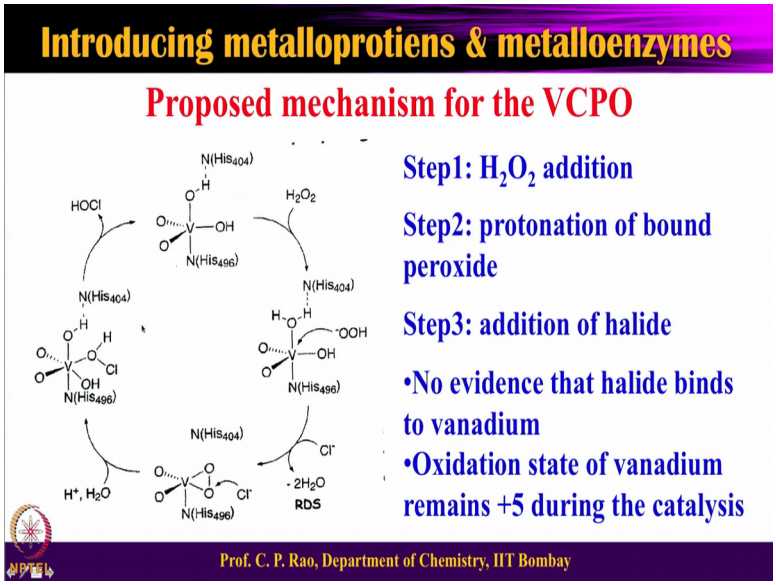

On the next slide I have tried to put all the possible steps that one can visualize, when the haloperoxidase act on the substrate. If you recall that one another thing I have forgotten to tell you is in all these things the vanadium is in vanadium 5 oxidation state ok.

So, therefore, in the enzyme when we talk looked at here, this particular part of the enzyme this is basically vanadium is in the vanadium 5 ok. Now let us look at the mechanistic aspects of this particular enzyme a chloroperoxidase, bromoperoxidase it does not matter, one of these we can a general mechanism or a generic mechanism for this that we can understand. If you recall this enzyme functions 1 you need H 2 O 2 other you need halide. The 2 components which are required in addition of course to the substrate and in addition of course to the enzyme.

So, the enzyme does catalytically is no question about it. So, this can be grossly looked at some kind of a steps. So, we can say the step 1 is the one where the H 2 O 2 is being added and then H 2 O 2 is added; obviously, we will add a protonation as well as a attack and the metal center. So, thereby form some kind of a peroxo kind of a component and the such a peroxide compound in the third step will activate the halide, to form a reactive halide species and such a reactive halide species very quickly dissociates by adding the halo ion halide species to the substrate and returns back to the normal part of it.

So, far the death evidence has not be seen for the any halide binding to the vanadium direct binding of this and still the hypo halide kind of a species is still being visualized. And the second aspect is the during the catalysis there is no change in the oxidation state, the oxidation state remains to be a plus 5; so therefore, these 2 are absolutely important aspects to keep in mind while building the mechanistic aspects. So, as you can see from here that this is the vanadate species, which is bound to the protein through this particular histidine and this is activated by a different histidine etcetera and this particular center of the enzyme is attacked by the hydrogen peroxide and that will add a protonation to this.

So, this will protonate this one and O HO 2 minus is; obviously, a kind of a nucleophile, which will act at the metal center ok. So, that will basically give a species of this kind of a peroxide species. Now at this stage I should take a little bit of deviation and explaining some things that I have noted. In fact, in the literature the small molecular components that people have prepared, they have looked at the addition of the for the vanadium 5 compounds they are looked at the addition of the hydrogen peroxide and peroxide bound species were isolated.

So, therefore, there is sufficient amount of proof of the evidence or support for the for the bonding or this peroxo species to the vanadium center. So, such kind of complexes are very well known in the literature from the coordination chemistry point of view. So, therefore, such a binding is very well visualized. Now at this stage of the thing the halide activation takes place. So, halide activation goes with natak on this peroxo species, sort of sort of break this peroxo species to form a species of the hypo chloric acid if it is a bromine case hypobromic acid and a hypo iodic acid.

So, hypo halide kind of an intermediate and suddenly this intermediate is not a very stable one and very reactive. So, at this stage, it will really stabilize the coordination in this the water as a coordination and then that will be setting with the histidine. So, in other words this particular species, when you have a substrate here and substrate gets halogenated. So, the product will be halogenated product and brings back this; since therefore, substrate is not shown over here, so therefore, it is shown as if it is a HOCl that is coming into this ok.

So,. So, the main mechanism is that you have the vanadium 5, vanadate base species the H 2 O 2 reacting to this and in protonation and H go to minus nucleophilic attack and a kind of a peroxo spaces for which there is a lot of evidence from the small molecular chemistry peroxo bridging peroxo binding and this is being further activated by the halides species to result in the hypo halogen as it and if you have a substrate at this stage, the substrate will get halogenated and the substrate is not there you can expect as a HOCl being liberated ok and this is how one can try to understand the mechanism of the peroxidase. So, let us look at one another evidence, that this is indeed is the reaction center.

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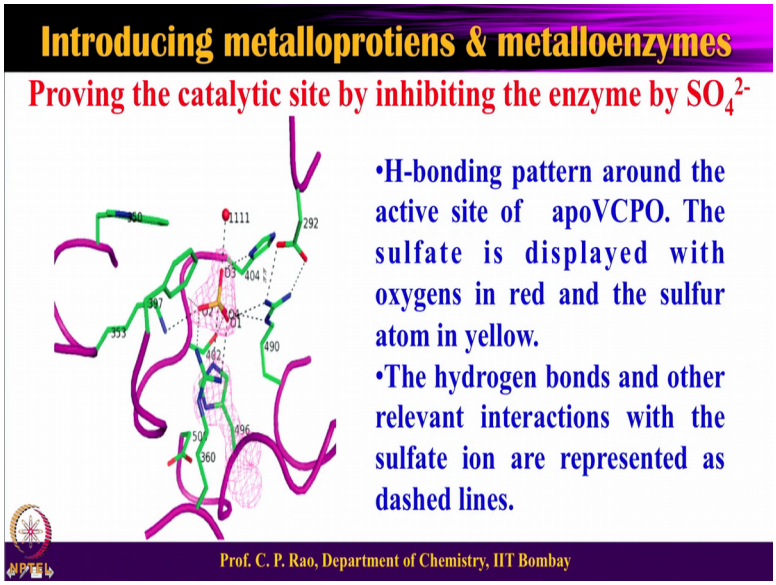

How do we know it? This is the actually the reaction site or active site. So, in that case what one needs to do? Now remove that vanadate part how will you remove? It is very simple, you can remove the whole vanadate. So, you reap remove then once when you remove this, what is the kind of a protein that you get you pour the protein you get when you remove the vanadate is called the apoprotein.

So, therefore, apo peroxidase you will get. So, now, there is no vanadate species to this you can add sulfate. In fact, sulfate ion S O 4 minus H B O 4 2 minus it is very similar and they go and fit into the almost the same region, where the vanadate was there and the sulfate goes. Now one of the sulfate goes as you can see here, the sulfate gets adjusted to

the coordination or to through the interactions with the protein and now no more reaction is taking place.

So, this is exactly the vanadate species, which was necessary for the reactivity. So, this will reconfirm that the vanadate center is indeed responsible for the reaction on this particular enzyme ok. So, let us look at overall, what we have what we have done in case of the vanadium story is, we have talked about the vanadium oxidation states, we have talked about the oxidation states, which are which are important and prevalent in the biological systems like plus 5, plus 4, plus 3 and then kinds of vanadium species that we have been able to see and we talked about the tools like vanadium 51 NMR is suitable and useful for vanadium 5 studies and whereas, the EPR is very highly suitable and useful for a vanadium 4 kind of a spices.

We also shown that the vanadium 5 is not very stable when it enters inside the cell, they get further reduced to vanadium 4 and probably vanadium 3 in some cases 2 for which certain specific cells, vanadobins and those kind of things were already vanadochrome have already been explained and shown. Then we have shown that the vanadium vanadate species are dependent on the concentration of the p H, then we have also shown that the vanadate acts like a phosphate and if it is present in the in the ATPase cycle during the phosphate removal, which is called dephosphorylation. At that stage if the vanadate is present vanadate will bind to the protein irreversibly and then stops the cycle of the ATPase that is a negative effect of the vanadium.

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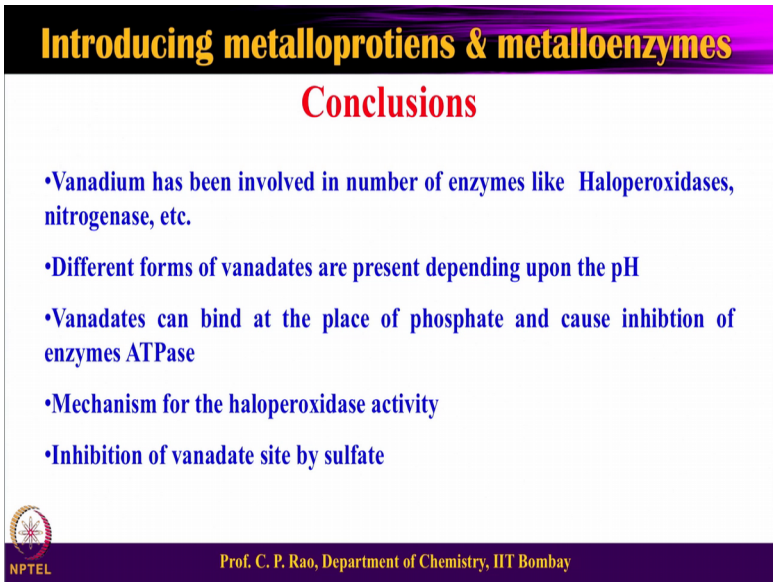

Positive effect of the vanadium we have looked at through the haloperoxidases; haloperoxidases are really a boon because they are the one, which are present in a algae in see algae, and they are the responsible in making the halogenated, brominated, chlorinated, idonated products of the organic, which are very precious, very expensive and which are very difficult to synthesize otherwise therefore. And then for such a kind of an enzyme we have looked, at in such an enzyme the vanadate species is suspended in the protein through one covalent link with one of the histidine, and as a large number of hydrogen bonds through the oxo species or a hydroxyl species present on the vanadium center.

And all this acts in presence of the H 2 O 2 and H 2 O 2 reacts at this particular center forms a peroxo species, which in turn is activated by the chloride and to form the hypo halous acid and that is the hypohalous acid, which is responsible for binding to the substrate and that is being shown and so, if the substrate is there it will bind to that and we have given the methods by which one can even you know as say the halo peroxidase activity of all this.

So, we also shown that this particular thing is absolutely true and correct the center the vanadate is the one which is responsible for a haloperoxidase reactivity, because we could show by removing this and putting the sulfate the enzyme is inhibited.

So, therefore, these are the aspects of one inhibition of vanadate, one enzyme and general features have been taught in this particular course I think that information is more or less sufficient enough in this for vanadium story and for the vanadium 3 instead of vanadium 3 I will take the molybdenum 3 for nitrogenous. So, in the in the story of nitros molybdenum, I will explain the nitrogenous. So, I hope you understand what I am talking about the how these metal ions form or metal ion species form, activate the enzymes and then the reactions too.

So, I think we are now geared to move to the next metal ion and the next metal ion that I would like to take in the biological systems of the bioinorganic systems is the manganese.

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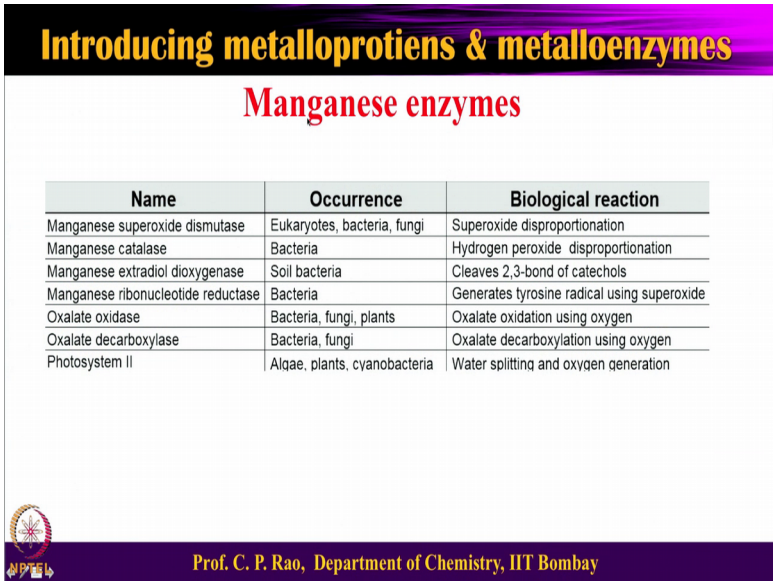

As you can see that also comes later, to the vanadium ok. Vanadium and then manganese the manganese also you know very well kind of a oxidation states, we know we have seen most of the oxidation state plus 2 and plus 7. Of course, some other chemically available oxidation states are also there too.

So, you can get large number of variety of oxidation states; however, not all of them are seen as you will see vanadium manganese 2, manganese 3 and manganese 4 or more or less seen most of the time 2 and 3 and 3 and 4 very rarely the 5 may be associated, but never 7 or never lower than 2, because lower than 2 or 2 reducing more than 5 is a highly

oxidative and you know biological systems never except take up the extreme kind of a species, if it takes up it will control it; then that is what we need to keep in mind.

Now, for a while let us look at this particular table to give a glimpse about the manganese enzymes and let me tell you manganese enzymes or divers. There are manganese enzymes where one manganese is present, there are manganese enzymes where 2 manganese ions are present, there are manganese enzymes where 4 manganese ions are present too and let us take some popular examples among this manganese superoxide dismutase I will come to this in while, manganese catalyst, manganese extradiol dioxygenase, manganese ribonucleotide reductase, manganese containing oxalate oxidase, oxalate decarboxylase photosystem in this name.

So, what is the manganese superoxide does? So, so superoxide does it is it breaks down the superoxide radicals into O 2 and H 2 O 2. So, O 2 your harmless H 2 O 2 is less harmful, then of then this O 2 minus. So, therefore, such H 2 O 2 is taken up by another enzyme called catalase and there are catalysis even on manganese and of course, on the iron too so, but I will be explain in the manganese case.

So, therefore, I may not explain this class of enzyme in the iron so much. So, manganese catalyst and this will convert the hydrogen peroxide to oxygen and water. So, overall the reactive species of oxygen, which is the O 2 minus which is superoxide radicals and peroxides these are disproportionated and dismuted to be less harmful oxygen and water kind of a species, occurrence is given you can always go through that. So, this the manganese superoxide dismutase is the case where one manganese is present, manganese catalyst is the case where the 2 oxygen, the 2 manganese are present I will explain that.

So, manganese extradiol dioxygenase and we will not be looking into this details of this enzyme. Manganese ribonucleotide reductase will not be looking into the details of this enzyme, but we look at their reactivity, the reason is that the details of this enzyme I will explain you and we come to the story of iron, iron is also involved in ribonucleotide reductase. But however, I will tell the reaction or function that is happening in this case in this story itself, then the oxylate oxidase will not going to take up oxylate decarboxylate now do not have.

Photosystem 2 where the 4 manganese are present and the water is oxidized to oxygen O 2. So, this is present in plant, this is present in bacteria, algae etecetera. So, in this case

we will study superoxide dismutase enzyme, catalysis manganese catalase enzyme catalysis, photosystem enzyme catalysis. These are the 3 cases that I am going to explain you under this manner of the manganese biological inorganic chemistry.

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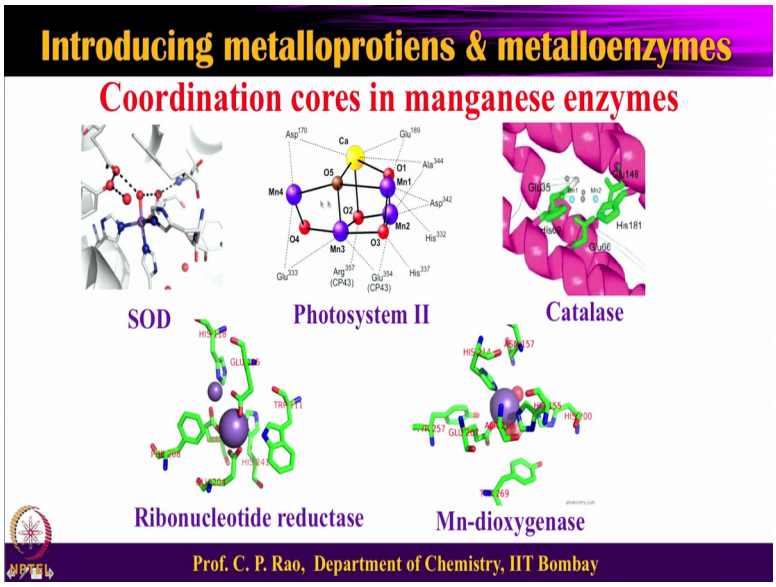

Before we go into the enzymes, I have put a slightly different mode of approach now, here I have shown 1 2 3 4 5 different kinds of enzymes, I have not shown the enzyme, but I have shown the metal ion or a manganese center.

So, you see the manganese center in the SOD there is only a one manganese here, this is bound by 3 histidines etcetera and this whole thing is protein, and this is photosystem there are 4 manganese the purple ones are 1 2 3 and 4 and there is a one calcium this is called a cluster, tetra manganese calcium cluster. Of course, more details will be explained later in this class itself, in this particular under the story of the manganese itself. Then look at the catalase enzyme, which disproportionates the hydrogen peroxide to water and O 2, and this has 2 manganese centers as you can see here, there is one little manganese over there another little manganese here, this has a core and this has a core, I will explain this too and mechanism too.

So, all the 3 enzymes I am going to explain their mechanistic aspects. Then if a one other enzyme which is ribonucleotide reductase, I will give just the function, but I will not be giving the reaction the mechanism of this because I will explain under the iron story. Manganese dioxigenase; I will explain this under the copper case and the iron case

dioxigenase copper dioxigenase. So, we will give away with this. So, out of these 5 reasonably well known enzymes, these 3 of the enzymes I will be I will be handling in under this story of the manganese.

Let us look at look at the kind of reactions that you do.

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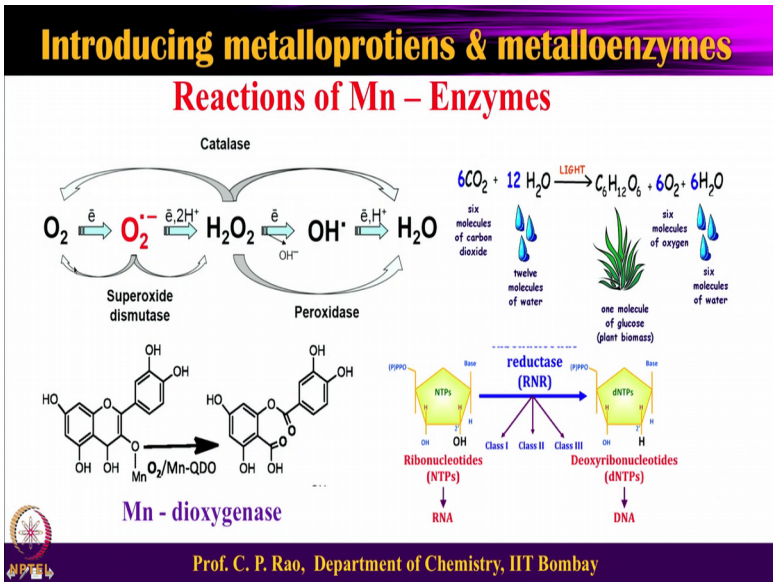

You see the oxygen, oxygen if you add 1 electron it will become O 2 minus, and this is what is called superoxide and if you put 1 more electron and put 2 protons, it will become O 2 2 minus put 2 protons H 2 O 2. So, the superoxide what it does? O 2 2 H 2 O 2 and what catalysts that? From H 2 O 2 to H 2 O so the right side part this is catalase the left side part is the superoxide and these kind of a the systems.

So, the H 2 O 2 then further breaking to OH minus and then H 2 O ok. So, you can use this and in the peroxidase enzymes also you can go through this kind of a step. Manganese dioxigenase I told you that I will explain this when I come to the copper, when I come to the iron so, but just I will show the example here and this the O 2 has got 2 oxygens, these 2 oxygens are added up over here see that the 2 more additional oxygens have come here and these are coming from here.

So, this is called dioxigenase whenever an enzyme adds 2 O parts to the substrate it is called dioxygenase. So, you will understand this much more better when we come to the iron case, when we come to the copper case. The other enzyme is RNR it is called

ribonucleotide reductase what is ribonucleotide reductase thing? Let me tell a little bit in this you know the systems have got RNA and DNA. RNA has got ribosal and DNA has got deoxyribosal and the to the body what is input ribosome never a deoxyribose.

So, if body has to synthesize DNA, it has to synthesize the right deoxyribonucleotide. To make the deoxyribonucleotide, it has to make deoxyribose. So, that particular enzyme which does this deoxyribose what is deoxyribose? Ribose has got a 2 prime hydroxyl, deoxyribose has 2 prime hydro hydrogen. So, this is what the differences. So, converting the finally, I converting the 2 prime hydroxyl to hydrogen So, this kind of a conversion is called the ribonucleotide reductase enzyme and after making the ribonucleotide ribonucleotide, then only the deoxyribonucleotide the other system of enzymes will make the DNA which that is not a part of this particular story.

So, first part of making a DNA is to make the deoxyribosal moiety or deoxyribosal nucleotide moietyk so to make that take ribonuclease time and make the deoxyribonucleotide. So, and the mechanism of this I can I will explain when we come to the story of a iron and probably even the story of the cobalt too. So, let us see what all we have done in this particular class, is I have explained you a several aspects related to vanadium and I have already made the conclusion of the vanadium.

And now I talk to you about the manganese entry, manganese got several oxidation state, but most oxidation states favor or plus 2 3 4 and very little towards a 5, never very highly oxidized never very low oxidized ones because they are either strongly oxidizing or strongly reducing there are I manganese enzymes with 1 manganese, 2 manganese and 4 manganese, I will be taking one example of each, one is superoxide dismutase, other is per the catalyst, the third one is photosystem, where the oxygen is evolved from the oxidation of the water center.

So, the kind of reactions are shown over there and the last reaction here you can see, that it is in the photo carbon dioxide going to the carbohydrate and the water going to the oxygen and I will be explaining the details of this as well. So, all these 3 cases I will be explaining in the next class.

Thank you very much.