Biological Inorganic Chemistry
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Lecture -11
Introduction

Hello, good morning everybody, so where we are today? So, we will start today the module 3, where will be talking about the different physical methods or the physical techniques, which are basically required for the characterization of any particular metal ion site in all these metalloproteins and metalloenzymes. So, the lecture 11 will be devoted to the introduction part how we think and how we tackle these particular physical methods that we will see.

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So, if we consider that the concepts what we can cover briefly in this particular class is that the different analytical and spectroscopic techniques. We all know because we are very much expert from your school and college days, that you know what is analytical chemistry. So, this analytical part basically, it has two components; one is the qualitative aspect and another is the quantitative aspect. We all know that when you go for the titrimetric method for analyzing any metal ion, say iron sample is there in your test tube. And we know how to detect first the presence of iron, and then quantify it.

So, it will have definitely two different components; one is the qualitative aspect and another is the quantitative aspect. So, similarly in this world of biology also, if we find to get some of these

informations based on different spectroscopic techniques, because, the concentration of these metal ions will be very less. When you talk about iron in the typical solution, what is given to you in your analytical chemistry class, you know the concentration is very high. Sometimes it is in the decimolar or the decinormal solution. But, when you have this biological sample, say iron bearing metalloprotein or metalloenzyme.

We definitely must have some understanding that the concentration of iron available from that particular species, even if you can take out that iron from that particular protein or the enzyme environment, the amount of iron you will be getting is very, very less. So, the technique what will be using for identifying that metal ion center must be very much sensitive.

Then, if we can apply those techniques or the analytical methods for the determination of structures, and the mechanism of a particular type of reaction, and what are their general applicability and few examples, basically not the very basic one what we know, but the high end one though, so highly specific ones how we can use or how we can utilize it for this identification.

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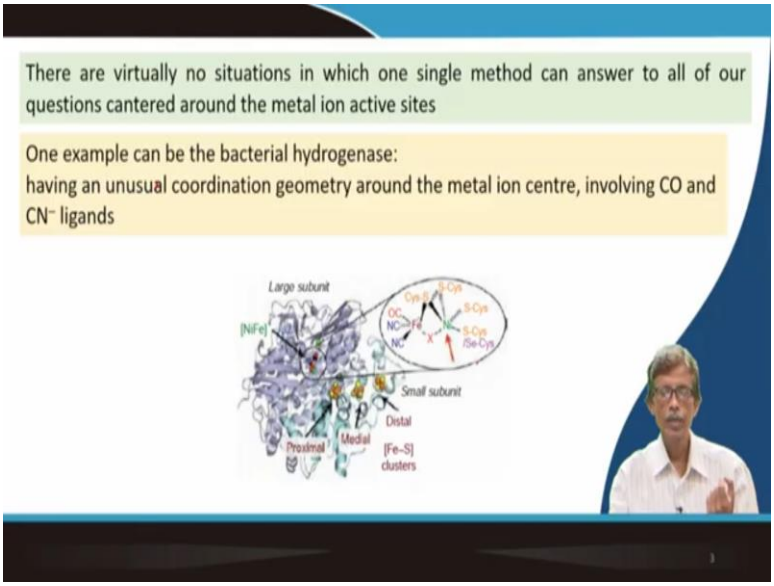

So, there are virtually no situations, in which one single method can answer all of our questions centered around the metal ion active site. So, if your iron is there, if we consider that we have iron in microglobulin environment. So, what is the immediate environment of that metal ion that we find with the knowledge of your bio-coordination chemistry. But, in the bigger way how

much do you know and how many of these proteins are there? Particularly, if we talk about the corresponding globin part. So, how the globin which is ligand, but to your true definition we can consider that globin chain as a monodentate nitrogen donor ligand.

Where, the terminal point is your histidine residue and the immediate site chain of the histidine residue will be coordinating, to the fifth coordination site around the iron. Only to we have seen that one, two, three, four; four of these sites are occupied by the nitrogen of the porphyrin ring; so, fifth will come from the bottom, as the corresponding globin chain nitrogen. So, if we try to understand everything, it is not, is not always a possible way to find out these through one particular technique; so, we will take many examples of these techniques. If we take that a little bit complicated one, but to understand that in a different way; we know the hydrogenase.

So, hydrogenases are nothing but the reversible assimilation of hydrogen, hydrogen molecule or the reduction of the protons to hydrogen; but, it has a very unusual coordination geometry. So, first challenge to a biochemist or a bioinorganic chemist is that to identify the environment; because during the last 60 years or so, we know through the structural determination of the myoglobin and the haemoglobin molecules, we know now definitely the typical coordination environment around this iron center in myoglobin and haemoglobin molecules. But, these are the most recent advances during the last 30 years or so, people have identified first the catalytic site, where that the metal ions are present.

So, the next challenge would be definitely to identify coordination geometry, which is associated with this bacterial hydrogenase. If it is unusual one that means you do not have any typical example, when you go for the coordination chemistry in a laboratory; or many other related coordination environment in the around the metal ions in the biology also. So, here we find for the first time people have identified that no biological ligand is there; but the typical inorganic ligands or the ligand anions what we know from our school days again, the metal carbonyls we all know the nickel tetra carbonyl we have learnt in school days also, and the tetracynonickelate type of things.

So, these two ligands are the first time people have identified in the biology also that okay, the biological part, the protein part or the amino acid residues are there around the metal ion. But, apart from that you can also have the corresponding involvement of the carbon monoxide and the CN minus ion. So, how to tackle this particular environment? So, clearly look at this particular

one. So, this is a huge molecule, many other things are there; so, it has been zoomed, this basically this particular smaller part inside the cavity, where you find that you can have a heterobimetallic system.

So, immediate challenge was there to identify the nature of the metal ion. The way I was telling you that if you have something some unknown sample is given to you for your inorganic qualitative analysis, a solid sample, a solid salt is there; we call an inorganic salt analysis. A solid sample is there and you have to identify whether you have iron there, or any other metal ion. Sometime we go beyond that much more complicated fashion, we can have a mixture of two salts or salts which can have four radicals, we call radicals, the cationic radicals and the anionic radicals.

So, if you are able to handle such samples, now you are able to handle this particular environment also; where you have to see two different metallic centers; one is iron, another is nickel. And immediately people identified what, people identified this particular iron center as well as the nickel center, and then the biological ligands first. So, the biogenic ligands which are coming from the huge protein environment; so the in square bracket, the NiFe environment, so Nife site is this site; where you have this iron and this nickel. So, you see that the terminal nickel, you can have the cysteinate sulphur; the cysteine amino acid residues, where you have the S-H group.

And when it is deprotonated, it gives you S minus; and two such S minus groups can bind to a particular nickel center; so, this is on from the right hand side. And the left hand side, you see iron. But, how you stabilize that particular iron center in close vicinity of the nickel center? What you can do the biological manipulation is very nice that you bring two other cysteine sulphur residues. Two are already bound to your nickel center, you bring two more. But, these two are not only corresponding to your nickel center, but it can also bridge the iron center.

So, that way you are trapping the iron site close to your nickel site. So, these bridging ligands are very important and identification of these bridging ligands are also very important, to bring the iron center close to the nickel. Then, we have this unusual coordination, coordination in terms of typical inorganic anionic ligand or one neutral ligand. So, carbon monoxide is your neutral ligand and you CN minus CN minus are the anionic ligand those are there. So, apart from these

also you have ion sulphur clusters and all other things. But, interestingly we will come back due to this, what a particular technique we can use as this X.

So, during this catalytic activity or the catalytic cycle of hydrogenases, where if you consider that okay we are simply reducing the proton, that means H plus. You have to supply the electron to H plus o get one H dot or simply hydrogen atom. And two such hydrogen atoms are binding together we know that the isolated hydrogen atoms are unstable energetically. So, two of such atoms will collapse together, forming the hydrogen gas or hydrogen molecule itself. So, how this x can be there? So, during this catalytic cycle also if hydrogen can come over there and we all know not that H plus, but H minus.

The hydride ion, this hydride ion can function as a very good ligand. H plus is a Lewis acid, but H minus is a Lewis base. And all we know that the Lewis bases are very good ligands and these are anionic in nature. So, if we find that this H minus is bridging again, that means stabilizing this FeNi system. That means you require this bimetallic site for this activity, for this hydrogenase activity. And the binding of this H, because you may not get it when you determine the x-ray structure.

Because at the end only will come to the x-ray diffraction technique and the x-ray structure determination technique, where we will find that in the native state, because this is a catalytic intermediate; where this H minus can come and bind and occupy this position of X, which is designated over here. So, what other instrumental technique or the analytical technique can be useful to determine some of the intermediate step or some of this particular active state, where your species is responsible for your catalytic cycle to complete.

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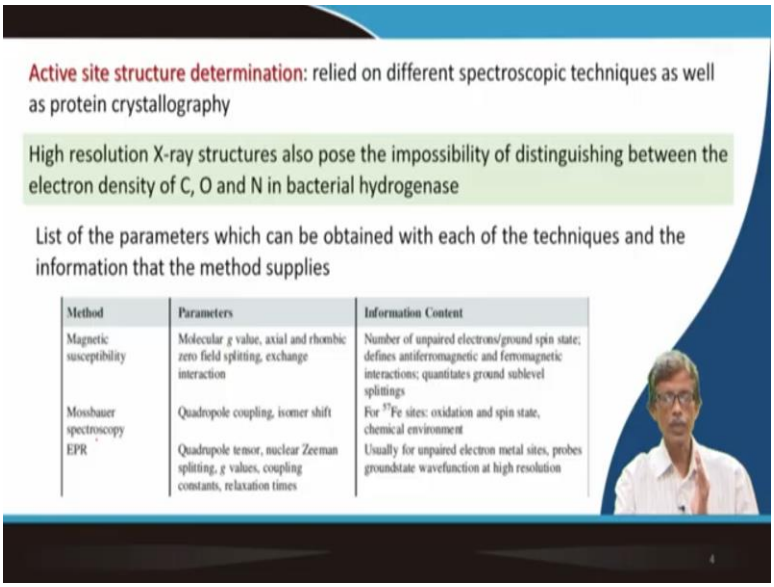

So, we will see that how this active site structure determination is important and this is relied on different spectroscopic as well as protein crystallography. So, at the end will go for the crystallography; that means the three dimensional full structure the way we determine the small molecule structures also. Because that is the ultimate picture; what we can have through this protein crystallography. But is a difficult technique, because you have to get good crystal, particularly the single crystals of these protein molecules. Then you have to take the diffraction data, the diffraction pattern and then ultimately we can examine.

But, before that we just simply find that the simplest procedures or the simplest technique. So, high resolution X-ray structures are also pose the impossibility sometimes, to distinguish between the electron density of carbon, oxygen and nitrogen in bacterial hydrogenase. So, even if you go at the end to determine everything through your crystallography, you will find problem, to level CO as C only and CN as CN only; because that electron densities are very close. So, we can have some time the other facilities also, like neutron diffraction or any other technique can be useful also.

So, the list of parameters, which can be obtained with each of these techniques, and the information of that particular method, which can be supplied to you. So, we can have from the left, so you just quickly go through it. And again this are the different techniques and is not possible for me also, to go for all these individual techniques nicely, only three, four or five

techniques, because I will I definitely have the time constraint. But, what you can see that a typical part of this, the analytical chemistry is one and you read little bit about the analytical chemistry once again; and then you go for the instrumental methods of analysis.

When you go for the analysis, it is not that your sample is simply the solution sample from the iron from the test tube. But, you can have the biological sample also. So, how you can tackle those biological samples by the different methods, where you can gather some parameters and the information what we can gather from that particular analysis? So, this is again from some reference book, what I am giving every time. But, you see quickly here that if we talk about the magnetic susceptibility, what you can understand? Because, you are studying again from your school to college days what is that particular magnetic susceptibility measurements?

So, we can have the gouy balance, we can have the susceptibility balance. But, how we can apply to this particular information to find out the exchange interactions; this last one you see not that all. So, the exchange interaction, so you can find out the number of unpaired electrons in these two metal ion sites; because what we are talking about these hydrogenases, the iron site and the nickel site. And all we know that both of them maybe paramagnetic or not; because iron site we all know the ferrous site in the low spin state is diamagnetic. Similarly, nickel in square plane environment is diamagnetic.

But, if you get some magnetic moment through this measurement by doing the susceptibility measurements, you can have some understanding that no these two sites are not only typical iron and nickel, but you have some good idea, guess, some rough guess work you can have. So that you can find out the corresponding oxidation state, whether iron is the plus 2 state or the plus 3 state. Similarly, nickel the most important and the most easily accessible or easily available state of nickel is nickel plus 2. But, you can have nickel in the nickel plus 1 state, and the nickel plus 3 state also.

So, magnetic susceptibility is you can have to determine this particular individual magnetic moments for these centers; and also any kind of coupling, when these two are close by. Because, in this hydrogenase example what we have seen that the nickel and iron centers are bridged by two cysteinate residues. So, if they are close by, they can have magnetic interactions. Then we will talk about the Mossbauer spectroscopy, then EPR- electron paramagnetic resonance. So,

where we can use this and what are the informations we can gather; and how we can apply those to the corresponding biological sites or the biological environment.

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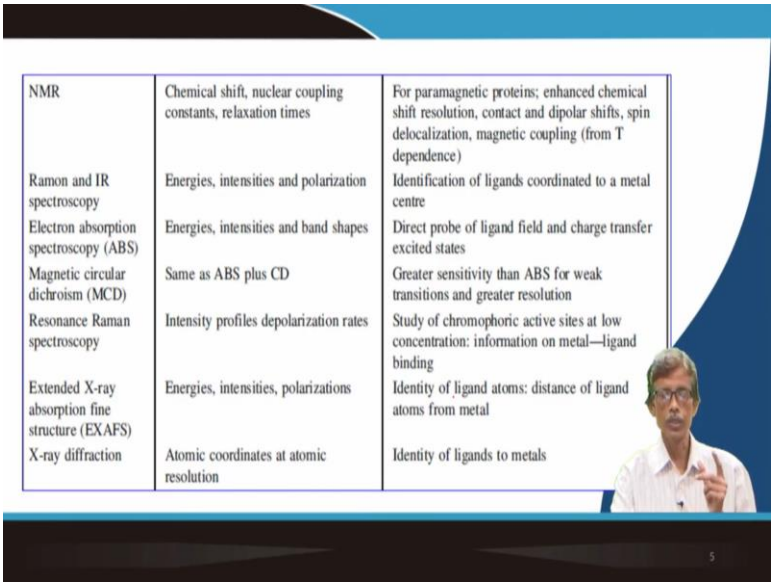

So, do not worry about the big list, because it will be with you; and if time permit occasionally will come back quickly to these particular systems to find out this. So, NMR today will not talk EPR and NMR will talking, we will be talking afterwards. But, today we can have the Mossbauer spectroscopy, then CD the circular dichroism. And one advanced level of thing is that the magnetic circular dichroism technique, which is similar to that of absorption, electronic absorption. We know why the solution is colored, any solution we when we see in a bottle or in a test tube or in a conical flux is colored, say methyl orange; we all know is indicator.

Why it is colored? What you can do to identify that, to analyze that? Similarly, where we can go for the circular dichroism spectroscopy? Then, some resonance techniques, the Resonance Raman not the nuclear magnetic resonance, or electron paramagnetic resonance; then, the EXAFS technique, where you can have extended X-ray absorption fine structure, not X-ray diffraction. So, we can find out energies, we can find out intensities and the polarizations; and that can be applied to the (last), see the last column. The distances of the ligand atoms are the donors, and the metal ions.

Suppose you have Fe, so what is the distance between Fe and Co? Just we have seen the example; and what is the distance between Fe and CN minus and what is the distance of FeS?

So, once you know this, once you know that you can have five such distances around the iron center, you know that it is only penta coordinated iron site. So, not only the environment, but also the coordination number, and sometimes we can find the corresponding nature of these groups. That means whether you have sulphur of cysteinate origin or carbon of carbon monoxide, or carbon of cyanide.

Then, finally definitely you can have the X-ray diffraction technique, where the typical atomic coordination can be find out; and atomic coordinates also the typical positions also and we can fully analyze that with respect to the ligand environment and the metal ions.

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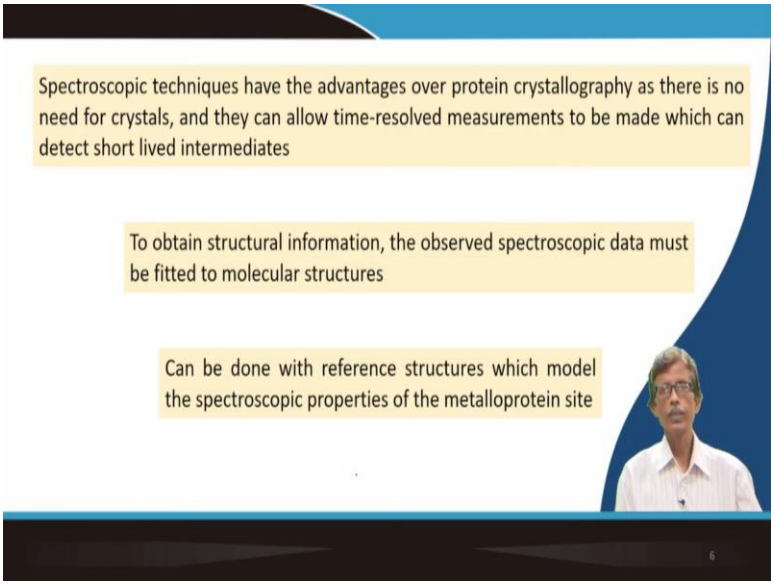

So, these different spectroscopic techniques are not always very much useful; but these spectroscopies has some advantage over protein crystallography; which is most tedious part of this analysis. But, we do not need any crystal, if you go only for the spectroscopic measurements. And sometimes we can have the time resource management that is with time very fast scanning we can do with time, what is happening there spectroscopically also. That means when you bring the time that means the kinetic thing; we are bringing that how the system is changing when you have a catalytic thing. The catalysis is going on within a time scale of, say some nanometer, nanosecond order, picosecond time.

So, how the system is also changing, how the metal on coordination environment is also changing; such that you can have many short lived intermediates during this particular catalytic

process. But, if we want to know the structural information from these spectroscopic data set what we can do? We can have small molecule analogs. We try to find out any such iron compound or iron complexes, or the nickel complexes; and try to correlate these spectroscopic signatures to that of your model compound. Why we call it the model compound? Because you can compare your data set what you are getting from your unknown sample and then compare.

So that is the very simple technique. What we always use that you can have some model substrate or a model species. And that model species we can use, we can take in our hand, such that you can have a hexamine iron complex. And you can have the corresponding iron nitrogen distances in that compound. But, that compound since it is very small molecule one, we can fully characterize it in terms of its oxidation state, coordination number, spin state and the Fe ligand distance; that means ml distances we call mostly that metal ion and the ligand distances.

So, if we find that iron, nitrogen distance in this particular range, and knowing the fact that iron is in this particular oxidation state as well as the spin state and the nature of the nitrogen. If it is amine only ammonia, which is a neutral one; which is completely different from the nitrogen which is coming from the amino acid residues. But, you will have a very good understanding that this is the range of the corresponding Fe and distance. If we find some informations through this EXAFS method only, that ml distance you can correlate it; and you can have some good idea about the nature of that particular iron site.

What is there? So what we can have now can be done with the reference structures, which model the spectroscopic properties. So, the spectroscopic signatures we know that when you go for the electronic absorption spectroscopy, we determine the lambda max and epsilon max values. So, these two quantities is true for your methyl red or methyl orange also; so these two quantities, the lambda max values and epsilon max values can be correlated with some unknown sample; say, metalloprotein active site. Let us see what is its corresponding lambda max value and the epsilon max value, considering the corresponding concentration of the iron center present in that particular enzyme or the protein.

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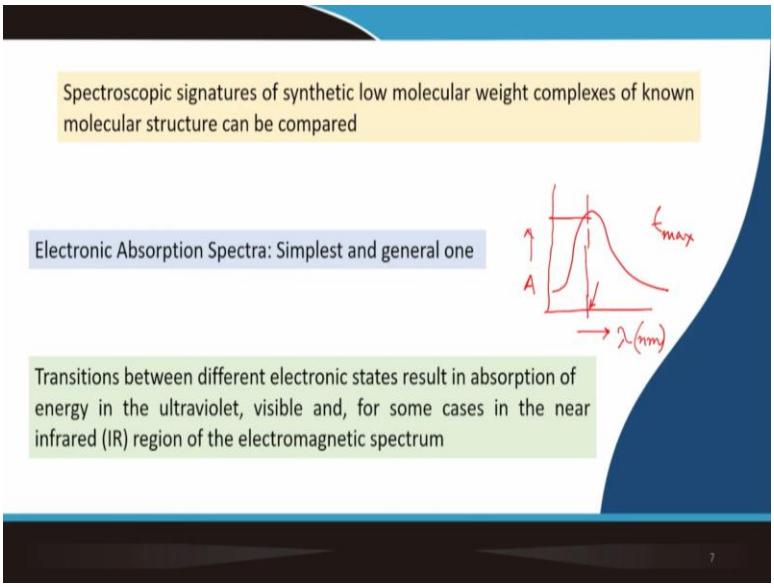

So, these spectroscopic signatures for the synthetic molecular weight complexes on having known molecular structure now can be compared. So, if you compare the epsilon value or the corresponding lambda max value, for the metal ion center in the synthetic molecule or the model compound. That we can compare for the iron site in hydrogenase or iron site in haemoglobin or myoglobin. Them we can have some idea whether we can have a corresponding d-d transition or a charge transfer transition. So, not only the position because the energy levels basically the electronic excited states we know.

So, these electronic excited states and the corresponding levels we know, and what energy you require for that particular type of transitions we can understand. So, for that reason only, the electronic absorption spectra, which we all know is the simplest and the general one. So, if you have the solution in your hand, so this particular one that means if you get that; and then you try to measure the spectra. We all know that the two axes definitely you can have and you plot it; we can have the calorimeter in your hand. Because our eyes are not useful to identify the corresponding lambda value for the solution what we are seeing as a red in color.

Say, solution of methyl red in water is given to you, is red in color; but what is its corresponding lambda max value? And two things the lambda max value and the molar absorptivity we have to determine. But, if we measure that particular thing and if we find that I will get a corresponding absorption like this, where this is your wavelength axis which is in lambda in nanometer. And

this is your absorbance A-value; from this particular situation you find the epsilon max value. So, at the maximum point, where you have this; so this is the point where you get this is your lambda max value.

And from the absorbance, you find out the corresponding epsilon max value. So, that gives us two quantities for characterizing that particular sample, whether you have a red color methyl orange or methyl red or red color in your blood. So, the red color of your blood how to quantify; so typically we should always look at the thing that you can have the corresponding color. So, I want to measure the color initially you detect; there maybe some smart students in our class that who can detect. So, this red is something like that; it is towards blue or towards red itself, or towards orange or yellow side.

So, you can identify through your eyes only. But, when you quantitatively measure by using a spectrophotometer, you can find out this particular one; and these are related to the energy value. The separation between these two levels the ground state as well as first excited electronic state. You have E1 and E2 and the difference between these two, the delta E value can be measured with that particular lambda max values. Because sometime you find that your solution is colorless; that means where it is the absorption is taking place. But, it can be beyond your visible range; visible range all we know that is 380 to 780 nanometers something like that.

So, it is either in the uv range or in the NIR range; that is why your eyes cannot see its color. But, the electronic transitions can take place, if they are excited with the exciting energy which we can gather from your corresponding lamps, which are used in this particular spectrophotometer. So, transitions between these for the different electronic states, what we are seeing here as the E1 and E2; that results in the absorption. So, this absorption this absorption for this particular peak is important; and that can span from ultraviolet visible to your NIR region or IR region.

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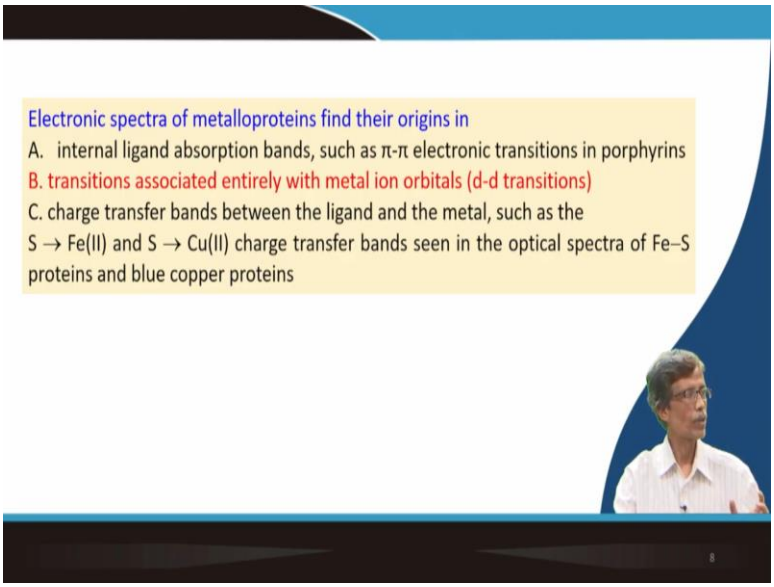

So, this particular electronic absorption spectra gives us for all metalloproteins, and one after another will come whenever we find in the typical examples. Because you can have the absorption bands for the pi-pi type of transitions. Then you have the d-d transitions; because the two levels based on the iron centers also and sometimes the charge transfer transitions. What we are looking for here also that you have sulphur environment around iron, and you can have the sulphur environment around copper. So, these charge transfer transitions can also be easily identified from your iron sulphur proteins or the blue copper proteins.

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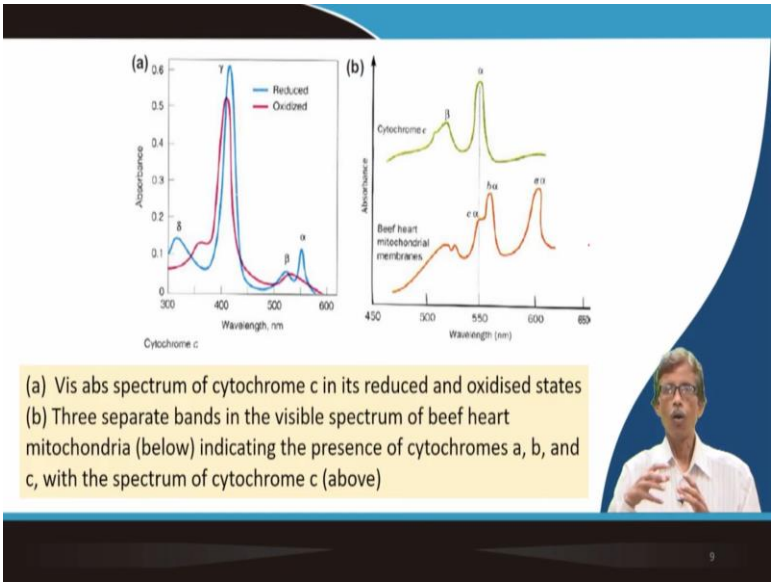

So, this particular example again what I have drawn just now is the corresponding one for the different cytochrome. So, the left hand one, the a is the visible absorption spectra and sometime we call it as uv visible absorption spectrum. So, this uv visible absorption spectrum what we can have for cytochrome c is a biological molecule having iron there. So, in is oxidized and the reduced form. So, you see if you quantitatively determine all these things, you know which one is for the oxidized form and which one is for the reduced form.

Similarly, three separate bands for the visible spectrum for the mitochondria indicating the presence of cytochromes a, b and c. So, if you have a, b and c; so the spectrum of the cytochrome c can also give us very good informations, that whether you have a cytochrome c or a, b for mitochondrial membrane. So, the origin where we are getting those samples is very easy to understand through this particular electronic spectroscopy only.

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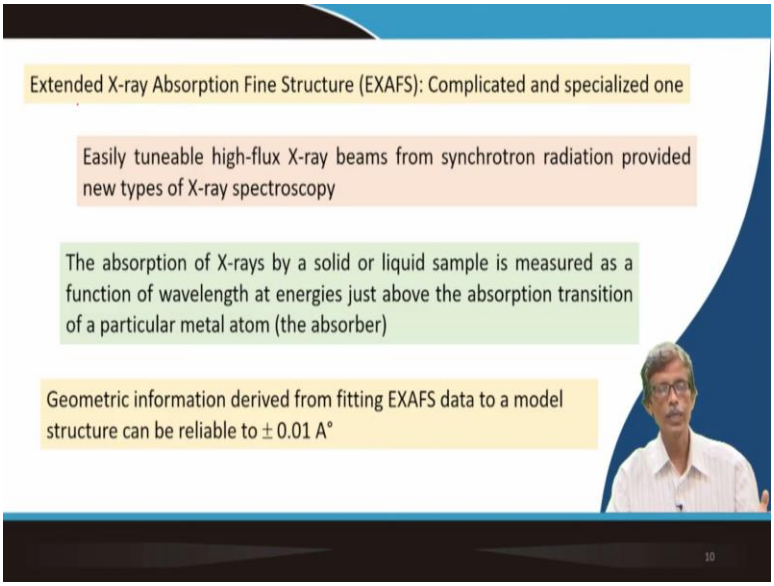

So, electronic spectral patterns are very useful and also the related one; but that is most difficult one. So, most easiest one and the most difficult one we are talking about the extended X-ray absorption fine structure, which is known as the EXAFS. And which is very much useful to know the metal ion and the metal ion environment; and the ml distances. So, these are you need X-ray beams and the typical X-ray spectroscopy. This is X-ray spectroscopy, not diffraction. So, the absorption of X-rays can be measured and the transition can take place from a particular metal ion, which is the absorber of X radiation or X-rays.

So, we get ultimately that particular case that the geometric informations basically the metal ion or identify the metal ion is typical environment; but again that fitting of that data from the known samples or the model structure. So, you gather the EXAFS data like your epsilon value or the lambda max value for the electronic spectra. Similarly, you have the corresponding EXAFS data; but you have to compare it such that that comparison will tell us, we can have the FeS distance or FeN distance or FeO distance. Whether you have sulphur in the environment in the first coordination sphere, or the nitrogen in the first coordination sphere, or sulphur in the first coordination sphere.

But, you see the good reliability, it is 0.01 Armstrong plus minus, compared to your X-ray structure. So, this particular determination is very easy very quick; and no such sample preparation, no such time consuming process is there. But, only the instrument is very high end instrument, is a very costly instrument. And many of us do not have those, different institutions cannot have this particular technique available to us.

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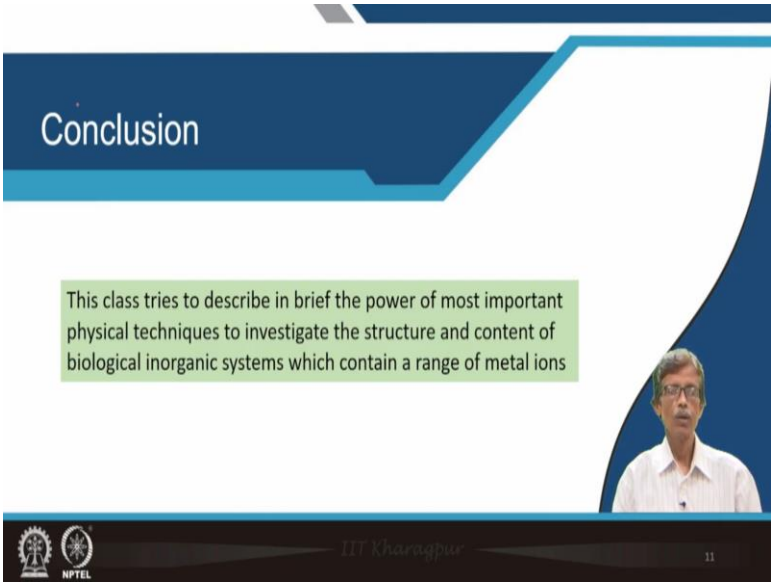

Then, you can conclude now by saying that what we are trying to understand from this particular class is to find out the power or the efficacy of these different physical techniques. And we have big list, and one for another we will discuss quickly to investigate the structure and the content of biological inorganic system, or the biological metal ion environment; where you can have a range of metal ions present in it.

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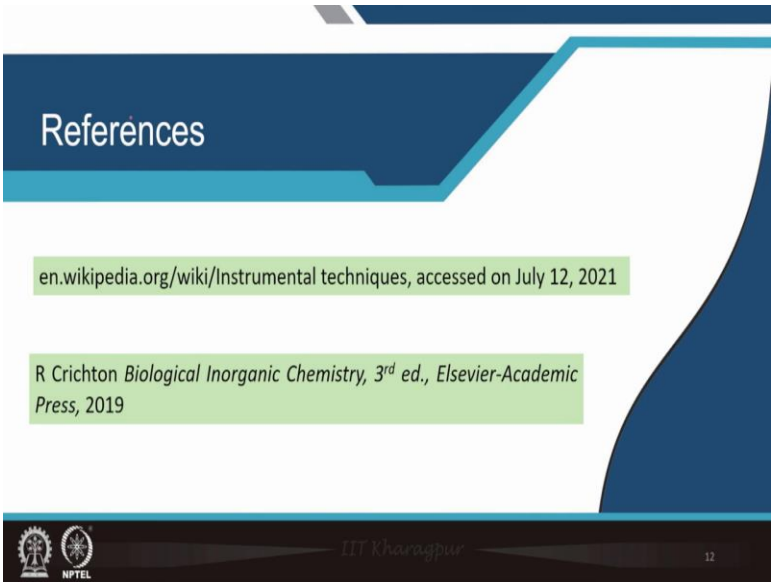

And lastly the references, the different instrumental techniques you can find out from the wiki and the respective techniques also. And also we should consult the corresponding book on Biological Inorganic Chemistry. Thank you very much. Thank you all.