Inorganic Chemistry of Life Principles & Properties
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Lecture - 57
Tutorials – Part I

Welcome you all to the next class, as you noticed that I have completed the main part of the syllabus as well as the highlights in the last few classes. So, in this class we will start with the tutorials covering over. Not only this class maybe next 2, 3, 4 classes we will try to cover the tutorial parts. So, that it will help students a lot how to read, what to look for, how to understand the subject when they read from my slides and they lead from my lectures. So, therefore, this is a kind of thing.

So, initially I have given very simple ones, then I have taken to a little bit more tougher side. So you can you know see the level that it grows from simple to the do somewhat serious kind of things too ok. Look at this particular slide I have asked a very simple things in this just a kind of a warm up kind of an exercise.

Expand the following EXAFS; so what is EXAFS? I am sure you all know; Extended X-ray, absorption, fine structure spectroscopy and expansion of MALDI ok.

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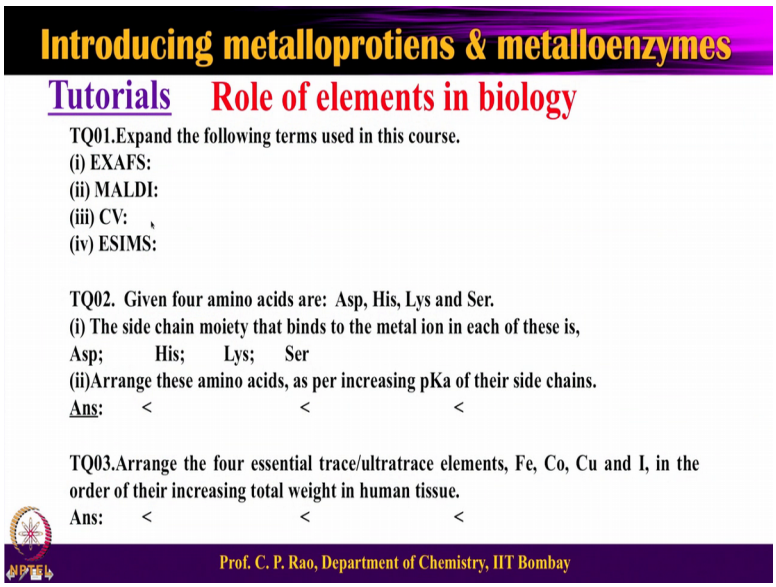

I am sure you know that Matrix Assisted Laser Desorption Ionization; CV it is used for Cyclic Voltammetry, ESIMS Electro Spray Ionization Mass Spectrometry.

So, there are variety of these things just few I have given for as an example; this does not mean only these are the ones. So, you should familiarize yourself, but some of the important cases this kind of short reform usage. Also you need to know primarily what are the kinds of side chains are present in the amino acids particularly those which are important in the bioinorganic chemistry, what do you mean by important in the bioinorganic chemistry? Those which extend coordination to the metal center so which are those things, we have already looked at in the during the lectures aspartic acid, glutamic acid, histidine, lysine, serine etcetera.

So, all of these have got the side chains which are very important. You can easily see these things from my notes and you can fake out the aspartic is the one where you have the carboxylic histidine is the one where you have the imidazole etcetera. I have also given you the pKa's and you know that the alcoholic OH will have very high pKa, while the COOH will have very low pKa and imidazole and the phenolic will have in between and the SH will have slightly higher too.

So, this is another question that is arranged these amino acids that aspartic histidine lysine serine as per the increasing order there pKa; how will one know this? If you know the side chains; so, how will you know the side chains? You need to familiarize yourself. So, if you familiarize yourself with the side chains at least about 10 amino acids, half a dozen definitely and about 10 amino acids, if you can be familiarize yourself with that it will be very nice.

Go to the next carry; so, this is something to do with the essential trace elements I gave how much they are present for a body weight of particular thing etcetera. So, based on that these are all essential trace and ultra trace elements iron, copper, chiron cobalt copper, iodine etcetera. So this you can easily make out these are all present in the human tissue and will be the highest among all these things followed by copper and then followed by iron, followed by cobalt.

So, these are the kinds of things you can look at the actual values you do not need to worry about what I say in this particular tutorial. This is just to warm you up with the

kind of a questions that would come later on. So, I repeat that you need to read all these things in the perspective on this.

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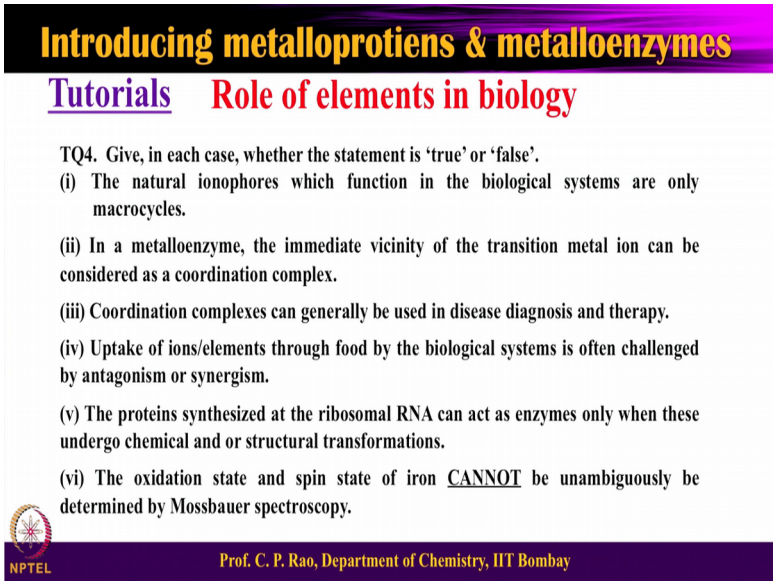

So now you are going to feel something's what do we read, let us get some more idea from this another one. Again very simple one of thing; so, I have designed some queries for a simple things like to say true or false. The natural ionophores which function in the biological systems are only macro cycles; is it only micro cycles? No there are macro cycles a lot, there are non macro cycles also ok. So, you have both macro cycles and non macro cycles therefore this statement is not correct.

So, in a metalloenzymes the immediate vicinity of the transition metal ion can be considered as a coordination complex; that is what how I started within the from the beginning. So, you look at the metal center in any metalloprotein, it is connected to the side chains of several amino acids one or more amino acids, sometimes even the heme followed my amino acids. And I told you that so therefore, this can be considered as their coordination complex. So, what is the answer? You understand now I told you already that ok.

Coordination complexes can generally be used in disease diagnosis and therapy; yes absolutely I have given initially in the introductory case itself a couple of slides, which I have listed the elements, their make compounds and in what kind of a diseases they are

found all of these. So, I am going to repeat all these; so, you can see that you can study all of them, so therefore, the yes kind of thing.

So, uptake of ions and electrons through food by the biological systems is often challenged by the antagonism and synergism. Yes, absolutely we know that a lot of elements entry of one of the element is opposed by another, entry of one of the element is supported by other. Opposed by other is called antagonism suppose by the other is called the synergism. So, therefore we have all the antagonist, synergistic all these things is; so, true statement.

The next query is the protein synthesis; I am doing more elaborate answer than required true false. True false is for you to write, but this is where I am giving reasoning. So, therefore, kindly listen carefully when you are listening to this particular part of the lectures, make note of the things too.

So, proteins synthesized at the ribosomal RNA can be; can act as enzyme. Only when these undergo chemical and the structural transformations; absolutely correct reason why? The reason is when it is made at the ribosomal RNA; so it just connects one amino acid with next one amino acid the next etcetera forms a chain.

So, we do not have enough sufficient time to completely structurally fold. So, there may be a partial folding; unless a complete folding is occurring, you do not expect a function to happen you cannot expect. That is what I told you a protein and amino acid joining together polypeptide into a protein by the kind of a 3 dimensional structure. Once you have the structure well folded then you will have a function then this is an enzyme; I explained to you several times that ok.

The oxidation state and spin state of iron cannot be unambiguously be determined by Mossbauer. You know that is not correct, it can be very unambiguously determined because this is only the technique where you can measure both identify both oxidation state and spin state of the iron.

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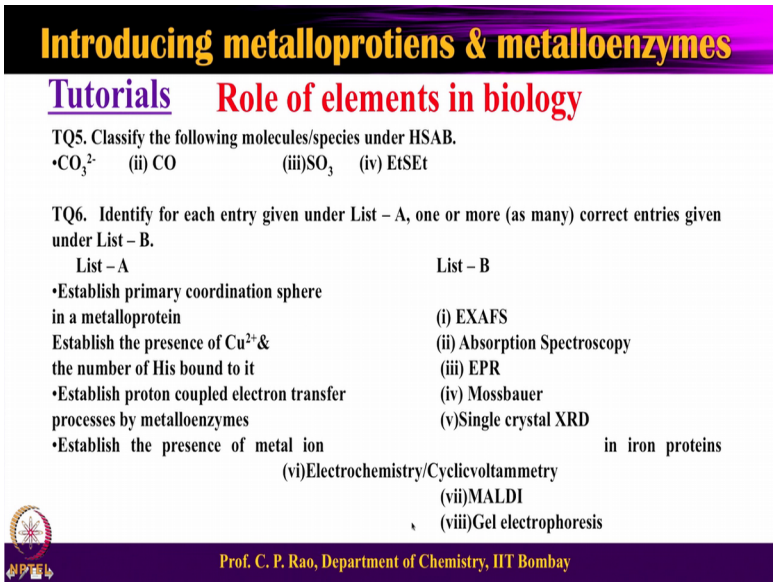

There is some on the exercise on the Hard Soft, Acid Bases you know the this is dependent on the; if you have a species then you have to identify which atom would be binding etcetera, first thing; then that atom whether you its oxidation state or formal kind of oxidation state, size, parameters you need to think in the mind before you can make whether it is a hard or soft.

Then before you can make acid and base you need to make whether it has; it can donate electrons and it will be brace a if it can it has some empty orbitals can accept electrons then it will be acid. So, therefore, Lewis acid Lewis base hard and soft I think you can sit at a home and do all this because I have given all the things for example, carbon monoxide maximum number of cases it is through the carbon; so, therefore, that you need to take ok. So, therefore carbon whatever you know the things.

I have given another thing to just warm up that between the techniques and the and the phenomenon that you understand the phenomena you observe or given here. So, the phenomena you can establish is given on the list A and the technique is given in B and sometimes you can have more than one also.

So, establish primary coordination sphere; so you can establish by EXAFS; of course, you can also establish by a single crystal XRD ok. And primarily these are the 2 where you can fully identify the things in that. See this is establish primary coordination sphere in a metalloprotein; establish the presence of copper 2 plus and the number of histidines.

Establish the presence of a copper 2 plus and the number, now the presence of the copper 2 plus suppose you take a apoprotein you add a copper 2 plus you can look at even absorption spectroscopy; absolutely no problem.

And you can also look at the EPR spectroscopy to find the; find that particular copper is there etcetera. Absorption you can use, EPR you can use also; of course, single crystal XRD and all can be used for all. So, therefore, let us not take that as a common, but the thing in this and if you want you can use that as one of the answer too. And the number of histidines can be formed from EPR; how? You have a super hyperfine coupling, then you have first thing is spin cup the nuclear spin.

So, the electron spin coupled with the nuclear spin; so nuclear spin S 3 O 2 therefore, the FO 2 into 3 O 2 by plus 1; that will be your 4 lines. And each of the 4 line can be further 2 Ni plus 1, i is the nuclear spin for example, nitrogen present for the histidine. So, from that we can make out how many lines you can calculate and get it. So, I hope you understand that everything can be formed from single crystal XRD ok.

Establish proton coupled electron transfer process by metalloenzymes. Proton coupled electron transfer you can find primarily and by the voltammetric. So, electrochemistry or voltammetric everything no other thing I will give all; you can do the some extent NMR etcetera, but you have to do huge amount of iterations, but it is not a direct method that method is electrochemistry.

So, establish the presence of a metal ion in iron proteins, in iron proteins, in the iron proteins establish the presence of an iron Mossbauer. So, if you have a Mossbauer here; yes absolutely you can find straight away. Of course, you can also use EPR etcetera and you can also use for that matter some stage the absorption, but mainly of these kind of things ok.

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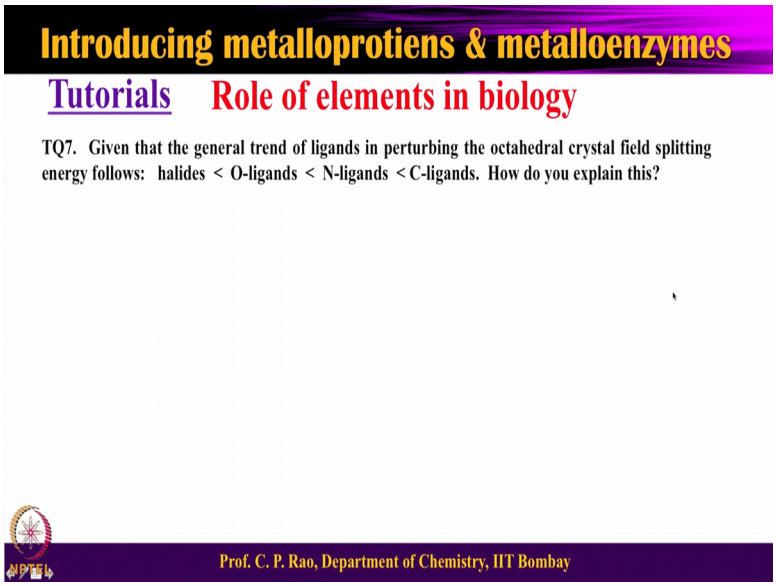

I also explained to you at some stage that the octahedral field splitting is being the T to G to EG level splitting can be reduced or increased by the type of the ligands that you have, by the nature of the metal ion you have, the oxidation state in the metal ion you have.

For example, if you take a particular metal same and then use highlights and oxygen ligand, nitrogen ligands, carbon ligands this is already given to you; highlights will be splitting less than the oxalyase and oxoligands less than nitrogen, less than copper ligand. So, how do you explain this?

So, this is not by the electronegativity; it is by the pi acid character, not this sigma part of it, it is the pi aspect of it not the sigma aspect of it. All of the ligands will bind to the metal center through sigma; so, the sigma bond characteristics are not so, important, but the pi bond characteristics whether the pi base, pi acid. So, it is called pi acceptor, pi donor kind of things that is where you can see.

So, with this kind of a warm up; I will take you into a bit of involved type somewhat a little bit more involved ok. Few of them would be easier, but some of them would require you to think, you can only think after you understand my lecture and that is how it is ok.

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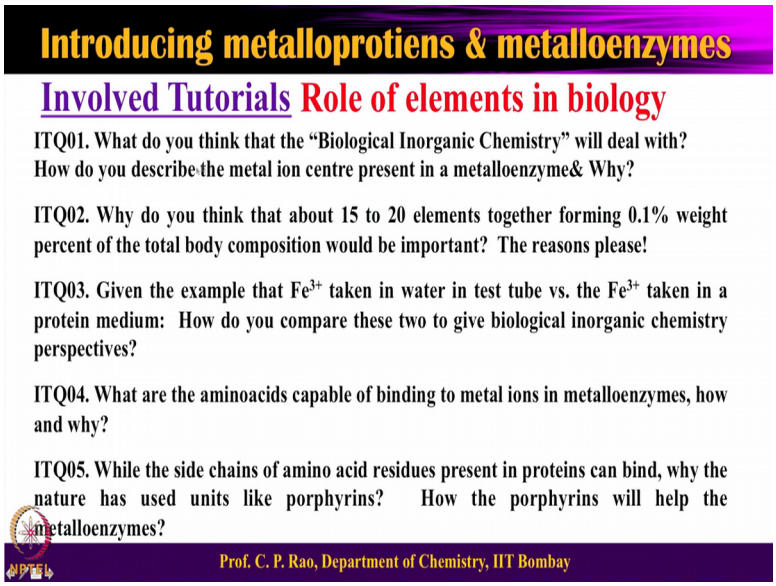

Let us see this some of the. Let us see what the question I have here for you; what do you think that the biological inorganic chemistry will deal with, how do you describe the metal ion center present in a metalloenzyme and why?

So, what do you think that bioinorganic chemistry will deal with bioinrganic chemistry deals with? The role, interaction, triggering aspects of activations and triggering aspects of inorganic elements on the biological systems; most relevant one is the protein or enzyme. So, you will that is what one would expect in fact, that is what all we have covered also.

And also you can see that not only the enzymes proteins, you can also have nucleic acids; you can also be there other biological molecule. To extend this, you can also say their interaction with the cells, their interaction with the organs as well; so these are all one can expect in this.

So, already I have answered the second part of this question; how do you describe the metal ion center present in this; in the metalloenzyme why, I have already explained to you that it is can be considered as a coordination complex because the metal ion is bound to the side chains of the metalloprotein. Side chains will form a small coordination complex and now it is a coordination, metal ion, primary co-ordinations and the secondary and other aspects of the protein enzyme protein.

So, therefore, metal the co-ordinations sphere of a metal center influenced by a protein that is what ok. And this is the reasons or that there is an influence by the primary coordination, there is also influence by the secondary co-ordinations and other things too. Let us look at another query not so difficult one, but somewhat easier. So, why do you think that about 15 to 20 elements together forming 0.1 weight percent of the total body composition would be important?

So, what is the reason? So, there are some 15 to 20 elements which are they together is present in less than point together; not each one is 0.1 weight percent, together is it is very small quantities. Because these are present in the enzymes their total quantity will be not so high, but important because without these enzymes; the body will not function.

So, therefore, even though they are very small; the total 15 20 elements together is only 0.1 percent, but still they are very vital. Because they make the entire enzymes to work, cell signals to function; all these kinds of things or the biochemical processes go to go on in the body. So, all of these are triggered or initiated or processed or favored or supported by the metal ions in metalloproteins, metal ions, anions various kinds of things.

So, let us come to the third one; the given the example that the iron 3 plus taken in water, in test tube versus the iron 3 plus taken in protein medium. How do you compare these two to give biologically inorganic chemistry perspective? This is something which you need to think a bit, I would say this is a bit of involved; probably for that matter even the previous question also a kind of a involved one.

So, all that I am saying is you take a iron 3 salt put in the test tube, live in the water within a short period; you will find the small precipitated then over a period of time this will become insoluble kind of a precipitated. As you know very well the iron 3 plus getting hydrolyzed initially to hydroxide and later on to oxides, where the iron ions are bridged by the oxo species etcetera are nonreactive.

In if you take the same thing and put the iron 3 plus into a protein or an enzyme; no it will not allow the iron to get hydroxy, iron to get iron oxide etcetera no. And the enzyme will or protein will bind to the iron center; so, that it is hydrolysis part; if the hydrolyzing capabilities are reduced unless you have a few coordinations opening. Even then you will not find Fe 2 O 3 etcetera, you will find some other kind of a reactions in that too.

So, therefore the biological inorganic chemistry is grown when the iron is put in a protein, but in the same iron 3 plus is put in water, it dies off as Fe 2. Fe 3 O 4 going via other oxides; hydroxide oxide Fe 3 O 4 finally, as their dead end and this is absolutely the beginning and this will be flourishing the whole thing etcetera.

So, I hope you understand this is how you need to really think and address. So, what are the amino acids capable of binding to metal ions in metalloenzymes how and why? A partly ions are in the previous questions something like aspartic, glutamic, serine the arginine, then you have cysteine, then histidine, then tyrosine and tryptophan some of these things can bind. So, therefore, you sit the sit and see I have also given in one of the slide a list of these amino acids which are involved in the primary coordination sphere. And a few of them may be involved in the secondary coordination sphere of that. Those which are involved in the primary coordination sphere can also be involved in the secondary coordination sphere too.

So, these are all kinds of things and so these are all reason is the kind of a side chain that you have and the side chain can bind to the center. Let us look at this particular a query you have here; while the side chains of the amino acid residues present in proteins can bind, why the nature has used units like porphyrin, how the porphyrins will help in metalloenzymes? This requires a much more comprehension, this we can understand and explain only when you have gone through my lectures to good extent.

The first one is yes; there are metalloenzymes, metalloproteins and are they not happy? That they are all from the side chains; why the nature has chosen porphyrins, what could be the thing? The porphyrins will provide a different kind of a stability, different kind of a redox character to the metal center. Therefore, in some cases the nature has chosen the porphyrin like molecules; in other cases it has to chosen just the side chains.

In third category, both porphyrin as well as the side chains all of these are there and modified side chains also. So, there are variety of these things; so all of these to control, regulate the reactivity at the metal center; particularly its redox properties, you can say in the black box catalytic properties; instead you can say the electron transfer properties too. So, therefore that is how these will help; I have already answered ok.

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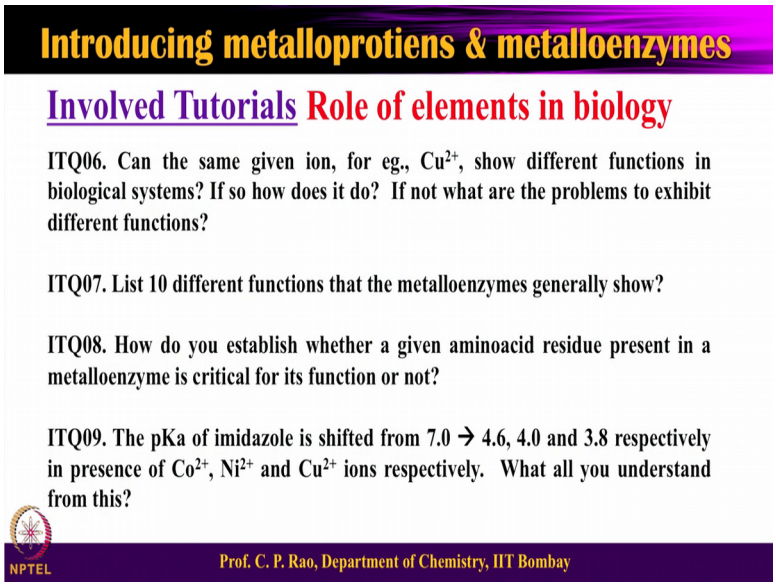

Let us look at a bit more this is that the previous one just now I completed in the on the porphyrins the role is; obviously, an involved kind of a query. Let us look at another one; can the same given ion for example, copper 2 plus show different function in biological systems, if so how does it do, if not what are the problems to exhibit different functions, what are the problem in order to exhibit the different functions; so see that.

So, a same ion can be involved in variety; if you take iron there are several hundreds of enzymes are there and at least a dozen classes of enzymes are there. If we take zinc there are several hundreds of enzymes are there; at least more than half a dozen classes of enzymes are there. Take copper there are several hundreds of enzymes are there; dozen different classes of enzymes are there.

So, therefore, same ion can show different functions in biological systems; provided the protein is different, provided the protein mantle and the immediate surrounding; the primary coordination, the secondary coordination and the whole protein is different. So, therefore, in such cases this can be this is possible. So, if you think it will not do that then there is no meaning of the extending half a dozen proteins, half a dozen enzymes for the same metal ion; nothing of that kind would be there too ok.

So, go to the next one is a list 10 different functions that the metalloenzymes generally show; this is a routine question you can just look at my slides like electron transfer, proton transfer, oxidative reactions, reductive reactions, group transfer reactions,

hydrolysis kind of reactions and ligase kind of reactions; where you break the bond without using the water, break the bond with using the water, hydrolase and you can use ligase, you can use lyse all those kinds of things variety kind of things.

So, there you can always make the things there. So, that is all possible that you can straight away get from my slides. And this is not an involved one; this is just to try to make yourself familiarize that is the reason why I gave; so, you can warm up yourself with all those things.

Now, how do you establish whether a given amino acid residue present in the metalloenzyme is critical for its function or not? How will you establish? Anything is critical or not. So, when somebody claims something is critical you bring absence of that, you make an absence that; you remove that one. If you remove that one that is not there; if that is not there if the function is still there; that means, that is not required, that is not essential. Another hand you remove the thing and if the; when you remove the function is not happening then that is obviously required.

So, similarly in this as well; so, what do you do; in a metalloenzyme, a metal ion is there and an amino acid is bound to that now you your query is that whether that amino acid is really essential for the function or not. So, what you need to do? You have to replace that amino acid by something else; this is what is called the mutagenesis. I have already talked to you site targeted mutagenesis; you can have one size, you can have multiple size these are all known very well.

So, using the site targeted mutagenesis; you can replace that particular amino acid by node by in the protein in its genetic version and then express then you get a protein in the protein in which that particular amino acid is not present. And as some other amino acid, which does not have the same side chain is present; therefore, and at that stage you check the function of the enzyme. If you check this function of enzyme and if your answer is the function is stopped and function is changed then you say; yes that was required.

Same thing is true even if I ask a question instead of one amino acid in a metalloenzyme of iron, I will replace the iron by some other ion; how do I know that the iron is essential for that particular? So, I just remove; I put some reagent like the EDTA or some other chelating agent by which I can take out the metal ions from the protein and make a apoprotein.

And see whether the apoprotein gives any function or not; from that you can be establish ok. I come to the next question in the involved type the pKa of imidazole is shifted from 7 to 4.6, 4.0 3.8 respectively in presence of cobalt 2 plus, nickel 2 plus and copper 2 plus ions respectively.

So, what all you understand from this, so what do you understand from this? In the water you have the pKa with a 7.0 mix down. So; that means, these for example, the imidazole; the imidazole group is activated by these metal ions. They make, they try to displace the proton; the imidazole has got NH; now the metal ion competes for this NH; so, it will become N and the metal ion NH 2; N metal ion ok.

So, and in the process it brings down the pKa from 7 to 4.6; so let us take as a 5. So, 2 units difference; that means, about 100 fold kind of thing 7 to 4 3 10 power 3 7 to 3.8 roughly. So, that is the kind of thing for cobalt, nickel, copper; the smaller the pKa that brings that is the stronger that it will bind also, so all these things are important.

So that is why the side chain PKA's are modified in presence of the metal ion and metal ion will displace the proton and then bind to itself and thereby coordination sphere is formed. Some more examples of involved tutorials ok

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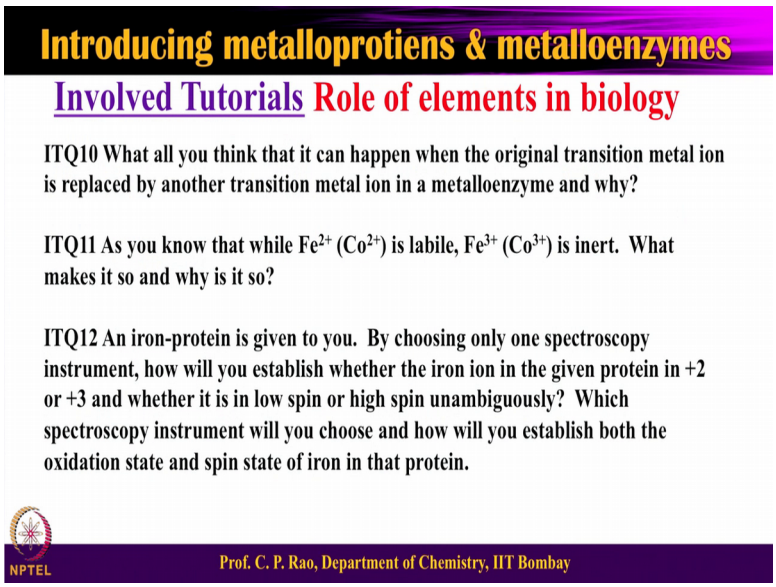

What all you think that it can happen; when the original transition metal ion is replaced by another transition metal ion in a metalloenzyme and why? I have already answered this question previously when the amino acid has come.

So, now you take let us say a copper zinc enzyme and remove the zinc put back, put copper what will happen? Check the activity ok. So, the activity may be stopped; it may show a different kind of activity or less activity; there are 3 possibilities, the activity is reduced, no activity or altogether new activity; there are 3 possibilities in this. So, therefore, you can easily make out whether that is; so, what happens etcetera everything.

In this process, if you are a researcher you should be excited that you take some metalloprotein from natural source, change the; remove the metal ion put a new one and start playing with it to get a new enzymatic activity. That is how your research will go and you can publish all the results for the future. And for the future utility of the same and that is how it goes. You can use for some bulk preparation of certain molecules, materials by using such kind of things too ok. As you know that while iron 2 plus and cobalt 2 plus for example, a labile, the iron 3 plus or the cobalt 3 plus is inert what makes it so and why is it so?

So, I am sure I have given you the plore; I giving you different ions and their liabilities, inertness etcetera. As you know the liability can be identified from the crystal field stabilization energy of the of the ground term minus the crystal field stabilization energy of the activated complex; if this were to be positive it is a labile if this were to be negative, it is inert kind of thing.

And although it depends upon the spin state and this size of the ion all of these; in alkali alkaline earth is only sizes ion in main group elements its only the size of the ion, but in transition metal ions both the size as well as the spin states; all of these we will be playing a role ok.

Let us look at another question an iron protein is given to you; by choosing only one spectroscopy instrument, only one spectral instrument not more. How will you establish whether the iron ion in the given protein is plus 2 or plus 3, whether it is in this low spin or a high spin and obviously, I have already answered and the earlier question in a different form of the question. Which spectroscopy instruments will you choose and how

will you establish both the oxidation state and spin state are the iron in that particular protein ok?

So, this is I am sure I have already answered to you earlier; it is a Mossbauer spectroscopy. In the Mossbauer spectroscopy based on the isomer shift and quadrupolar coupling; so, you can easily find out, you can look back into the things of the type.

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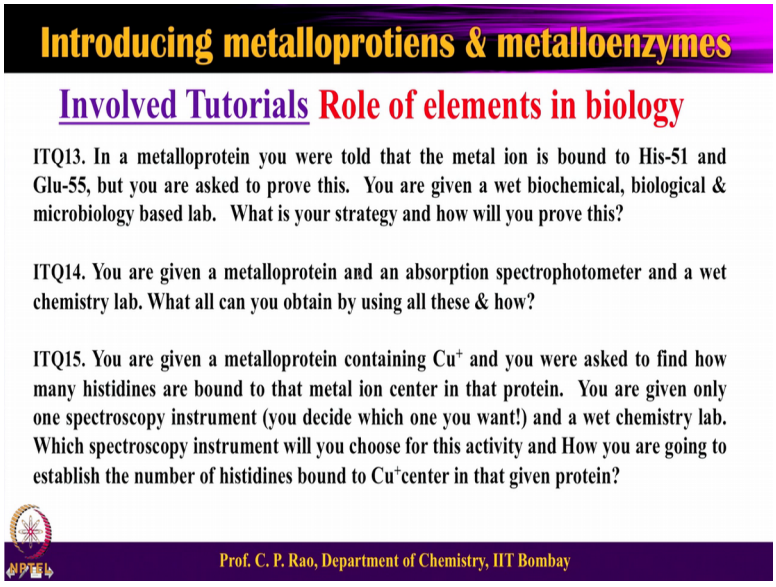

In a metalloprotein you are told that the metal iron is bound to histidine 51 and glutamic 55, but you are asked to prove this. You are given a wet bio chemical, biological and microbiology based lab; what is your strategy, how will you prove this?

If you answer this, I am sure you will be able to design some research problems for yourself or for your supervisor; that is where the thing is. Answers are there in the question; earlier I used to when I give histidine, glutamic I do not use to give the number now given number; that means, the position histidine 51, glutamic 55. Now you know in the protein in that 51 there is a histidine, in the 55 and there is a glutamic; bound to this.

So, therefore, one can do; so you are asked to prove this particular thing in that. So, you can do by wet chemical methods and biological methods; so biochemical biological where you can replace this particular amino acid by a different one and show the property that it does not show it is required by this. Similarly for a glutamic, you change the thing by genetically or even chemically.

So, in some of these can also be converted chemically; the histidine chemically, the glutamic and then look at the function and then you can try to prove that. So, these are the kinds of strategy for this what you require? Wet lab; you require the bio chemical biological kind of things that you require that is why in the question I have given that ok.

You are given a metalloprotein and absorption spectrophotometer and a wet chemistry lab; what all can you obtain by using all these and how? So, if I given a metal a metalloprotein, you can remove the metal ion, you can add the metal ion, you can add different levels, you can study absorption spectrum.

Now you can also remove that metal ion; keep adding the other metal ions and look at the change in the absorption spectroscopy or even as a function of concentration, even a function of pH, then you can establish whether they are binding, they are not binding. If they are binding, they are binding in octahedral center, they are binding into tetrahedral center; all of these information can be obtained from these ok.

So, we will continue in the next class further with the several more questions of this kind, which is was some of them could be involved, some of them could be simple, some of them can be could be very involved. Now you understand you have to apply mind and thing in these ones.

Thank you very much.