Inorganic Chemistry of Life Principles & Properties
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Lecture - 58
Tutorials - Part II

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the last couple of classes, we have been looking at various questions in the form of tutorials. And let us continue for this maybe one more class also based on this particular thing.

Towards the end in the previous class we were looking at some examples yes.

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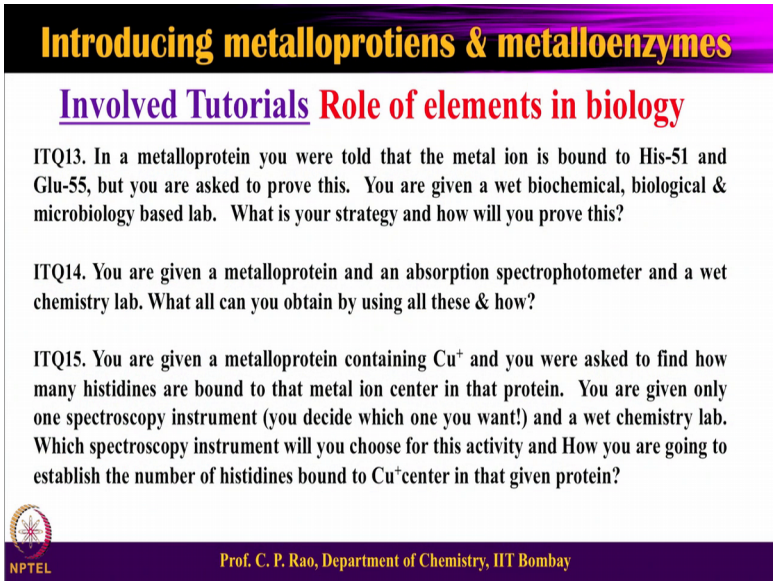

Let us say you look at this particular question in a metalloprotein you were told that the metal ion is bound to Histisdine 51 and Glutamic 55, but you are asked to prove this ok.

You are given a wet biochemical, biological microbiological based labs. What is your strategy and how will you prove this? So, the fact that is given the position so; that means, you need to sort of take this position into a different kind of amino acid. So, how do you change one amino acid by other? And that is what is referred as a site targeted mutagenesis.

So, therefore, by using the site targeted mutagenesis you can replace one at a time the histidine 51 at one time, glutamic 55 at another time. You can also do both of them as well multiple mutations too and from that you can definitely prove, where the reactivity is present, reactivity is not present all these things.

For this you require lot of PCR work and the nucleic the plasmid based work; that is why we talked about biochemical and biological kind of a work. Then once you change this you have to express; so, you express in micro box; so, therefore, microbiology. Then you synthesize you get the product which is in the form of the enzyme and there enzyme activity you can study. So, that is how one can approach for such a kind of a problems ok.

So, the next question the tutorial because these tutorials are supposed to be making you to think is how to respond to the questions. So, you are given a metalloprotein and an absorption spectrophotometer; I would say you are given different metalloproteins and you are given absorption spectrometer and wet a chemistry lab. So, what all can you obtain by using all these and how? So, when you say wet chemistry lab you should be able to do chemistry modifications; for example, you are given a metalloprotein you can take out the metal by using some collect agents, then you get a apo protein; you can check the apo protein absorption spectrum, then you start adding the metal ion; so, as a function of the metal ion concentration; as a function of the pH all of these you can study.

So, from that you can establish the metal ion, its coordination, aspects such as geometry other things and many of these kind of things can be identified. So, therefore, the same question will come in another form later on; so, we look into that as well. So, this 2 what I talk now actually I have already talked in the previous class towards the end ok.

Let us look at the next one you are given a metalloprotein containing copper plus; copper plus and you are asked to find how many histidines are bound to that metal ions metal ion center in the protein ? So, you are given only one spectroscopy instrument.

So, you decide which one you wanted to use and a wet chemistry lab which means you can play with it. So, which spectroscopy instrument will you choose for this activity and how you are going to establish the number of histidines bound to copper plus center in

that given protein? So, you are allowed to things anyone spectroscopy technique and you are allowed to do some chemical modifications; true because chemistry lab is given.

So, suppose you want to go suppose you think that your answer is absorption spectroscopy. See the absorption spectroscopy is not going to tell the number of histidines at all. You may say argue that the shift the shifts of these very broad bands; suppose you have few histidines or more hestidines the positions could shift to the very similar to that. So, therefore, absorption spectroscopy is not a right method rather EPR. How will I say EPR is copper plus? Everyone knows copper plus is d 10 and d 10 is a non magnetic one, but try to see in the third line a wet chemistry lab. So, what will do? I will add some agent which converts the copper plus to copper 2 plus.

. So, what will I use? I can use ion oxidizing arising agent. So, I will oxidize to copper 2 plus; once it is oxidized I have a copper 2 plus which is the d 9 system and now this d 9 system; I can tackle through the EPR spectroscopy. And if I go for a high resolution EPR spectroscopy, I can even establish the number of the historians being present in this one and that is how it is ok.

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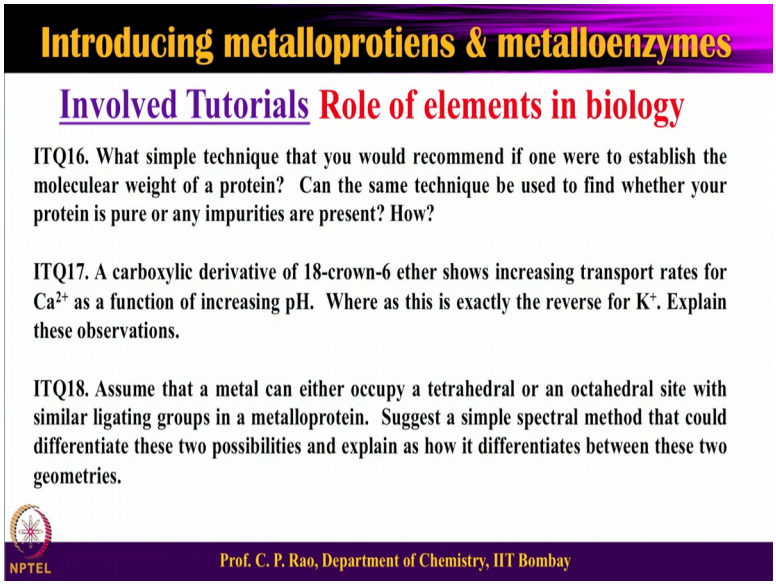

So, now, let us keep looking at more and more questions of involved type; what is what simple technique that you would recommend if one were to establish the molecular weight of a protein? Can the same technique we use to find whether the protein is pure or

any impurities are present? How? First of all to find a molecular weight, the best technique is mass spectrometry.

And for protein what is the best mass spectrometry? Is the MALDI; Matrix Assisted Laser Desorption Ionization spectroscopy. So, therefore obviously, MALDI and the MALDI sensitivity is so high it can identify even the concentrations of 10 power minus 9, minus 10 in that range too. So, if you have a impurity which is at low concentrations, then your protein still you can find out. So, therefore, MALDI is absolutely good thing I am not just telling answer I will be for every question, I am giving the reasoning; so you should also think first the reasoning of this ok.

Let us move on to the next query next query in the tutorial; a carboxylic derivative of 18 crown 6 ether shows increasing transport rates for copper calcium 2 plus as a function of pH increasing pH. So, it is a carboxylic derivative has a 18 crown 6 and it increases as a function of pH for calcium. Whereas, this is exactly reverse for potassium plus what that means? As we increase the concentrations of potassium plus instead of calcium 2 plus; so, it goes down; so explain these observation.

Now, what are what we know is what happens when the pH is added or pH is increased; sorry pH is increased for a carboxyl group what will happen? At very highly acidic pH, carboxylic group will be COOH and as you keep increasing the pH slowly some portion of carboxylate will come at neutral almost most of the thing could be carboxylate and in a basic minimum also it will be completely carboxylic.

So, that self is a half of the answer or in other words main clue has come. So; that means, the calcium is binding very well when you increase the pH, when you increase the pH you have more and more of carboxylate. And we have studied in the chapter that carboxylic groups have a very specific affinity towards calcium. And in fact, nature has modified even (Refer Time: 08:16) acid to one additional carboxylic group be being present.

The same thing at low pH the potassium plus will have a better transport because at low pH; it is completely CO OH and COOH is neutral and that binds vector to calcium potassium plus and that is the reason absolutely. So, always try to analyze the question and try to understand what we know first, then what we wanted to do discuss and debate.

Let us take the next question; so, assume that a metal can either occupy a tetrahedral or occupy a octahedral site with similar ligating groups in a metalloprotein; let us assume that. Suggest a simple spectral method that could differentiate these 2 possibilities and explain is how it would differentiate between these 2 geometries. I have already explained to you; absorption spectroscopy where tetrahedral versus octahedral; tetrahedral versus octahedral; tetrahedral gives a completely allow transitions.

Therefore you have a very high absorption coefficient whereas, octahedral it is both spin as well as a laporte forbidden therefore, you will have a very little absorptivity. So, therefore, one can easily find out by using absorption spectroscopy whether the metal ion has gone into your case of the octahedral or whether into a core of a tetrahedral. So, tetrahedral versus octahedral can be easily found out by absorption spectroscopy ok.

Now, let us look at more and more questions based on each metal low enzyme kind of thing.

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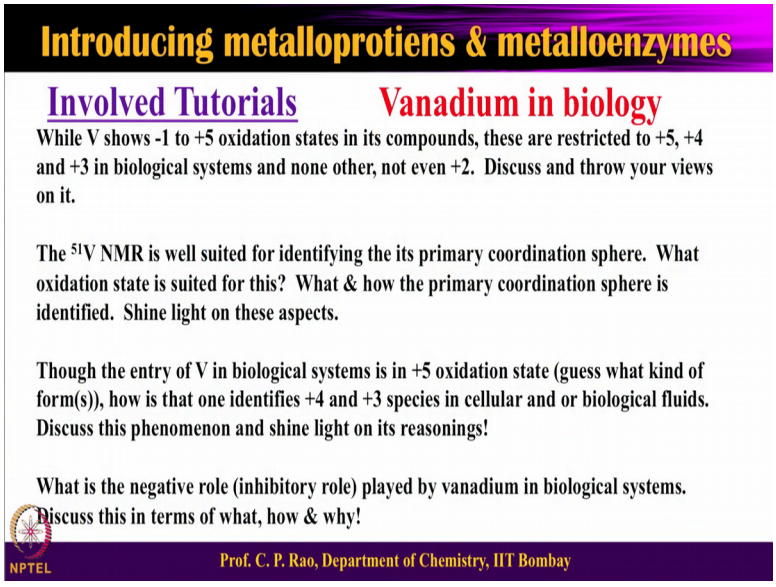

So, let us look at this particular slide the first these are based on the vanadium ok. So, let me read the first question in the top in this while; vanadium shows minus 1 to plus 5 oxidation states in its compound; these are restricted to plus 5 plus 4 plus 3 in biological systems.

And none other not even plus 2; discuss and throw your views on it. So, we know when you open that chemistry compounds; chemistry aspects of vanadium what you will find? You will find all kinds of oxidation states minus 1 to plus 5; you know the carbonyls etcetera will show 0 and minus 1 minus whereas, the other compounds can show plus and oxides can show plus 5 and some extent plus 4 halides etcetera etcetera.

So, therefore, in chemical compounds you have all through minus 1 to plus 5, but whereas, in biological system is only plus 5 plus 4 plus 3 y; so, that is the question. So; that means, there must be some problem if it goes to plus 2; if it goes to plus 2 what could be the problem? It will immediately you know reduce this; this environment or surrounding and get self oxidized to plus 3 or plus 4 or even plus 5.

So, therefore, the lower oxidation state or are strongly reducing therefore, the system biological system does not accept such kind of extreme kind of a things and hence I think it is reasonable to say that this is the reason why the plus 3 plus 4 plus 5 only are used in the biological systems not so, much so plus 2 and lower oxidation states ok.

Let us look at the next question the vanadium 51 NMR is well suited for identifying its primary coordination sphere. What oxidation state is suited for this? So; obviously, any NMR when you look at; look for a non paramagnetic or preferably the with the one which is diamagnetic. So, which of the vanadium is diamagnetic? Vanadium 5 Why? Vanadium 5 is d 0 ok.

What and how the primary coordination sphere is identified? Shine light on it. So, if you go back and look into my the story that I have explained in vanadium in biology; one plot you will see x axis is the vanadium chemical shifts having 0 on one side minus, other side plus and the y axis you have a sigma chi by n; sigma chi is the total the electronegativity of all the atoms by n is the number of ligating centers or the coordination number and that you have seen pretty much.

So, you go to more and more the negative know more and more electronegative atoms, the value will be more and more negative and the more and more positive will have the positive. And now I will say I will not do that although answer I have activated you to look into that particular plot and you can get with the answer very nicely and easily.

So, now by if you know the vanadium 51 NMR; you can draw simply a line from the y axis on to the straight line and try to get what kind of groups are there on this. Yes, I hope you can do that and it is not very difficult, you should be able to do that.

Let us move on to the next one; though the entry of 5 vanadium in biological system is plus 5 oxidation state that is the one. So, what are the species kinds of species for us 5 oxidation states? Vanadate and other validates too; depending upon the concentration and pH. Guess what kind of forms, how is the one identifies plus 4 and plus 3 species state in the cellular and biological fluid?

So, discuss this phenomena and shine its reasonings; see actually though the entry in the biological system is plus 5 when it gets in inside the cells, they get into 4, they even get into 3 because there are cellular reducing agents the cellular reducing agents will reduce this one. So, this can be identified by EPR, when it comes to the vanadium 4 which is d 1 can be absorption come EPR also plus 3 all these kinds of things; when you have plus 5 you can use from the NMR. So, variety of these techniques can be used and identified.

So, it is a basically the cellular reducing agents which will convert plus 5 to plus 4 plus 3 and they are stabilized bicellular molecules, they are complex bicellular molecules after being reduced. So, therefore, they their existence in the cell is not only 5 can be flow plus 4 can be plus 3 ok.

Let us get to the next one what is the negative role or inhibitory role played by vanadium in biological systems? Does this in terms of discuss this in terms of the what, how and why?. So, negative role I am sure you understand in the movies negative role is by the villain; so, it is the same thing here too. So, what kind of a inhibitory role; in case a negative role in biological systems is nothing, but the inhibitory role.

So, you know for this the answer is that you need to compare vanadium as vanadate and the phosphorus as the phosphate. And why we have to compare because it is the phosphorylation; which is most important to make the enzymes to be active which I have told several times in during variety of enzyme reactions; when we are discussing.

Therefore, in the case of the sodium potassium ATPs; a 0 in the second cycle where the potassium binds and the enzyme undergoes a dephosphorylation; at that stage if the vanadate is found is present, the vanadate will bind to the enzyme and make an

irreversible contact with the enzyme, therefore, vanadate will be will be acting like an inhibition in the second part of the cycle. And this also I have given in the course, in one of the slides; you can have a see and try to understand that too.

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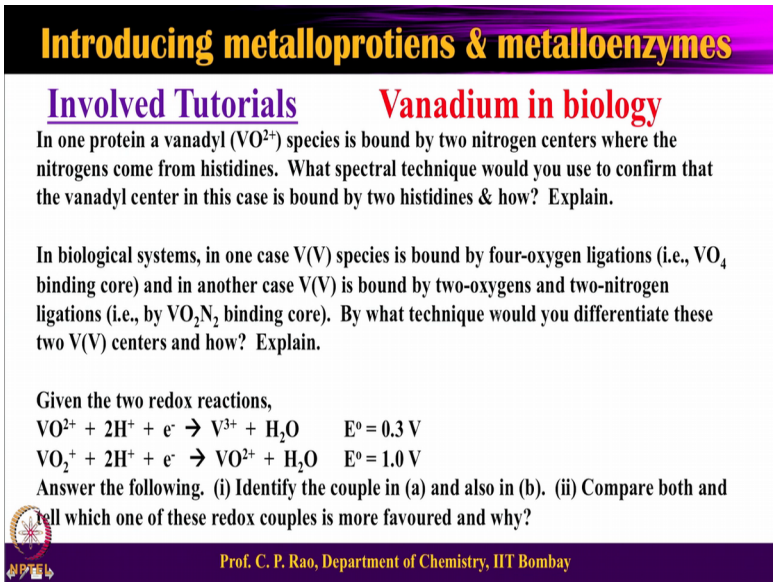

Looks like there are some more questions in my slides; let us go into that. So, in one protein a vanadyl VO 2 plus species is bound by two-nitrogen centers; so, you have a vivo and VO is bound by the two-nitrogen centers where are the nitrogen centers? Primarily histidines and it is also written in the question as histidines what spectral technique would you use to confirm that the vanadyl center is in this case is bound by two histidines now?

Absolutely so, VO 2 plus what is the oxidation state of vanadium? It is 4 plus; what is the d electron system? D 1; so d 1 is a best system for EPR and no other complication and if you go for a high resolution EPR; the nitrogens which are bound, which is having I is equal to 1; will further split and from the number lines, you can make that. The same kind of a question has come earlier in different form; so, the answer is the same.

Let us come to the next question; in biological systems in one case vanadium 5 species is bound by 4 oxygen ligands; VO 4 binding core. In another, the vanadium 5 is bound by two-oxygens and two-nitrogen ligands; VO 2 N 2 binding core. So, what is the technique that you would use to differentiate this? Just a while ago, I talked to you

vanadium 51 NMR, I talked to you it is dependent on the summation of the electronegativities.

When you have all 4 nitrogens; the summation electronegative is greater as compared to the VO 2 N 2 two-oxygens and two-nitrogens, the summation of chi is different; therefore, from this straight line you can easily find out and there NMR positions are absolutely different; therefore, you can differentiate ok.

Go to the next question, there are 2 redox reactions are given; now VO 2 plus plus 2 H plus plus electron giving rise to V 3 plus plus H 2 O; the potential is plus 0.3 ok. Let us get to the next question where we have a redox potentials VO 2 plus plus 2 H plus electron V 3 plus plus H 2 O which has a plus 0.3. And the other equation given is VO 2 plus plus 2 H plus plus electron VO 2 plus plus 2 H 2 O, which is the one electron volts.

So, what is the first; so, identify the couple in a also in b compare both and tell which one of these redox couples is more favored and why? So, for this is what we need to look at? You need to look at first of all what is the couple? So, on the left side VO 2 plus vanadium is 4 plus, right side is V 3 plus; it is a couple V 4 plus versus v 3 plus.

In the next one VO 2 plus; so, that is that is what 4 then plus is that 5; vanadium 5 and then on this side VO 2 plus vanadium is 4; so, 5 to 4. So, 5 to 4 is plus 1; 4 to 3 is plus 0.3; that means, 5 to 4 is more favored as compared to the 4 to 3, but both are favored because both are positive.

And this is you have to take delta G is minus nfe since the potentials are positive it will be still minus. So, more negative value less negative value 5 to 4 is more negative value; 4 to 3 is less negative value of delta G therefore, they are corresponding the feasibilities etcetera ok.

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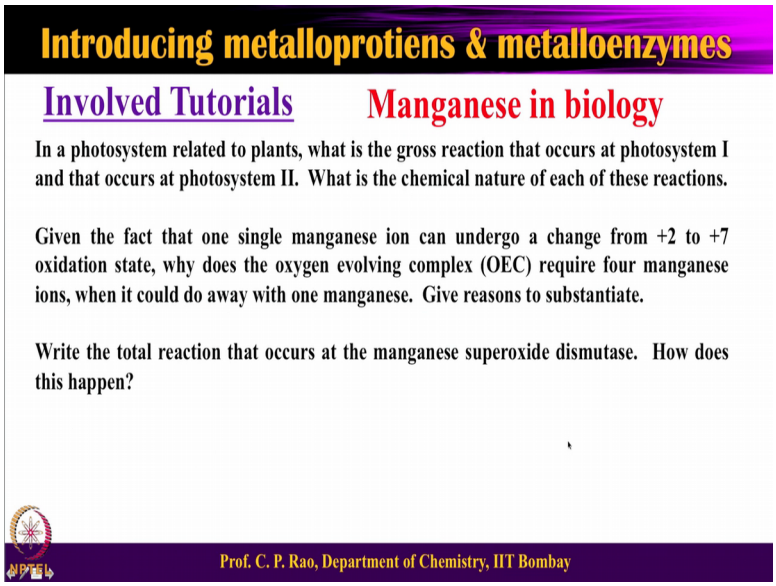

Let us get into another arena of the enzymes which is manganese based; look at the one of the questions first. In a photosystem related to plants, what is the gross reaction that occurs in at for the system I and that occurs at the photosystem II? So, it is already given in the in my slides; the photosystem I, you need to do reduction and bring the carbohydrate.

In the photosystem 2; you need to do the oxidation of the water to O 2. So, so you can easily do that; so, what is the chemical nature of these reactions? The first one is reduction, the second one is oxidation. So, in photosystem I always you have a reduction and photosystem II; you always have a oxidation.

What is the next question; given the fact that one single manganese ion can undergo a change from plus 2 to plus 7 oxidation. We know very well even from high school the teacher tells that the manganese has a large number of oxidation states and in the school level; we will study this plus 2 plus 7 could you have other things are also possible. Why does the oxygen evolving complex require 4 manganese ions? First of all what is the oxygen evolving complex? Is the photosystem 2 which is a manganese complex.

So, what is this reaction? This reaction is basically oxidation that is 2 H 2 O giving rise to O 2 plus 4 H plus plus 4 electrons; so totally how many electrons? 4 electrons; so, 4 electrons change if you have one manganese, if it is in 2, it can go to 3, it can go to 4, it

can go to 5, it can go to 6 or which is in 7 it can come to 6, 5, 4, 3 all of these are possible with one manganese.

But it does not happen; it happens by 4 manganese ions because in this particular enzyme; each reaction is by one pulse of light. And each pulse of light will activate 1 proton, 1 electron and therefore, these are all transferred to the each of metal ion that you have. Therefore, the whole thing is taken care by all the 4 manganese ions not by the one single; so, therefore one requires this; so not the just one.

Of course, where the actual reaction occurs; it is one of the manganese and a calcium center. So, between this calcium center and one of the manganese; the entire conversion of water is 2 OH 2s 2 waters; the OO has to come closer, the OO has to make a single bond, the OO has to make a double bond. The hydrogens which are attached to O should go away etcetera etcetera; then all 4 protons should be out, then electrons will be in to get the bond and both the things are there.

What is the total reaction that occurs in the manganese super oxide dismutase? For any superoxide dismutase, any superoxide dismutase it is the basically the O 2 minus; so, it is not 1, it will give 2 of this which will give one oh 2 and one H 2 O 2; one O 2 and one H 2 O 2.

So, in some enzymes first there will be a reduction; then in the second step oxidation. In some enzymes, first there is a oxidation next there is a reduction this is how it happens. So, it can be in the manganese, it can be in the iron, it can be in the copper etcetera etcetera or all the cases this is what the basic story is and entire thing very clearly given in my slides, I refer you to look back into the slides in these things.

Every one of these; so, you can listen to my lecture followed by you can use the one.

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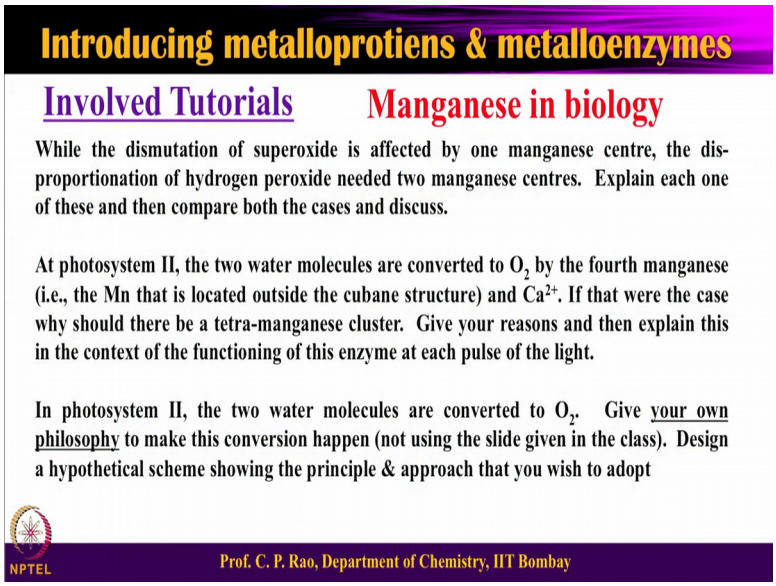

There is some more queries are there manganese in biology; while the dismutation of superoxide is affected by one manganese center; the disproportionation of the hydrogen peroxide needed to manganese centers. So, what is dismutation of superoxide it is called? Superoxide dismutase.

What is our disproportionation of the hydrogen peroxide? It is called catalyst. So, you have a the superoxide dismutase catalase; so, how many electrons in super oxide dismutase? 1 electron, how many electron in peroxide 2 electrons; therefore, you have one metal center in case of superoxide dismutase which can take care of the one electron and 2 electrons have to be taken care therefore, you have 2 manganese centers and what that is what will happen. So, both the electrons simultaneously will be transferring things can happen alright.

In the next questions, it looks like in the previous question itself I extended answer the answer, but we can look at again. At photosystem II; the two water molecules are converted to O 2 by the fourth manganese that is the manganese that is located outside the cubane structure. If you look at the my slide, you will find and calcium 2 plus these 2; these are the ones.

If there were if that were be the case; why should there be a tetra manganese cluster; I have already answered to you, give you reason and then explain this in the context of the functioning of this enzyme at each pulse of the light; I have already explained to you that

the manganese undergoes each time one oxidation state and that is transferred to the neighbor manganese systems etcetera; in order to have a total catalytic.

And the second thing is for each pulse of the light, there is a proton and there is an electron being generated and utilized. And these are being very well taken care with when you have the tetranuclear cluster, but actual reaction occurs in between the fourth manganese and the calcium and that is what we are looking at. So, that is where the reasons that we are going ok.

Let us see in the next in photosystem II; the water molecules are converted to O 2 give your own philosophy to make this conversion happen; not using the slides given in the class, design hypothetical scheme showing the principles appro; principles and approach that you would wish to adopt.

So, to form a O 2 from H 2 O and H 2 O; first of all what you have to do? You have to bring the 2 O s of the which 2 O close by; then what you have to do? You have to disconnect the hydrogens; when you disconnect one hydrogen from this, one hydrogen from the other water; then you can get the one bound. So, one proton out one electron; one proton one electron the 2 electrons is one bond.

In the next stage, one more hydrogen is removed as a proton and the for other water also it is removed; therefore, one more bond the second bond. So, therefore, you will find; you will make 2 bonds. So, this means I need to make an enzyme system or my enzymatic model system; where the 2 metal ions are holding 2 oxygen waters and they are in close proximity. And if I can put some kind of a conformational change to this of course, I am almost mimicking the enzyme and that is how it is. So, I hope you understand in that ok.

Let us start with some more tutorial problems; let us move into other enzymes too. So, the next one is iron in biology; iron case huge amount iron story I have already talked to you, let us start looking at this. Though there is about 1 percent of CO present in cells; the hemoglobin and myoglobin bind selectively 2 O 2 and not to C O how and why? Let us first look that part of it; 1 percent of CO always is present in ourselves because the red dead cells are decomposed.

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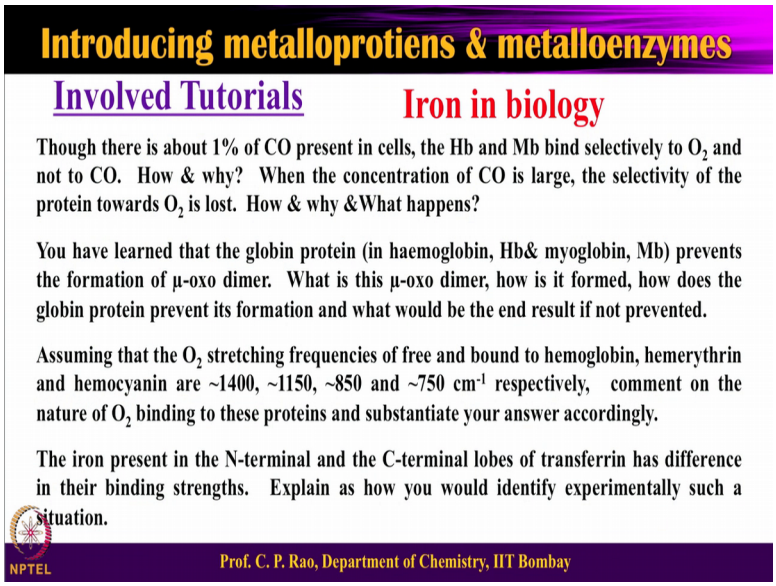

And that is why the reason; why you where you get 1 percent of CO.

Now, you have a CO binding; you have a O 2 binding at 1 percent of this; is the concentration of the CO is not very high. And the enzyme has a mechanism to differentiate the O 2 binding versus CO, which I told in the class; the CO binds straight, O 2 binds bend; the bend one is stabilized by the hydrogen bond with a histidine.

A distal histidine and which is not happening by CO; COP is CO is repelled by a carboxylate group. So, in CO cases it is repelled in O 2 cases it is it is attracted therefore, the system goes towards the O 2 and therefore, O 2 is recognized over CO at those concentrations. However, when the concentration co is very large then there is an equilibrium is shifted and the CO binds.

If the CO binds once; it will continue because CO binding is very strong and is as strong as 30 times to that other O 2. Therefore, the iron CO is more favored and therefore, iron O 2 is not favored. This is what exactly happens when I talked about the story, I also mentioned must have given the story that in most of the fire accidents, the death is not because of the fire burnings, burning skin burnings or body burning; rather than it is because the choking by the CO because there is a lot of smoke because you have a smoke, it is over takes us ok.

So, let us look at the next question; you land the globin protein hemoglobin myoglobin prevents the formation of muoxodimer. What is this mu oxodimer? How is it formed? How does the globin protein prevents its formation and what should be the end result; if not prevented? Mu oxodimer is 2 iron centers are coming closer and the bridged by the oxo and that is what exactly.

So, you have an iron with one O 2 another ion O 2 when both these 2 have come together, it will become initially iron peroxo iron and then iron oxo iron. Therefore, the as iron oxo iron will be the nu oxodimer and once that is formed; the iron centers are also oxidized to form that one. So, if they are oxidized to form that one; then what do you have? You have no way; it will reverse therefore, mu oxodimer formation is a dangerous.

And in case of the enzyme the 2 such units cannot come closer because the with the protein curves these 2 groups and they cannot come closer with their distance of. Because the 2 irons have to come within about 3 angstroms or so; they can never come. Therefore, muoxodimer is not formed and it is being prevented ok.

Assuming that the O 2 stretching frequencies of the free and bound to hemoglobin, hemerethrin and hemocyanin are 1400, 1150, 850, 750 respectively. So, comment on the nature of the O 2 binding to those proteins in the and substantiate your answer accordingly ok. So, assume that the stretching frequencies are this one 1400 quite high, little low, much low, very much low in these things ok.

So, when your O 2 is absolutely intact; we will get 1400 when some little disturbance of perturbance is there; it will become 1150, when it is particularly reduced to single bond; then it will become the 850 hydroperoxide. When it is completely peroxo, then it becomes 750 and that is what happens. So, in the hemoglobin very little perturbation and the hemerethrin a hydroperoxide kind of species and in hemocynine peroxide species; which is bridged between the 2 copper centers.

The iron present in the N-terminal and C-terminal lobes of transferring has difference in their binding strengths. Explain how would you identify the experimentally such a situation. Yes, if you go for release of these N-terminal as well as the C-terminal; you would be able to release one of them at a little higher pH.

The other one at a little lower pH; the one which is at lower pH is more stronger that is happens at the C-terminal. And higher pH will happen the N-terminal, which is somewhat weaker. And this can be done by electrophoresis; many kind of thing, many methods in this is the possible to do all this ok.

So, we have many more questions and then I will continue as we keep moving on across in the next class.

Thank you very much.