Inorganic Chemistry of Life Principles & Properties
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Lecture - 54
Highlights of the course - Part II

So, welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the previous class, I have tried to summarize the things relevant to the introductory aspect of that and let me continue with some more highlight speaking to you and in the introductory part, I also have talked about some techniques based on the spectroscopy techniques based on the microscopy.

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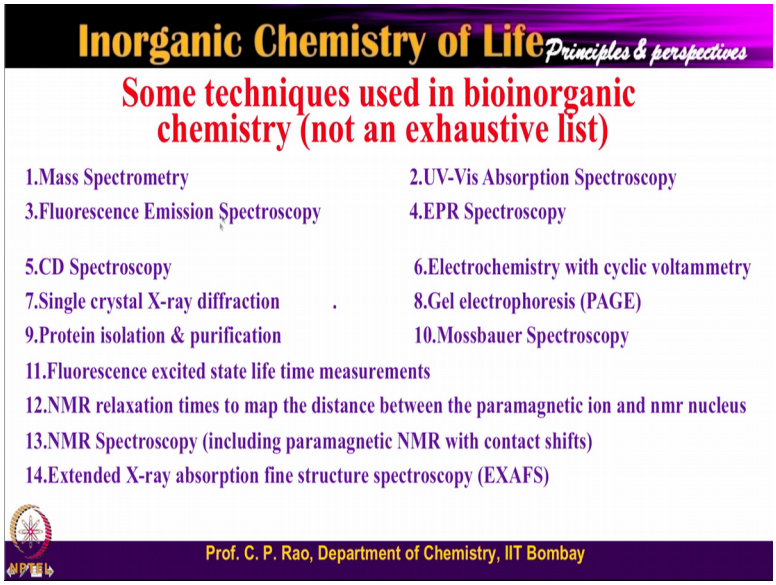

Techniques based on the diffraction and techniques based on the analytical kind of thing like mass spectrometry, etcetera; so, various things on lifetime measurements.

So, a host of techniques are there. So, each of this technique is a good in characterizing these enzymes not only characterizing their enzymes; when the enzymatic action goes on, you can also trap some intermediates, you can also study the mechanistic aspects all of these things like mass spectrometry absorption spectroscopy EPR spectroscopy fluorescence emission spectroscopy CD spectroscopy, and single crystal x ray diffraction protein, and many things like NMR relaxation times fluorescence lifetime and NMR spectroscopy including paramagnetism extended X-ray absorption, fine, structure

spectroscopy many of these things are there and mossbauer spectroscopy. So, all of these and voltammetry is one of the very vital kind of a technique.

Then, you can also have some several protein based techniques like protein gel electrophoresis where you can study not only the purity of the protein, you can also study the protein plus small molecule a protein plus drug protein plus something else binding, how strongly, they bind a protein plus another protein enzyme plus another protein and nucleic acid plus protein. All these kinds of interactions they are binding strengths can also be studied and proteins can be isolated, etcetera.

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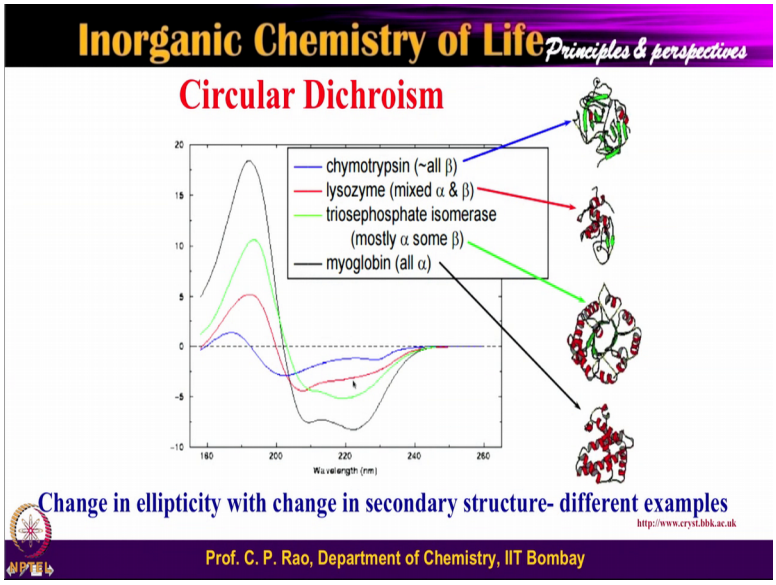

If you look at even a simplest technique which we may think circular dichroism as you can see that that you have features which are very characteristic of their alpha b alpha helical structures, beta sheet structure, etcetera, you can see from this figure for these for these for these etcetera this is just for as an example, I am showing, but that does not mean that is only this one and you know that protein.

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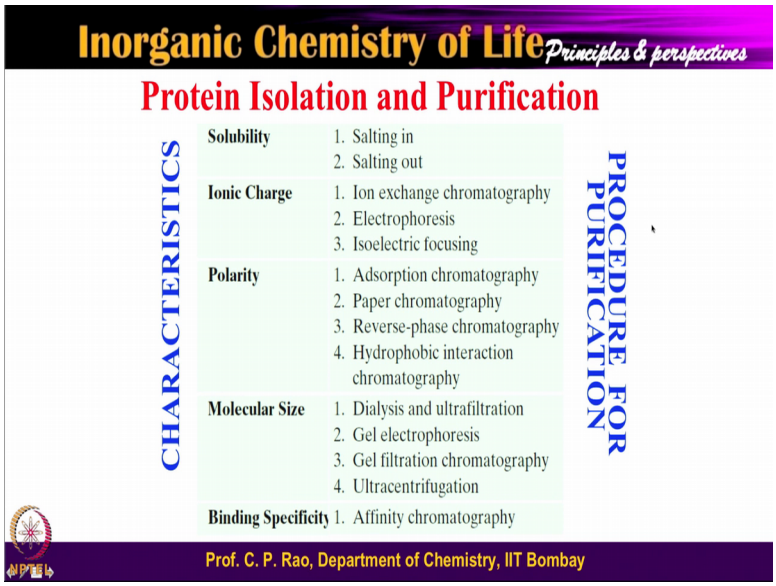

Techniques there are a lot of protein techniques are there; protein solubilizing protein salting out salting it out means bringing the protein out and taking the protein in kind of things. So, the ionic charge ion exchange chromatography electrophoresis isoelectric focusing variety of things.

So, these could be even based on the polarity based. So, that is their adsorption chromatography paper chromatography reverse phase chromatography ok; hydrophobic variety of these things based on the size or molecular weight dialysis ultrafiltration gel electrophoresis gel filtration ultracentrifugation variety of the techniques are there. So, you can do protein isolation, you can also do protein purification these are all from the natural methods, but sometimes you may not have much of a production from the natural then you have to do the genetic engineering and then express in the E-coli. So, that is kind of thing binding by affinity chromatography etcetera we will see that

So, this is a little bit extension of the chromatography equipment looks like this.

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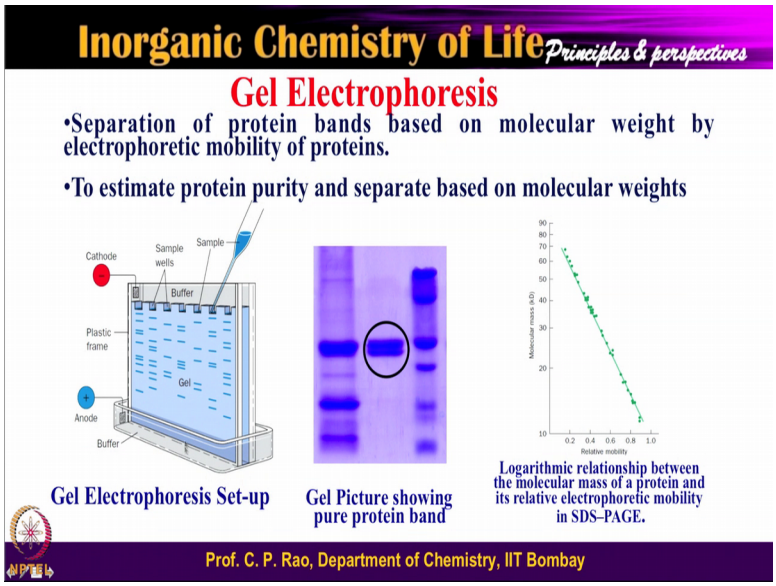

You have on one side the one of the charge, this is plus and the minus applied. So, between these two the flow will be happening, the flow of the proteins is because of their shape, because their size because there is charge, etcetera, etcetera. So, that will basic based on this you can in fact, bring a correlation between the molecular weight and the logarithmic relationship between the molecular size of the protein and it is the mobility the electrophoretic mobility electrophoretic mobility of these. So, this is a logarithmic scales people look at that.

So, you can find various things that besides these things.

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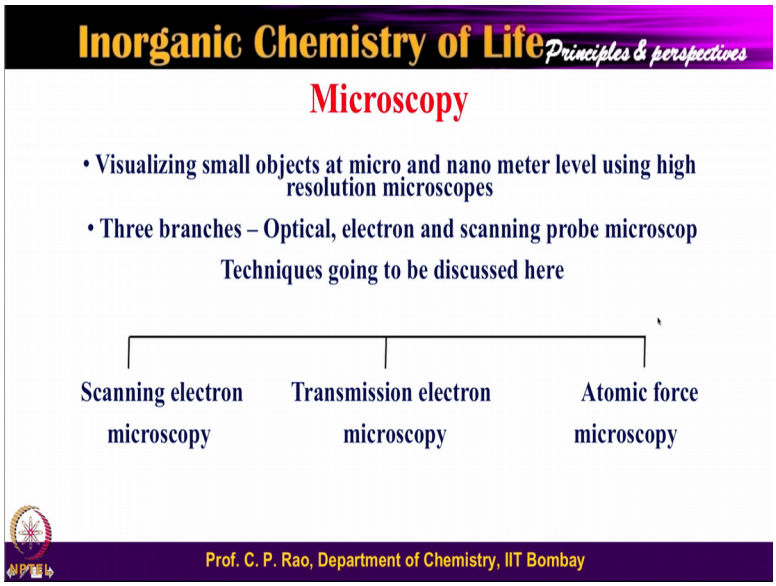

We also have microscopy techniques where you can use scanning electron microscopy transmission electron microscopy atomic force microscopy; this can be used at the biomolecular level.

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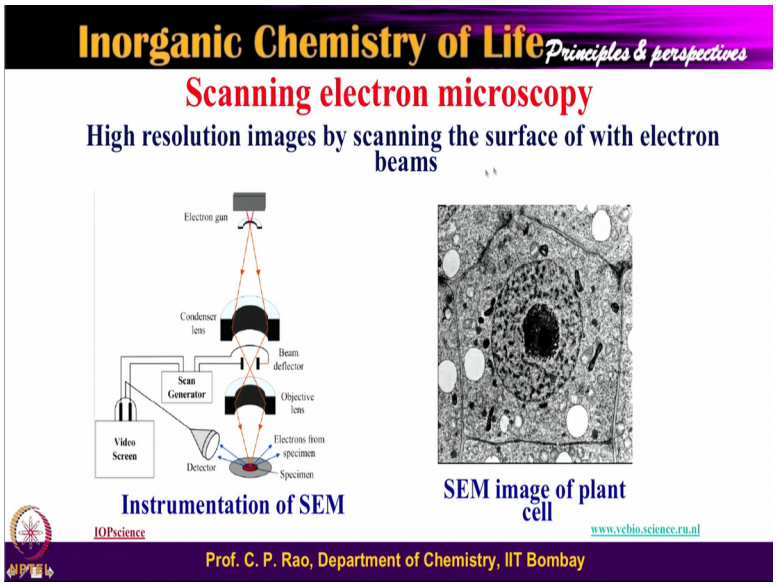

This can be used at a cellular level and you can see for the cell; the sem of the cell where it showing all different parts of the cell etcetera you can identify.

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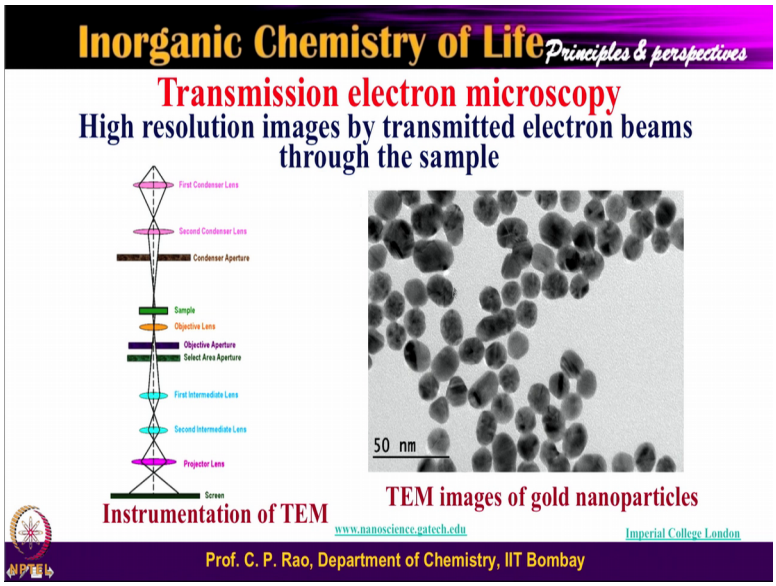

And you can also look at the beam here example is shown for the TEM of the gold nano particle and in fact, you can po coat a protein to nicely.

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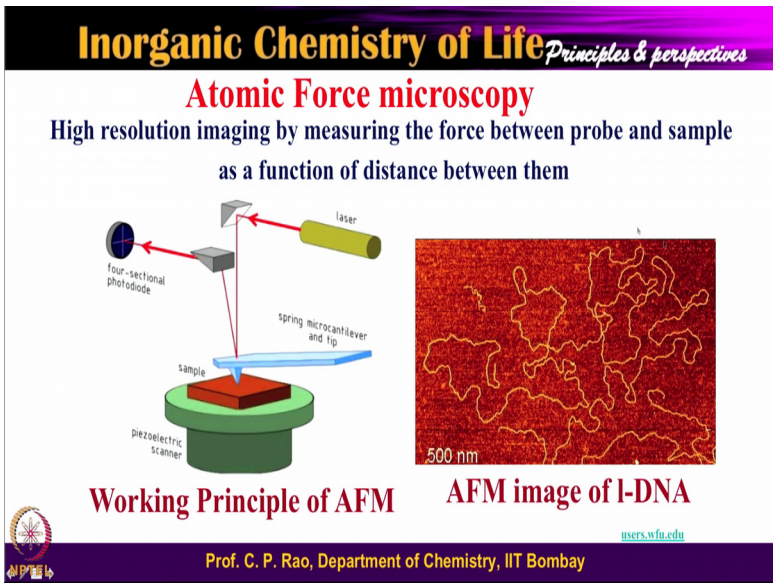

And you can only even see by atomic force microscopy these are nothing, but the DNA and the DNA imaging is coming within the atomic force microscopy. So, using this microscopy you can get their shape you can get their size you can get the height of the things in terms of the size, so many different factors, and when the enzyme substrate complex when the enzyme regenerated how the changes happen all these things can be

studied as well and so based on this that all these things. Now we are at a stage where we can classify the metalloproteins and metalloenzymes.

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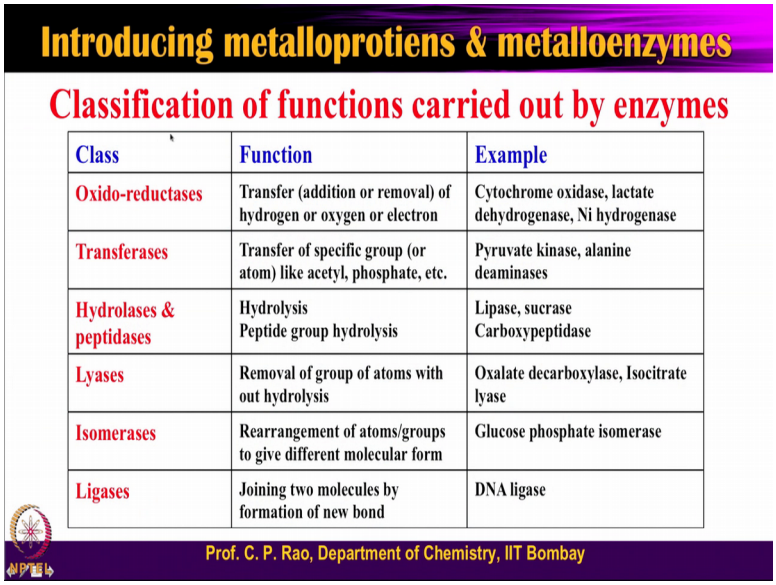

So, the metalloproteins and metalloenzymes can be doing simple hydrolase activities or it could be doing peptidase activities or they are can be doing oxidoreductase or they can be doing some kind of a transferring a group from one to the other or they can be doing isomerizing a particular moiety a group or geometry or they can be doing lyase which is removing without the hydrolysis part hydrolysis can also remove ligase take two parts of it and join together.

So, overall the whole bio inorganic enzymes biological inorganic enzymes the kind of functions that we perform these are the ones besides the storage transport etcetera I guess you understand this; these are all various kinds of a enzymes.

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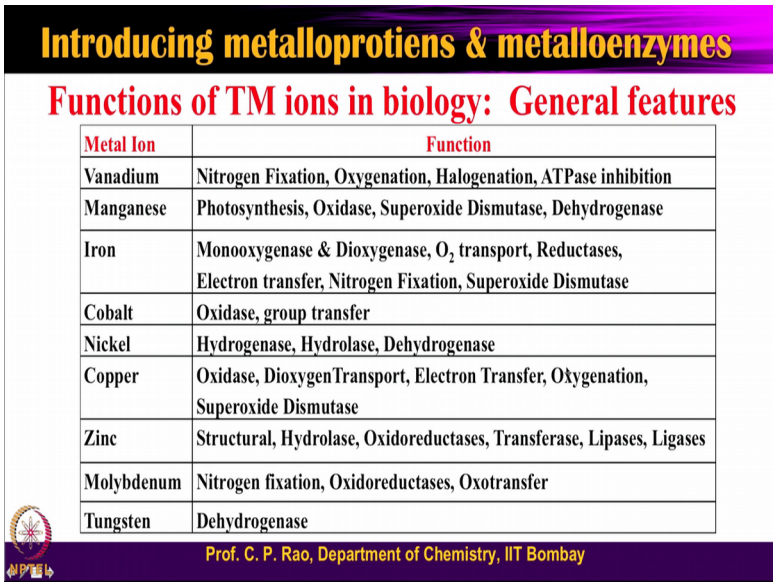

Metal Ion	Function
Vanadium	Nitrogen Fixation, Oxygenation, Halogenation, ATPase inhibition
Manganese	Photosynthesis, Oxidase, Superoxide Dismutase, Dehydrogenase
Iron	Monooxygenase & Dioxygenase, O_2 transport, Reductases, Electron transfer, Nitrogen Fixation, Superoxide Dismutase
Cobalt	Oxidase, group transfer
Nickel	Hydrogenase, Hydrolase, Dehydrogenase
Copper	Oxidase, DioxygenTransport, Electron Transfer, Oxygenation, Superoxide Dismutase
Zinc	Structural, Hydrolase, Oxidoreductases, Transferase, Lipases, Ligases
Molybdenum	Nitrogen fixation, Oxidoreductases, Oxotransfer
Tungsten	Dehydrogenase

So, what kind of things that we have already seen earlier that the transition metals like vanadium, manganese, iron, cobalt, nickel, copper, zinc, molybdenum, all these things; they can do oxidoreductase properties some of them can do electron transfer properties some of them can do hydrolase properties; some of them can do lyase properties some of them can do ligase properties.

So, variety of these things oxidation reduction as well as the hydrolysis group transfer isomerization and the various kinds of things of this; of course, in this electron transfer proton transfer kind of a things are also possible in all of these.

So, this is over all that I have talked to you about the overall introductory aspects of the metal ions in biological systems I have treated this is very well suited even if a BSC second third year student sits for this particular course, would be able to understand, because I have built the basics required for that from the point of view the metal ion elements in biology from the point of view of the coordination chemistry from the point of view of the biological molecules also from the point of view the techniques that are used.

Now, let us smoothly transit into the biological inorganic chemistry of alkali alkaline earth and then followed by transition metals.

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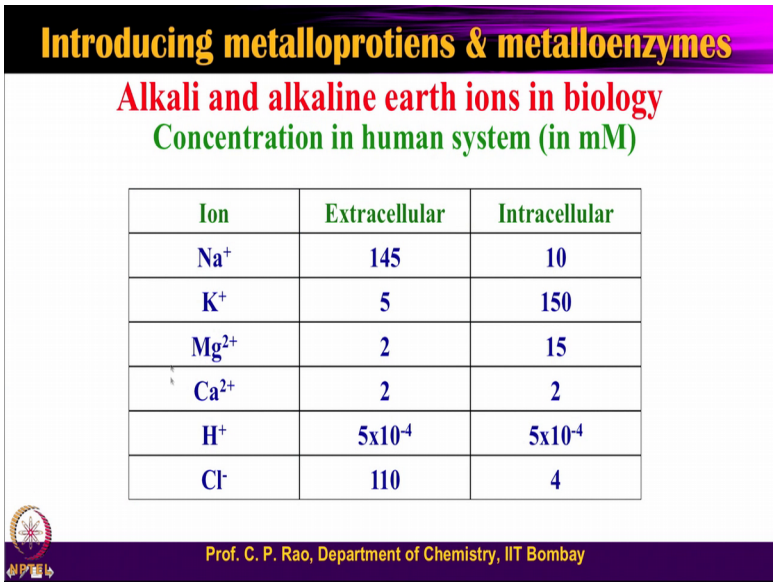

So, let us look at that I have talked to you about sodium potassium magnesium calcium; the very important sodium is very high outside low inside potassium is very high inside and low outside. Therefore, this sodium and potassium have to be transported across the cell.

Similarly, you have a magnesium is quite good both in external internal calcium is also, there the magnesium is found much more than the calcium inside the cell. Therefore, the calcium binding bound proteins have to be protected in some way have to be special feature in some way. So, that the magnesium ions will not trigger or activate the calcium proteins.

Now, how it is doing yes you know very well that the calcium proteins have a special affinity towards the carboxyl groups carboxylate moieties. Therefore, some of the post translational proteins give additional carboxylate moieties too and they are all will make this one.

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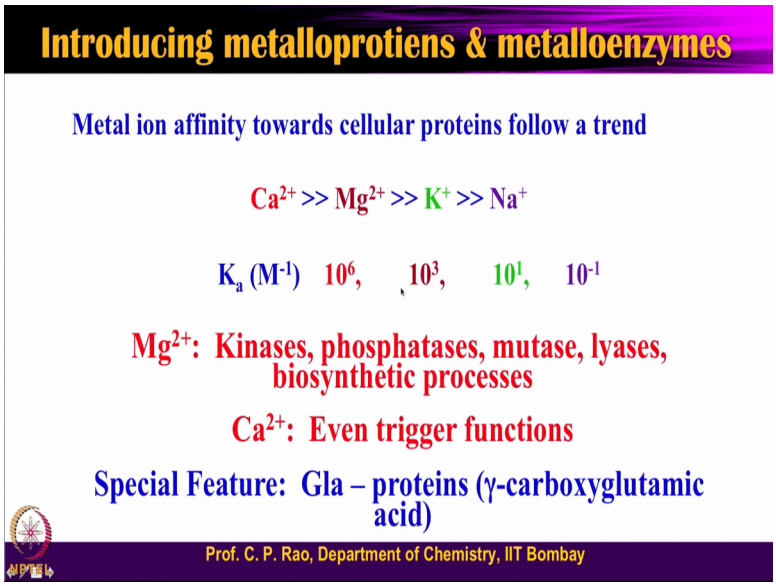

So, overall if you look at the in a very general fashion the calcium binds to the protein mantle or protein molecules or proteins much higher like a 10 power 6 range by new constant 10 power 3 range for magnesium 10 per 1 range for potassium and 10 power minus 1 range for sodium; that means, between sodium and potassium calcium, there is a 10 million 4 difference between potassium and calcium there is a million fold 10,000 fold a million fold 100, 1000 fold and a 1000 fold difference is there.

So, a magnesium of course, activates kinases phosphatases mutations a lyases and many involved in many biosynthetic processes the main thing is kinase and phosphatase absolutely these are the two ones which are running the whole the signaling process and all the things events that take place.

In addition, calcium will show many of the properties plus the triggering of this specific feature I talked to you is called the Gla proteins which are nothing, but carboxylated ones gamma carboxyglutaric glutamic acid are the ones calcium is a special feature.

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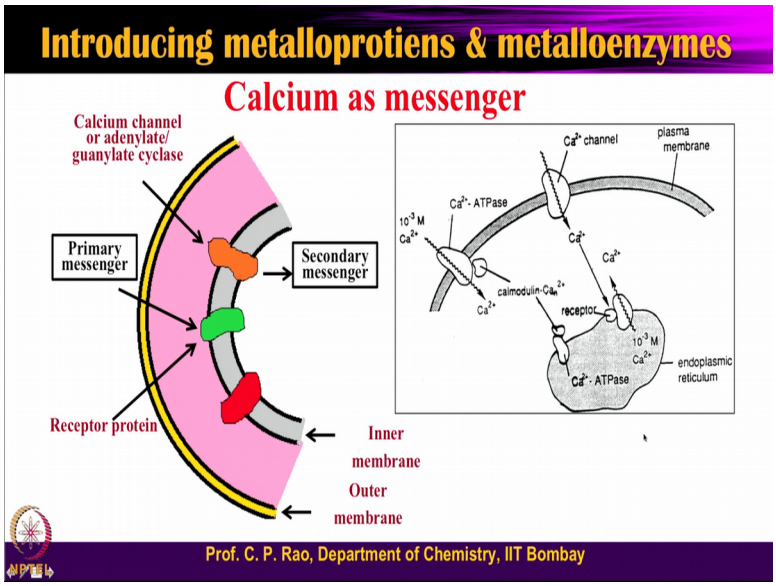

Unlike the sodium potassium and magnesium; that there are when the calcium gets enters into the cell membrane can be captured at the inner membrane and something else gets released over there. It is called secondary messenger. So, I mean various ways to calcium released and then that will activate some other kinds of things too there it can even activate some phosphorylation as well too.

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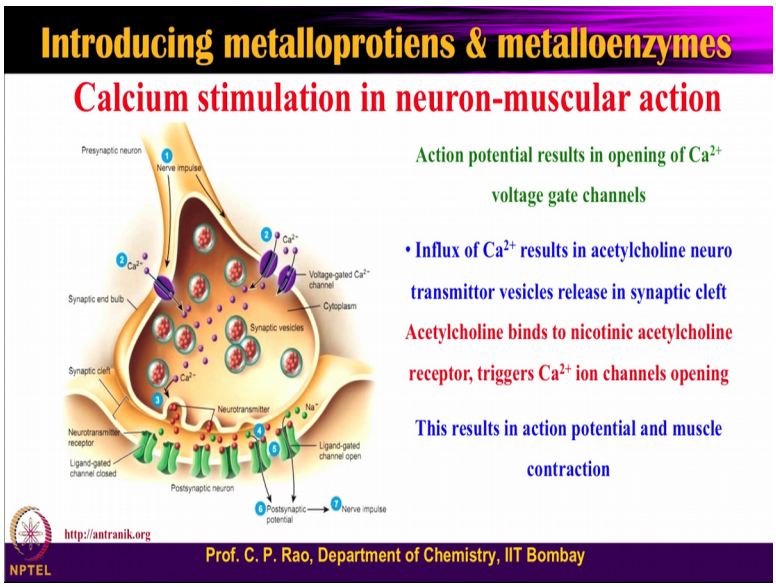

So, calcium can act as an not as a simple primary, it can also act as a secondary this can also be very well seen from its stimulation between the neurons and muscular junctions.

So, one is from the neuron another is from the muscle. So, the new neuron muscle at this particular junction the signals will be carried over and these are all fully responsible by the calcium ion concentrations calcium released components, all these kinds of things; I hope you understand that ok.

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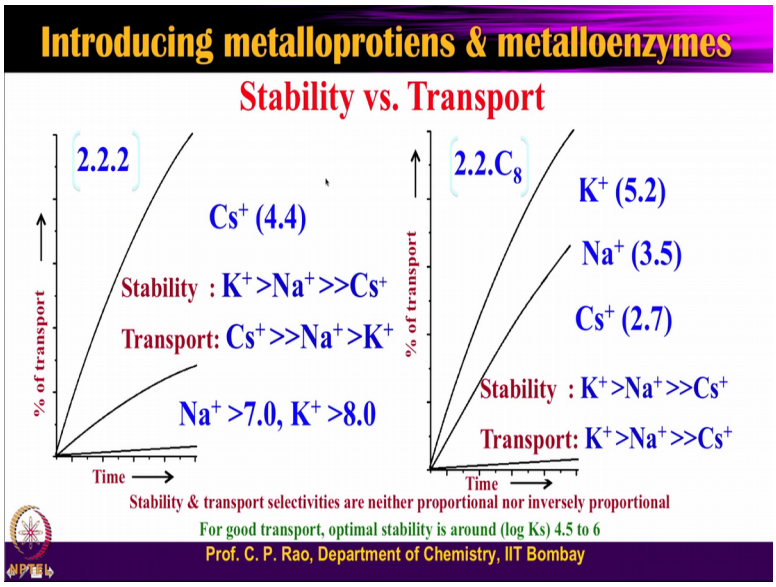

In the process, I have also talked a lot of small molecules, how they bind how their stability constants; how their transport characteristics all of these. So, what we found from all of this is that the size of the pore or the of the ionophore size the ion is important, but not sufficient enough, then the nature will ligands present in the ionophore and the number of ligands are present in the ionophore; the outer surface of the ionophore lipophilic hydrophilic all these kinds of things; how well it can; it can cover the metal ion all of these factors. So, we have seen all of them in the transport, they can be passive transport, they can be active transport we have seen all of those parameter.

Let us look at one highlight of the example here one of this curve; there is shows here the sodium transport by a particular this molecule called 2 2 2 krypton. So, that is 2 oxygens in the linker another 2 oxygens in the other linker, 2 oxygens in the other linker besides the nitrogens and this is called 2 2 2; in case of 2 2 2; if you use the sodium will have a very high binding greater than log case greater than 7 potassium is having greater than 8 and the cesium is having cesium is having the log cases around 4.

So, if you see that the transport; this is the cesium is transporting maximum than the sodium than the potassium you see that and then if you look at the stabilities stability is sodium is greater than or the potassium is greater than the sodium greater than the cesium. So, therefore, these two are not directly proportional, it could be you can say reverse.

Then, instead of that you take 2 2 and 1 more is carbon chain 8 carbon chain and no two oxygens, then what will happen. So, then what will happen is the cesium shows very little sodium shows somewhat better potassium shows maximum. So, potassium has got a 5 log case and cesium sodium has got 3.5 log case and the cesium has got 2.7

So, in this case the transport is greater than potassium is greater than sodium greater than cesium. Similarly, potash stability is also potassium is greater than sodium is greater than cesium. So, in one case it shows the stability and transport are inversely proportional in other case, this is no this is seen as the stability and transport are inversely prop directly proportional. So, what we will take we cannot take either of these.

So, there is something else which I have brought to your notice the highest the optimal seem to be around 4 to 5; 4.5 to 6 is the log case. So, whenever you have the 4.5 to 6 log case; you get a optimal transport a maximum transport with optimal stability. So, very low binding will lead to a disruption of the transport, before it is reached the destination very strongly bound case it will reach the destination, but will not be able to release the ion.

So, therefore, either case, it is very dangerous very strong binding of the ion with the ion by the ionophore very weak binding of the iron by the ionophore both are dangerous whether, it is a ionophore whether it is a synthetic one or natural one or it is a protein binding to that because in some of the cases not only small molecular peptides the protein will also involve in transport phenomena too, so they will bind too.

So, that means, when you are talking about the ATP.

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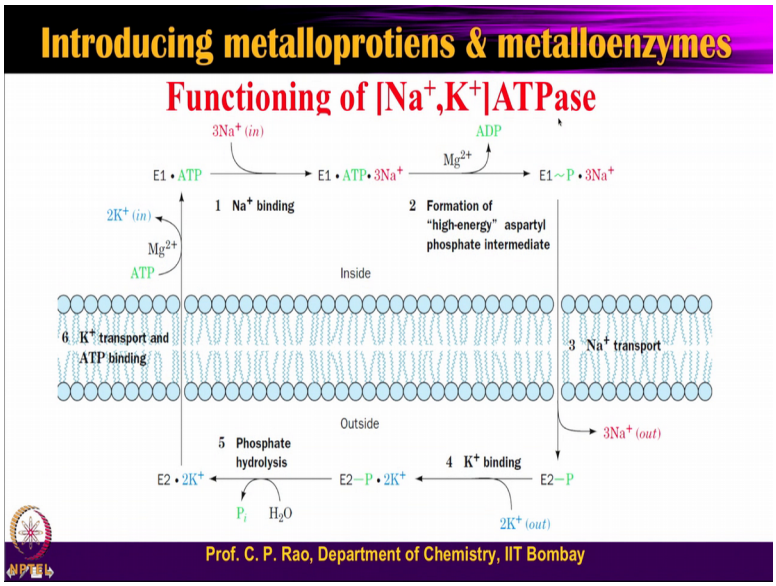

You know that the ATP is that enzyme which requires the ATPs; ATP hydrolysis and during the transport of the ions from the outside to inside of the sodium from inside to the outside of the potassium and in these two are triggered by the 2 conformational states of the protein and these 2 transformational states are triggered by phosphorylation and dephosphorylation and phosphorylation is activated when the sodium is bound dephosphorylation is activated when the potential concern close to that particular thing.

So, therefore, that is how in the transport of this the protein has as an affinity and core for sodium binding in the other confirmation in the phosphorylated one it has a potassium core and potassium affinity. So, therefore, the affinity changes; so, affinity of the protein towards binding to sodium or potassium switches over from sodium to potassium when you go from the ATPs enzyme in the normal to a phosphorylated enzyme and then back, etcetera, etcetera.

So, these are some of the things that I have talked in the context of the transport aspects of transport aspects of this enzyme. So, the transport acts aspects of the transition the alkali and alkaline earth enzymes ok.

So, now I think we have looked at a lot of aspects pertinent to the pertinent to the elements in biological system. So, elements in the biological system their relevance to the bio coordination chemistry, their relevance to the proteins, they relevance to the

stabilization their relevance to the liability and the transport aspects etcetera we have tried look at.

Now, we will look at some highlights the highlights is highlights of the transition elements; how these transition elements are involved in the enzymes; this is where we will look at the highlights.

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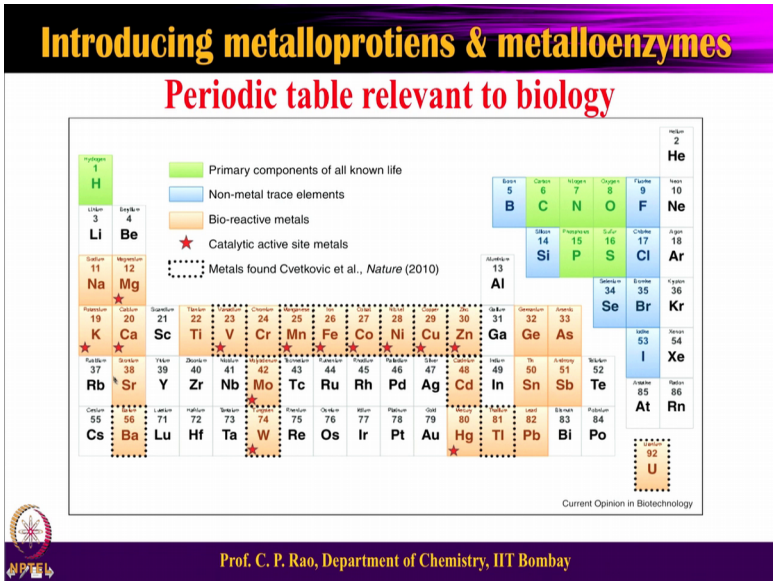

Now what are the transition elements where the enzymes are present vanadium, manganese, iron, cobalt, nickel, copper, zinc and molybdenum, then we also look at some on the mercury and some on the selenium. So, this comes from the cysteine selenocysteine and this is a mercury reductase. So, removal of the mercury from the environment, it could be even from systems as well too ok.

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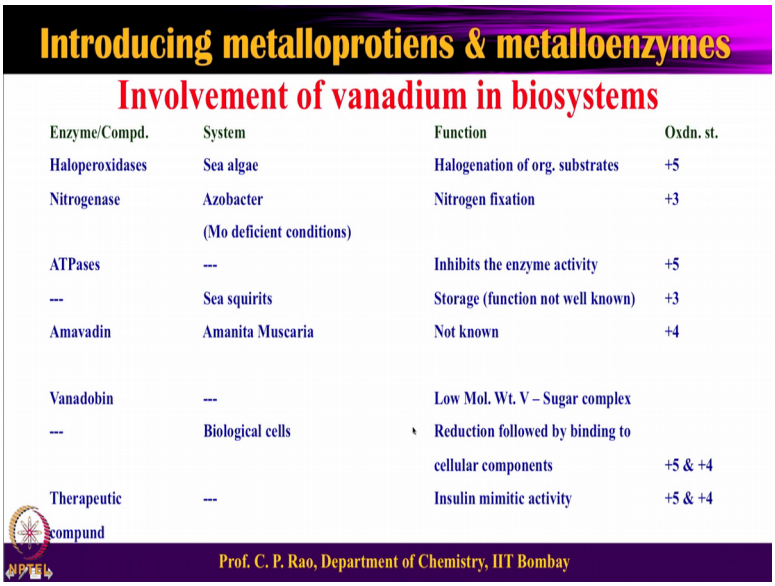

Coming to the first system which is the vanadium; vanadium as we know, can have oxidation states from minus 1 to plus 5 in the chemical systems, but the oxidation state which are very important our or possible in the biological systems are plus 5 plus 4 plus 3 although, I explain to you why not the lower like plus 2, etcetera, you know the plus 2 is a strongly reducing, it will easily go back to the oxidized 2 and the many things below as well they are not stable. So, therefore, plus 3 and below are not found plus 3 plus 4 plus 5 and these are of course, found they are being identified in various organisms and they do variety kind of a functions.

So, this is also boring these guys plus 5 can be studied by vanadium 51 NMR plus 4 can be studied by the EPR and things of this kind one can try to look at these ones.

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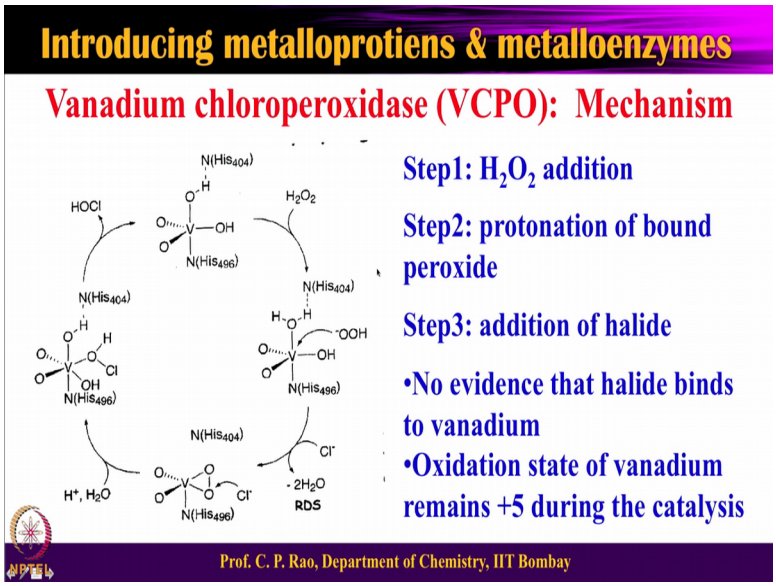

So, the things that we have explained to you was the phosphate can inhibit the vanadate can inhibit the phosphate in the phosphorylations in the sodium potassium phosphatase cycle at the second half of the cycle where the potassium comes activates to remove the phosphate group at that stage if the vanadate is found, it will bind; it will be it will be stopping the cycle of the ATPs, therefore, the ATPs is completely inhibited therefore, ATPs inhibition is possible by vanadate.

Now, that is the negative aspect of it let us look at the positive aspect of it. So, it is the peroxidase activity; it is actually called hallow peroxidase example, I taken is a chloro there can be chloro peroxidase bromo peroxidase iodo peroxidase, it is a kind of an enzymes these are the enzymes which are found in algae C algae; these are the enzymes which are responsible for converting the organic molecules into halogenated organic molecules.

So, in these cases, the enzyme in the enzyme the vanadium is found like a vanadate which is suspended in the enzyme and one of the coordination is coming from the histidine and from their protein and this is the protein histidine. So, therefore, it takes more or less like a trigonal bipyramidal more or less like that and this is a vanadium 5 form, you can very nicely study the coordination sphere coordination kind of a ligands using the 51 vanadium NMR and this in presence of the H 2 O 2 peroxide will try to interact with the peroxide and then the peroxide species will be bound this in presence of

the halide can make break this one and form the hypohalous acid and this is a species which interacts with a with their substrate and give halogenated substrate and then goes back to normal.

So, this HOCl is not just like that coming out it comes out and then reacts with the substrate so; that means, substrate should be bound very close to this in the enzyme and indeed and then you have the kind of an enzymatic action going on. So, H 2 O 2 addition protonation of the bound peroxide addition of the halide and these are the kinds of things makes.

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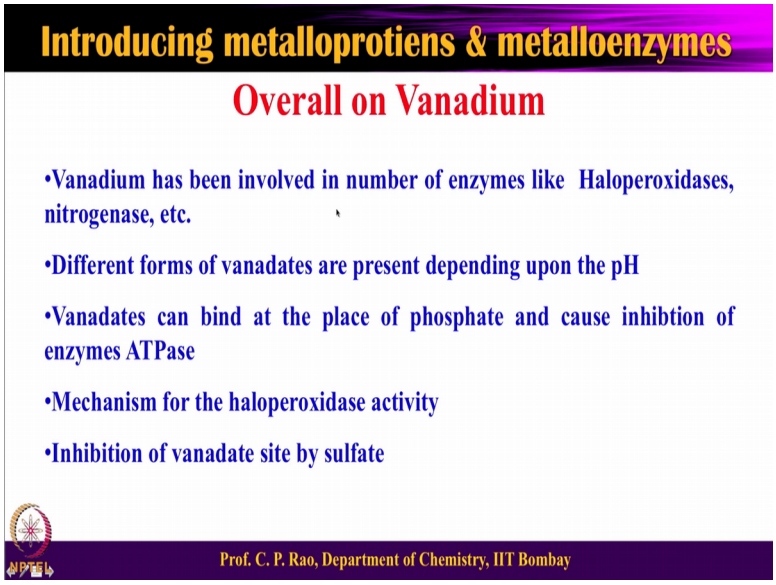

So, overall what we have studied at that time; when I talk to you the story of the vanadium. So, we I have explained to you that the vanadium has been involved in variety of enzymes like heloperoxidases nitrogenase, etcetera nitrogenase, I have not explained because I will be explaining completely under the molybdenum also the vanadium nitre nitrogenase details are not so well; very well known, but it is thought to be that the vanadium nitrogenase functions very similar that of the molybdenum nitrogenase, different forms of the vanadates are present depending upon the ph on concentration in the what are the biological medium are outside too.

So, vanadates can bind at the places of phosphate and cause the inhibition. So, I have explained the mechanism helo peroxidase, and in fact if there is a sulfate the sulfate can do inhibition and that is how this is being found in this kind of things.

So, this is overall highlights of the vanadium and let us look at some highlights of the manganese enzyme; once you open the doors for manganese you have a variety of things, it can act like a super oxide dismutase.

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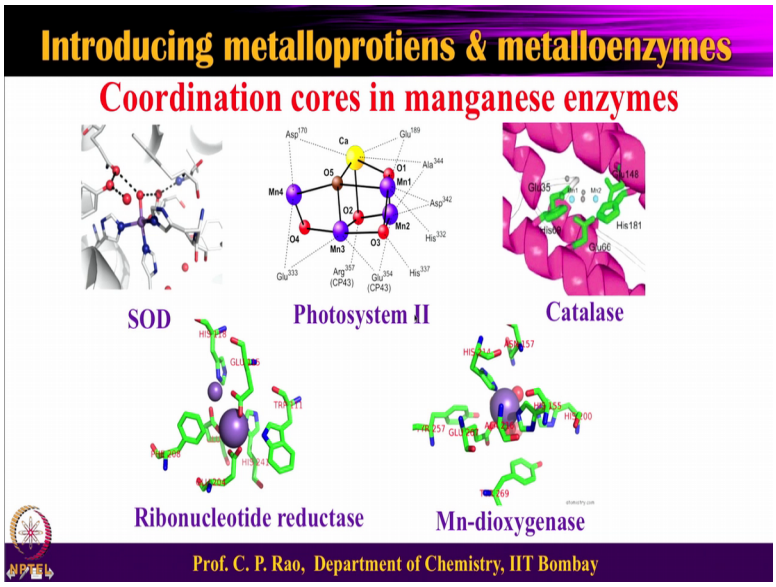

It can be involved in the photosynthesis in the photosynthesis this is involved in the photosystem 2; what is the reaction the photosystem 2; I am sure you are aware that it is going from water to oxygen water to oxygen what is the reaction oxidation and water to oxygen; how many oxidations 4 electron 4 proton oxidation.

So, therefore, from water you have to get O 2; that means, you have to remove 4 electrons and you have to remove 4 protons and that is where this is involved I will explain you in a while and catalase; what does catalase do; catalase does because sod converts the O superoxide radicals into water and H 2 O 2 that H 2 O 2 is further converted by catalase into water completely or oxygen kind of thing and we have the ribonucleotide reductase to. Of course, we will be explaining more detail of the ribonucleotide reductase in the iron story now and the dioxygenase dioxygenase will explain to you the more we already explained you more details under the story of copper as well as under the story of the iron, but not in the story of the manganese.

So, the main thing is explained is the sod and the photosystem to and the catalase.

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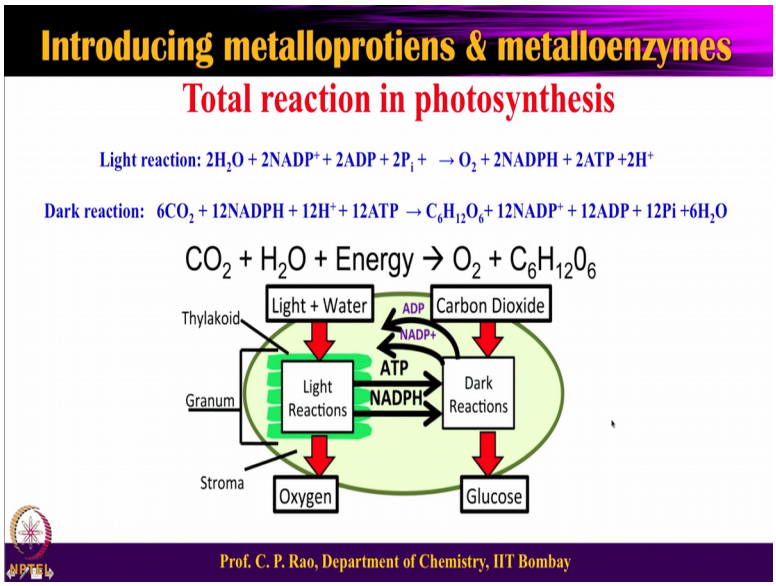

But in the highlights; I will take up the photosystem basically. So, what is the reaction that as you can see in the light based reaction a dark reaction co two getting reduced to a carbohydrate there is a lot of ADP, ATP involvement and the water to oxygen there is a lot of ATP ADP involvement of all this.

So much of ATP involvements of all this as you can see the light reactions and the dark reactions and these are all the coupled reactions that you have. So, these go at the photosystem one; this will go at the photosystem 2 and we as the bioinorganic chemists inorganic biological chemists; we are interested in the photosystem 2 reaction of converting water to oxygen. And as you can see that these are the kind of a light harvesting devices and the electron transport system and then you have the reactive center which is the manganese.

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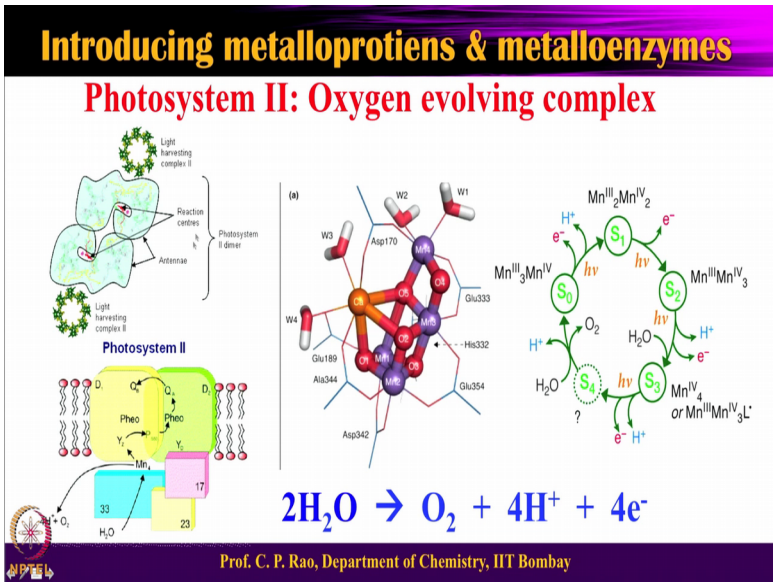

So, you have the light receiving which is called antenna electron transport a huge transport system and the reaction center manganese center where the reaction occurs. So, at the manganese center you can see here; it is kind of a cubine and there of the manganese oxygen alternate except one of the thing is by the calcium and that the fourth manganese is where here and this is bridged by the kind of an oxo species this ok.

Now, you see that. So, what is the kind of things that you require 4 electrons and the 4 protons see this is S 0 is what is a dark state. So, at the dark state is the S 0 state of the enzyme where 3 of the manganese 3 and 1 manganese 4 and this when the first pulse of the light falls on it. And then, light drives this reaction and drives out a proton and activates to S 1 state and this S 1 state will have on the one of the manganese 4, it is also converted to manganese 3 kind of a situation because the reduction.

So, one more electron one more manganese 4 two 3 this is one more electron out sorry electron is out. So, 1 manganese 4 manganese 3 to manganese 4, then one more electron more; 1 more manganese 3 to 1 more manganese 4 like that you have one more proton and electron, then the state comes in to the manganese 4 system or manganese 3 with 3 manganese 4, it is probably at this stage where the oxygen is evolved and the system is regenerated. So, overall you have. So, the protein goes through the S 0, S 1, S 2, S 3, S 4 and with the 4 photon signals, 4 electrons, 4 protons, photon signals, etcetera, etcetera.

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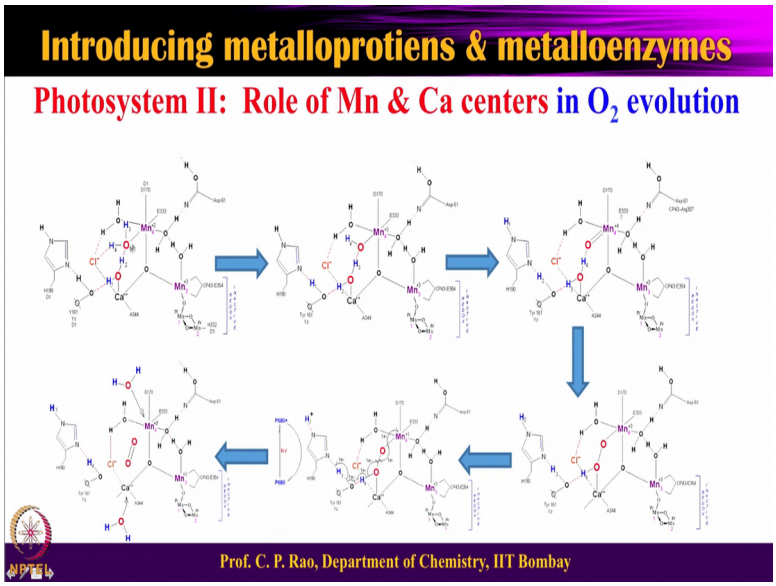

So, what is that you have the fourth manganese and the calcium both are having water molecules these are the two water molecules which are involved in the O 2 evolution; that means, 2 water molecules have to come closer and so, you have a water 1 over here and water 1 over there. So, there is a calcium 2 plus with the water.

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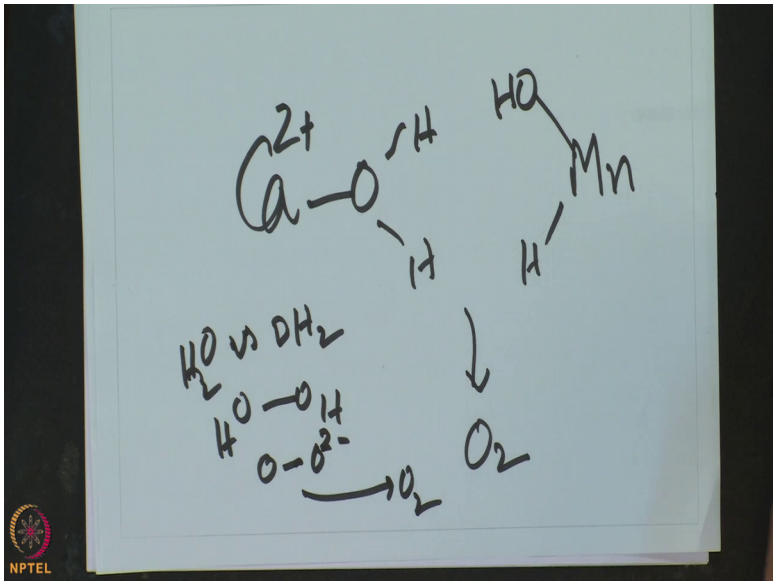

And then manganese with the water here you have O H and H. So, therefore, these are the ones where you have to have.

From here, if you have to make O two then OH O has to come, then OO bond has to form, then cut this bond etcetera and then finally, becomes a O 2 ok. So, OH; so, therefore, O H 2 versus O H 2, so, this will become OH and H and then becomes O O 2 2 minus, then it will becomes O 2 finally, 2 O 2. And these are the kinds of steps that you require in this kind of a thing that is what is so, 2.

So, the rest of the 3 manganese are not directly involved in catalysis and one of the manganese and one of the calcium the water two water molecules as you can see that and then with the first pulse electron they come closer than the metal redox undergoes that will become oho and then further oxidation will become manganese 4 and then you see manganese O with a O H, but still.

And now this will further convert to the redox here to the OO bond. Now once the OO bond is formed you have to start breaking them Mn O and here the Ca O kind of a bond a basically Mn O bond if it is not breaking this one, then you will get one electron from here 1 electron from here O 2, then the O 2 S come and then O 2 is released kind of thing. So, when the O 2 is released it will go back to the normal state. So, we could see a variety of these things very nicely O what 2 water molecules how they give O 2 ok.

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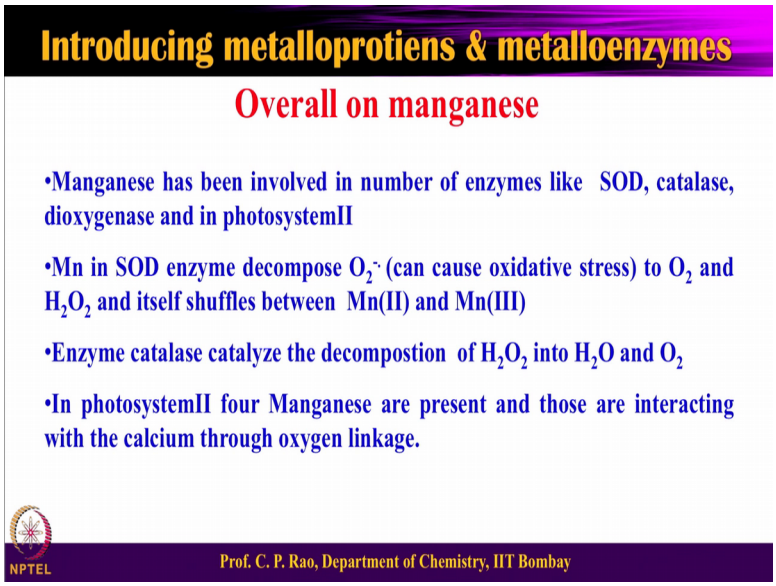

So, in the manganese involved in lot of enzymes like sod catalase dioxygenase photosystem 2 SOD case, it decomposes superoxide into the O 2 and H 2 O 2 and in that case, what it does; it goes through manganese 2, manganese 3, back to manganese 2 2 to

3, 3 to 2, kind of a thing. So, that is what happens and then whatever the H 2 O 2 comes that is being converted to H 2 and O 2 by the 2 manganese centers present in catalase; of course, I have not shown in the highlights, but I have shown in the regular lecture that you can see that.

So, in the photo system 2 just; now I explained to you the 4 manganese, but one calcium primarily, mainly there is one calcium and manganese which are involved to bring the two waters close by make the OO bond, then O bond that too that is the kind of a thing that we have tried to look at these ones and ok.

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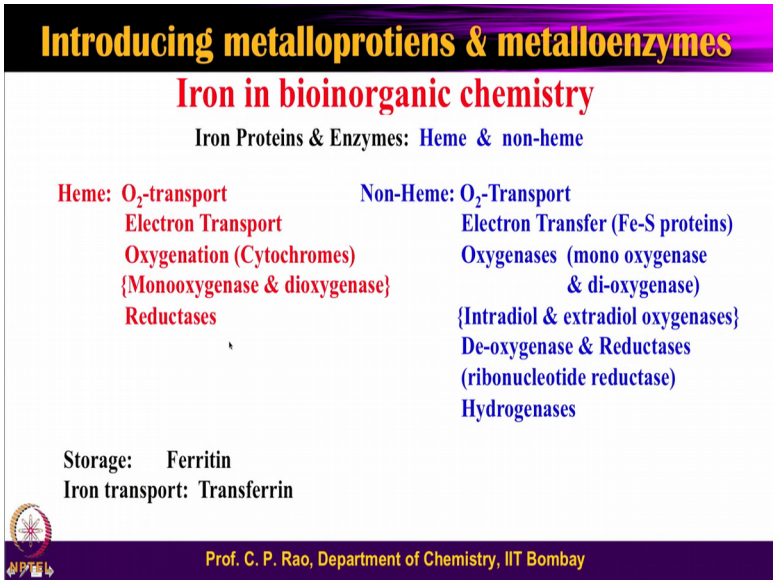

Let us get into the next aspect where I have taken after the manganese I have taken the iron in the bioinorganic chemistry probably, what I will do is I will take up this highlights in the next class because iron enzyme will have so many things because iron is in the transport iron in the non heme iron is a oxidative reductive etcetera all of these are there.

So, no, no, no problem and what I will do is, I will explain these ones in the next class and as I have talked to you talk to you about the transport of the sodium potassium ions, then I have talked to you about the vanadium enzymes where then I have talked vanadium enzymes and vanadium heloperoxidases etcetera how the bromination chlorination iodination occurs at these things; how the phosphate and vanadate analogues will play a role in place over the other. And then I have explained to you the manganese

in variety of enzymes with more details of the manganese in the photo system; photo system 2 where the water releasing the oxygen.

Of course, I will take up the iron enzymes and the rest of the enzyme highlights in the next class. Hopefully in one next class, I can try to explain all the highlights of this.

Thank you very much.