Biological Inorganic Chemistry
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Lecture -14
NMR and X-ray

Hello everybody, so we will start again where we have finished with EPR spectroscopy. So, in this class of lecture-14, we will talk about two techniques; one is again another resonance technique, which is nuclear magnetic resonance. Just now or in our previous class we have studied the EPR spectroscopy, the electron paramagnetic resonance study. But now we will talk about the nuclear magnetic resonance, whether the NMR technique is also helpful in understanding the environment, and the structure and the reactivity of the metal ion site particularly in biological world.

And then finally we can see also little bit remaining part of x-ray. We have seen also the EXAFS technique, now we will talk about that x-ray diffraction technique. Whether the x-ray diffraction technique will be helpful to us, for finding out the corresponding nature of this corresponding metal ion centers?

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So, what are the concepts that we try to cover in this particular class is the energy of NMR transitions. So, what particular energy we will be using for seeing the NMR transitions; because there are many spectrometers available, for our routine chemistry wise or the organic chemistry

studies also, starting from 60 megahertz to 500 megahertz NMR is useful to us. So, what sort of energy is useful for studying the biomolecules?

And there we have different parameters basically the chemical shift, the coupling or the coupling constants. And how the setup, the basic setup of NMR spectrometers are there, and the use of x-ray diffractometer. So, how the diffractometer can be used and single crystal? How we can generate the single crystal for protein crystallography? So, this will see one by one.

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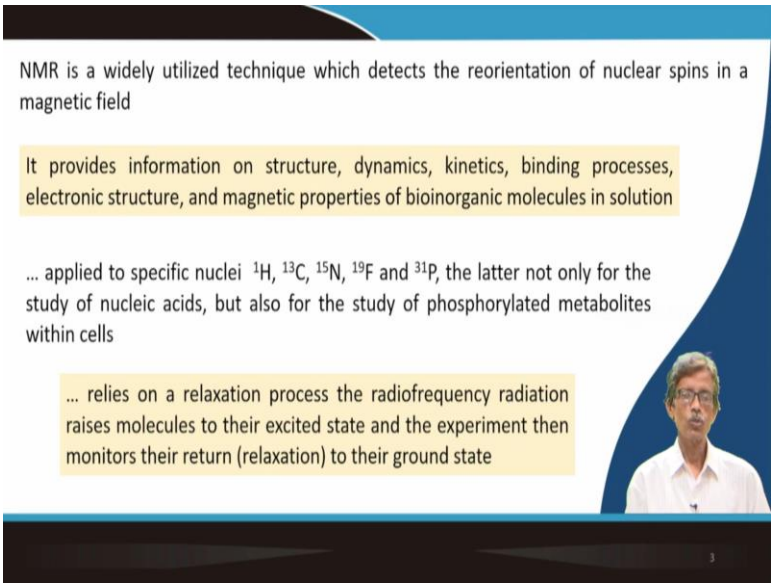

And what we see that is a very useful technique, and we can use widely to detect the reorientation of nuclear spin. So, we are talking in case of EPR that the sample is inserted again in the magnetic field; but, this is of different magnetic field. But, we will be looking for the change in orientation of the nuclear spin, that means the nuclear energy levels is not the electronic energy level, but, it is the nuclear energy levels that we are looking for, when you have an applied magnetic field. And is a superconducting magnetic field also, which is kept in presence of liquid helium as well as liquid nitrogen; so, that provides very useful informations.

The first of one is very useful which is the structure; because this another one in our health related studies also. We know about MRI, magnetic resonance imaging; so that is also resonance technique. But, is the magnetic resonance and we try to get some image of our brain of our heart, and all these things. But, that the basic principle for studying that or using that is your NMR technique. So, the structure of the sample, structure of the biomolecule, structure of the inside

brain or inside heart we can find out with this particular resonance technique. Then the dynamics: the structural dynamics or the reaction dynamics, kinetics for the reaction also we can find, and the binding processes.

If the substrate which is going to bind at some point, can change the NMR pattern of the system; then, we can find or we can determine, or we can study the corresponding binding process also. Then, the electronic structure and the magnetic properties of the bioinorganic molecules also in solution; that means if you have metal ion centers, whether we will be able to detect that metal ion, by studying this through these particular measurements. So, it is applied to specific nuclei, which are NMR sensitive or NMR active; like proton, the 1H proton, say a plus nucleus, and 13C, 15 nitrogen, 19 fluorine and 31 phosphorous.

You see these are bio relevant centers or the corresponding elements. So, biogenic elements in its corresponding numbers 1, 13, the corresponding atomic number; so these atomic numbers are very important, because the number of neutrons and number of protons are there in the nucleus. And they are EPS sorry NMR sensitive, and they can study. Because these are they are the basic constituent of any biological molecule, where metal ion is present like your blood sample, containing your myoglobin and haemoglobin, and also studying the nucleic acids. You see nucleic acids have carbon, hydrogen, nitrogen; sometime you can have the fluorine also and the phosphorous.

The phosphate ester bonds or the phosphates there. But, also we can study within our body that we know the phosphate transfer potential; some term we use the phosphate transfer, because the ADP is converting to ATP. So, phosphate group is transferring from one site to another site; then the sugar molecules or the carbohydrates can also be phosphorylated. So, these phosphorylated reactions are very basic reaction. In our metabolites also, when we burn our food material, we need to have the corresponding phosphorylation; the fructose 6-phosphate we know, we have studied it.

So, that phosphorylated reactions also we can monitor or we can study by NMR technique; and which is happening within the cells also. So, this process or this studies relies on a relaxation process is a resonance technique. But, again we are depending on the relaxation process, but not in the microwave region, but it is in the radio frequency region. So, raises the molecules to their excited states, so the nuclear energy levels can be excited to the upper energy excited state. And

then it monitors the return; that means the relaxation it going up, and then it is coming down. So, when it is relaxing, so the relaxation process we can monitor to the ground state; and that when we monitor or do we measure, we find the corresponding NMR signals.

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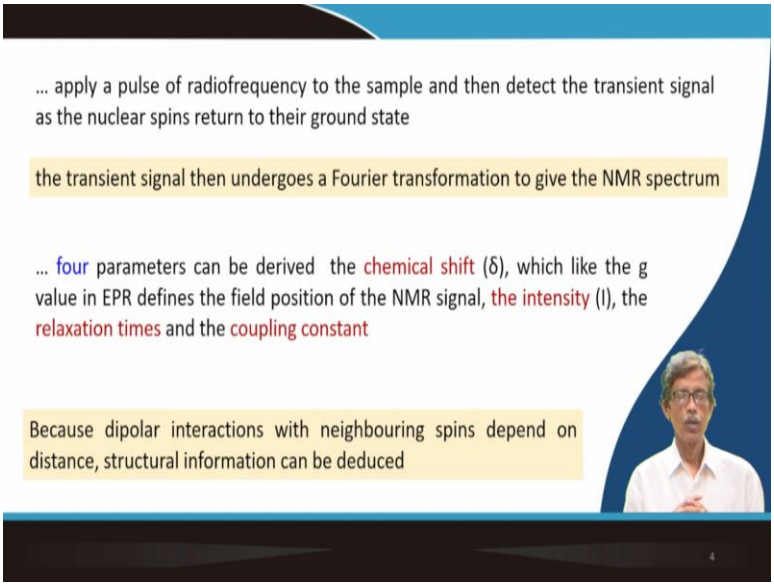

Then is not the direct one, so you can have the pulsed one also; so, you can apply a pulse radio frequency. So, the pulse radiofrequency in a particular range of frequency, we find that we can get it in the megahertz region; unlike your EPR which is in the gigahertz region. We expose the sample to that radio frequency electromagnetic radiation, and then detect the transient signal as the nuclear spin returns to its ground state. So, once you have it in the ground state, you give the energy; and energetically you excite it through the radio frequency energy, and it goes to the upper excited state.

But when it is coming down to the ground state, you will be able to record the signals. So, the transient signal then undergoes the Fourier transformation to give the NMR spectrum; because the Fourier transformation is a very important technique, is a mathematical technique all we know. Because this particular transformation is required, starting from your IR measurement also. Once we I told you that FTIR similarly it is FT-NMR technique; and this FT-NMR technique is useful. Because you record in particularly you record it initially from the time domain, is a very fast recording; you can go for a very fast recording in terms time scale.

If the process is very fast, the kinetic some kinetic thing is operating. So, you record it within some time, within few seconds, or within some few milliseconds; and then you plot it in your frequency domain. That means you have the corresponding frequency and the signal, the signal intensity. So, that particular transformation of the recorded material or recorded dataset from one domain to the other, is being done by your this Fourier transform. So, we get this through the Fourier transformation and that Fourier transformation gives us, and through analysis of the whole thing we will be getting something, where you can have four parameters involved.

So, when you record NMR, first thing what you know how you give get the sample? How to put the sample? Again like your EPR tube, you have the NMR tube. And you can have the some solvent also like the organic molecules we put in that particular sample of solvent and dissolve it, and we transfer it to the tube NMR tube. But, in this particular biological sample also, if some dilution is required, then neat sample is not given there. We go for some diluted solution with some solvent, and put inside the tube.

Then we measure the chemical shifts the delta values in PPM we record, the delta and PPM we record, even some very small organic molecules say, like phenol, the tyrosine radical when we are not getting anything, we are not able to get detect or any other thing, we should be able to detect the corresponding presence of the corresponding tyrosine radical or the tyrosinate ion; which is bound to say copper center just now we have seen. So, that particular signal giving you the corresponding C-H protons of the phenol ring. So, those C-H protons can have characteristic chemical shift values. So, by knowing the corresponding delta values, so this is in presence of the, in the aromatic ring.

So, it will be within range of 8 to 8.5 delta values. So, that delta value will definitely tell us that we can have a aromatic ring; and that can be a typical origin for you tyrosine, the tyrosinate ion bound to your copper center. But, some complication will again come, when you have the copper center, the copper center itself is paramagnetic. But since we are talking about all in the magnetic field; so that will shift the signal, so, that will be giving you the corresponding contact shifted NMR; that will be little bit complicated, but we can analyze still; so, like your g value. So, where we know that g value for the EPR, the corresponding signature for NMR is definitely the delta value, the small delta value which is your chemical shift.

Which will definitely giving you the position within the NMR spectrum. So, you have the NMR spectrum, the x-axis and one-axis in the 1-D NMR spectrum. One dimensional definitely NMR spectrum, because the other NMR spectral features are also known the 2-D NMR, or the 3-D NMRs are nowadays available. Then the intensity: how strong the signal is? Then how many protons are there we are trying to locate it? That means the signal intensity is also important. Then the relaxation time, how fast it is relaxing? We have promoted it from the ground state to the excited state.

But, how quickly it is coming down to the ground state, and then the coupling constant vales; that means the g values. That means the adjacent site or adjacent proton centers are there; so you can have CH-1-CH function, and another adjacent CH function like simply ethanol molecule. CH3CH2OH there are three different types of protons; you will get three different signals in terms of your chemical shift values. So, this coupling constant values are also important, and this gives also some other informations.

You can have the dipolar interactions with the neighboring spins the nuclear spins; try to remember do not forget anytime that here we are talking about the nuclear spins only. So, they are depending on the distance and the structural informations we can gather. So, the structural information can be deduced from that particular spectral measurements, with the help of your, this particular type of spectroscopy.

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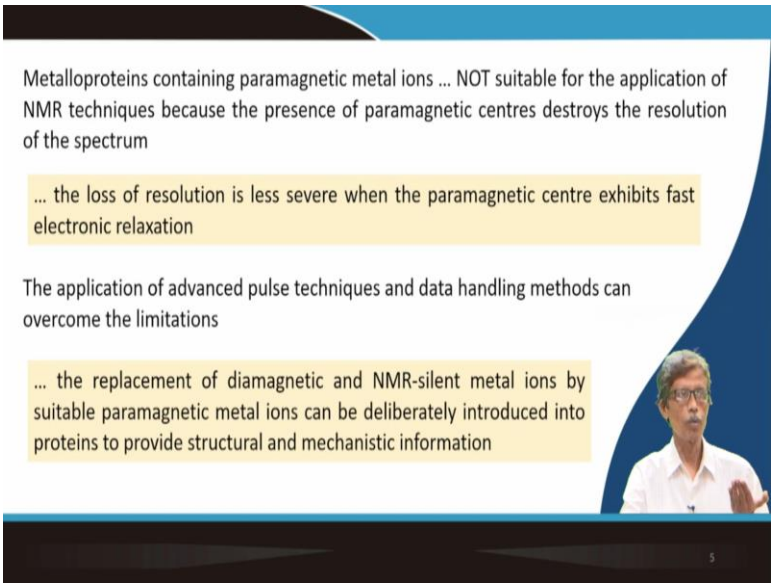

Then you come to the metalloproteins. So, how the metalloproteins are helpful in understanding using NMR spectroscopy? But, just now I told you that you can have the paramagnetic metal ion centers, like your copper 2 plus. So, if you have copper 2 plus, which is itself is a paramagnetic center with one unpaired electron; but we are talking in terms of a nuclear spin which is under the magnetic field. So, the local magnetic field out of your copper 2 plus will shift the signal from its original position. So, we get the contact shifted NMR signal. That is why these are not suitable for the application of NMR technique, because the presence of paramagnetic center destroys the resolution of the spectrum.

Not only shifted, but is it will also damage the type of resolution what we are expecting; for a diamagnetic sample, or from the pure organic molecule, or pure organic type of biomolecule. So, once you lost the resolution and it has a severe effect, when the paramagnetic center exhibits fast electronic relaxation; so it can also have fast electronic relaxation. So, the electronic relaxation is the responsible thing to destroy the corresponding fineness of the spectrum or the resolution. Then what we can apply the application of advanced pulse techniques, so that is why the higher or the sophisticated level of techniques for NMR measurements is there.

And the data handling methods can overcome these limitations. So, you can have the pulse technique, so that is why we call the pulsed FT-NMR technique. So, you can have the pulse technique and it is a Fourier transform technique and the pulse Fourier FT-NMR technique can

also be useful. So, if we go for the replacement of diamagnetic and NMR silent metal ions sometimes. So, once you see that metal ion is not there, you have one particular sample; metal ion is there, but it is diamagnetic. So, zinc proteins or zinc bearing metal ion complex; so the zinc protein or zinc bearing metal ion complex, can have some important role to play, to study the corresponding NMR spectrum for that particular sample.

But, sometime what we can do? We can do we know the zinc is present in carbonic anhydrase and carboxypeptidases. But, if we try to get some or extract out some other information, we try to substitute that by some other similar type of metal ions; say cobalt 2 plus, which is a paramagnetic metal ion. So, is a deliberate introduction, so deliberately we introduce into the protein structure to provide many other information, it can be of structural origin or can be of reaction mechanism origin.

So, these can also be studied, so these are the technique; because we are interested to know the techniques. Not the theoretical justifications or the theoretical background of all these techniques. But, how to use the thing when you have a sample of biomolecule containing metal ion, diamagnetic or paramagnetic whatever it is; but, how to use or how to handle the corresponding spectrometer?

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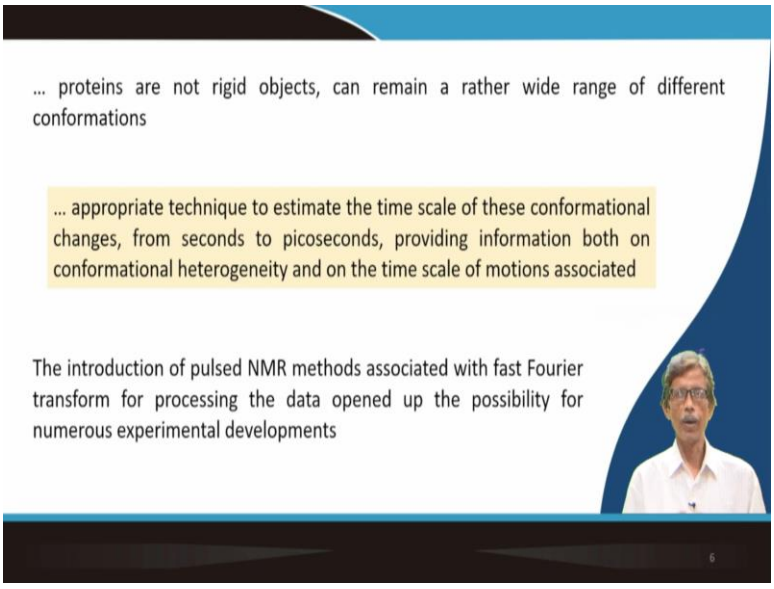

So, the protein structures which are not rigid we all know, and can remain in rather wide range of different conformations. So, is basically a dynamic structure and then dynamic structure if we try

to photograph, or try to get the corresponding spectrum of that; that will be a difficult task then. So, we need to have a corresponding technique to estimate the time scale of these conformational changes. So, within which particular time domain, one particular conformation is changing one form to the other. Whether it is changing within seconds time, or within picoseconds time. And the conformational heterogeneity is not a very uniform thing; but it can have a heterogeneous structure on the time scale of the motions, which are associated to it.

So, these informations can also we can have. So, the higher level of this particular technique which is the pulse technique and with the first Fourier transformation, we talk about the transform Fourier transformation, but the first Fourier transformation, because we need to have the data processing within a very short time scale. So, that has opened up the possibility of numerous other experimental developments. So, you can design, you can plan different new experiments with the biomolecule, such that you can extract out new, and very useful informations out of these studies.

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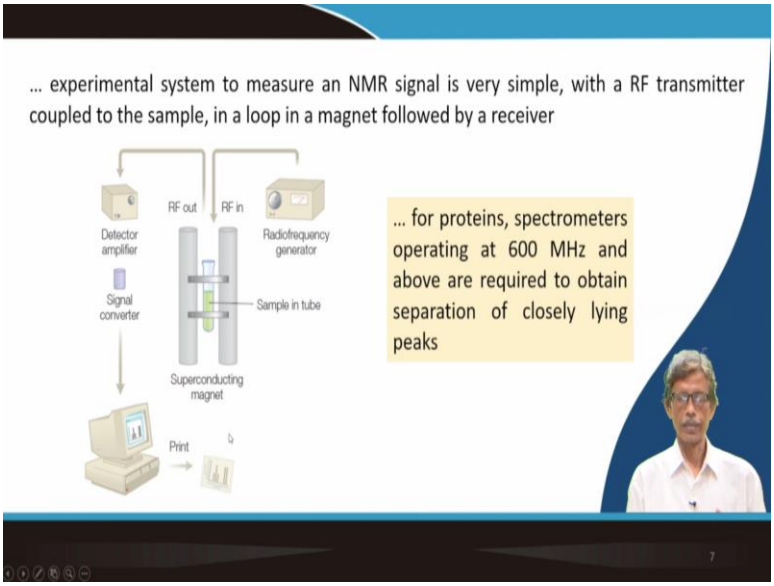

So, the experimental system to measure the NMR signal is very simple. What we can have the basic components, what we can have is the RF transmitter coupled to the sample. So, radio frequency transmitter you should have like radio transmitter we all know. So, this is radio frequency transmitter, which is giving the corresponding frequency to the sample, within a magnet followed by a receiver is like that electronic absorption. You expose the sample to your

electromagnetic radiation, then you have the detector. And detector will take up that particular information that how much radiation is getting absorbed by the sample.

But, to study the proteins by using these spectrometers, we need a higher level of spectrometers, say 600 megahertz type of thing. So, if you have a 600 megahertz NMR spectrometer we will be able to measure the biomolecules, we will be able to measure the different proteins. Because the separation of closely lying peaks because is a huge molecule, the macromolecules we are talking about. So, those macromolecules we have and those macromolecules have large number of CH, NH and other CoH, or other aromatic rings are there. So, they all have CH functions, so that CH basically those protons if we try to locate, they giving you clumsy structure or clumsy picture to it.

But, if you have strong enough spectrometer of 600 megahertz, it will spread the corresponding entire range. So, this is the block diagram, so you should only remember the block diagram all the time this instrumental part. So, instrumental part for a NMR spectrometer is that part only; what we have the basic component. So, you see you have the radio frequency generator on the right, so that is generating the radio frequency, and we are passing it towards the sample. So, radio frequency is coming in, so you have the sample that is nothing but your sample tube. And the sample is there in the tube within the magnetic field, so this is a superconducting magnet.

So, if you have the superconducting magnet like the two magnet poles, so you can have this particular cavity. We have the magnet poles over there and the radio frequency what is getting absorbed and due to the relaxation process or coming down the excited molecules to the ground state, you can have to have the corresponding signature that how much radio frequency is getting absorbed. So, RF in is giving the is the incoming radio frequency and RF out is the corresponding transmitted radio frequency after absorption. Then it goes to the detector and the detector is also connected to the amplifier; because the intensity you have to amplify.

Then signal converter or the interferometer, if it is some kind of that of your Michelson interferometer is there, then it goes to the computer. So, computer will have the printer option, or you can directly get on the computer terminal the corresponding signals, as your typical NMR spectrum. So, that way we get the corresponding NMR spectrums in our hand and we are happy with those signals, and we are happy with those measurements.

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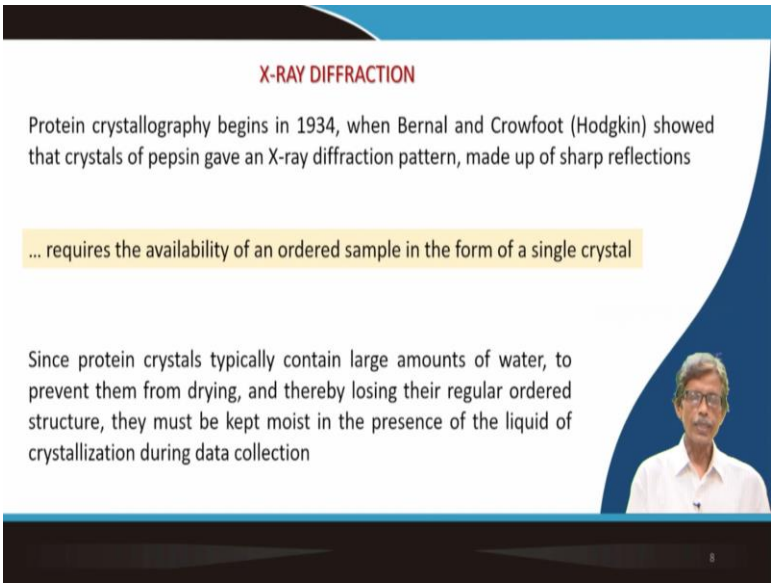

So, next quickly we just see the x-ray diffraction technique; the other second technique what can be useful and which is also a complementary one. And to know that we have a different crystallographic thing, which is your protein crystallography. Unlike your small molecule crystallography, again it is a single molecule or sorry, single crystal structures, which began during 1934, two people Bernal and, Professor Bernal and Professor Hodgkin showed that if the smaller crystal they crystallized pepsin, the small crystal they crystallize it and they go for the x-ray diffraction.

And they found out that the reflection pattern you get, and the reflections you can monitor the reflections in terms of its intensity you can monitor. So, what we can have that requires the availability of an ordered sample; why ordered which is very important. Until and unless it is disordered like the solution; the molecules or the species which are moving around the sample. But when you go or expose it to the x-ray; it will not impinging or not hitting one particular molecule, or one particular crystal. So, you need to have a block of definitely is a very small size, is a block of single crystal; which should be exposed to x-ray. So, we need a single crystal even for your protein molecule also.

So, the protein crystals are typically contained large amounts of water, and we should keep that water there, such that it is drying, this crystal will be cracking. To get a ordered structure, in this moist form what you can do? You have to preserve the liquid, particularly the mother liquor.

When you grow small crystals in our system, also small molecules or the metal ion complexes; if you take out the solvent, the crystal can break or crack. What you have to do, you just keep the mother liquor attached to that; and you put within the x-ray. Because when x-ray is impinging this sample you have the local heating also.

And the local heating will also evaporate that particular solvent which is trapped there. So, to good idea to do for that particular system is that you put the sample inside the capillary. So, inside the capillary if you sealed it with the mother liquor, or with the solution what is available. That means the mother liquor where you are crystallizing the protein molecule, such that you can keep it moist with that particular ligand. During the crystallization what you have got and during data collection also, it is present over there.

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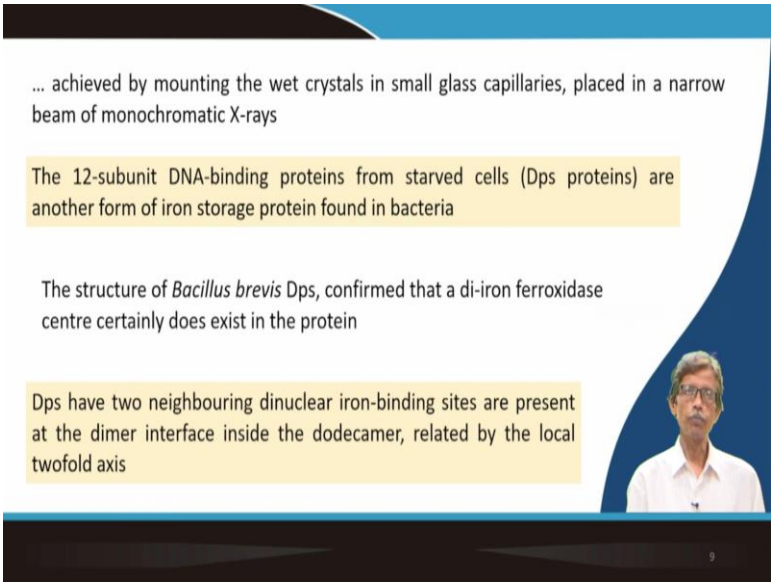

So, next you mount the crystal that wet crystal in small glass capillaries, and you seal it then. And expose your mount on the goniometer head that particular capillary, and capillary will have the crystal inside. And then you expose it through the x-ray beam. So, you focus from the x-ray source. So, monochromatic x radiation is then falling on it and that monochromatic x radiation can fall on this particular sample, and give you these structures. Now, we will take the example of one thing, where you can have a bigger molecule, where you can have 12-subunits of DNA-binding proteins. How we get that?

So, these DNA-binding proteins can be crystallized from starved cells of say E.coli or any other source; which is known as Dps proteins. So, DNA-binding proteins basically DNA-binding proteins are known as Dps proteins and another form of iron storage protein found in bacteria. So, these DNA-binding proteins are also useful in bacteria that we know the examples for (()) (25:26) force are the examples of iron binding sites, where they are typically coordinating the iron. But, for their survival, they also need iron. Similarly, much more complex ones DNA-binding proteins also can have iron storage.

Now, if the question is that whether your, this x-ray diffraction technique or x-ray structure determination technique, can be useful to find out or to locate the corresponding iron sites within this molecule. We have guessed it that iron is there for its function; so some other indirect information, iron analysis or iron another spectroscopic technique can give us the signature. But, what we want to know is that where you have the iron, and how iron is bound to that particular protein molecule. So, the structure of Bacillus brevis Dps is one particular sample, and this particular thing can confirm that we have a di-iron ferroxidase center.

So, not only one iron center we try to locate two iron centers within this particular Dps molecule or Dps protein. So, this particular di-iron ferroxidase center certainly does not exist in this particular protein. But, in other cases this di-iron site or ferroxidase site is present. But, instead of that these Dps have two neighboring dinuclear iron-binding sites at the dimer site, an interface inside the dodecamer; the 12-subunits. We are talking about dodecamer is the 12 number is 12. So, the 12-subunit DNA is there and related to the local twofold axis, because all these crystals can have some crystallographic symmetry.

The axis of symmetry or the plane of symmetry, all these things guide us to determine the structure for the unit cell only. Once you get the structure of the unit cell, you can replicate it with respect to that particular crystal symmetry. The crystallographic symmetry we call to generate the entire structure.

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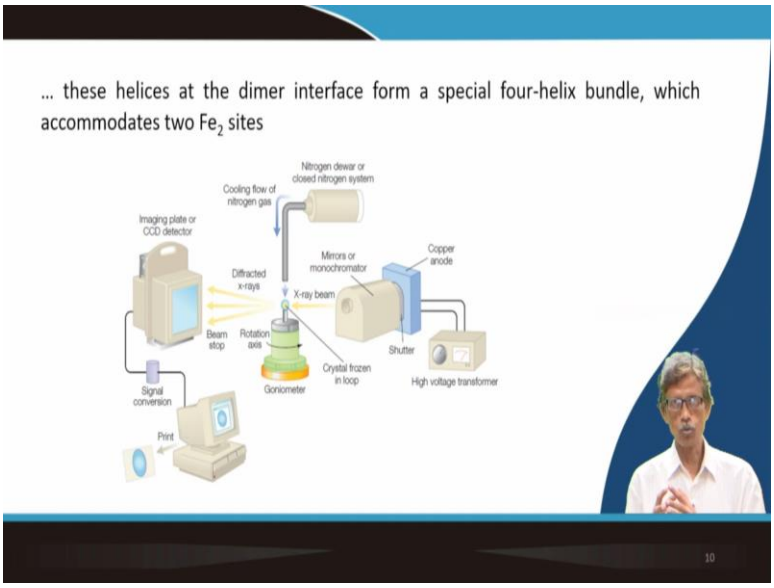

So, whether we will be able to get that particular information? How these helices are there at the dimer site? And at the dimer interface form a spherical four-helix bundles. So, one bundle having four helix, so double helix we know, is a triple helix we also know. But, you can have a four-helix bundle. And that four-helix bundle is responsible to trap or locate or take that particular Fe2 site. So, this is a typical block diagram for EXAFS structure determinations. Quickly, we see that and as I told you that you have the corresponding nitrogen Dewar, which is injecting the cold nitrogen. The cold nitrogen steam is there on that particular crystal frozen loop.

So, it can be on the loop or it can be in the capillary. Then x-ray beam is there; so, you have the high voltage transformer and the x-ray generator. And again like absorption thing, you have the diffracted x-ray beam, and we have a CCD detector. And that CCD detector is processing the signal, and then ultimately to the computer and the print out; so, all these things the modeling and all these things ultimately there. So, you have the source which is impinging on the sample; you have the detector and then finally to the print out. So, that gives us all these informations in our hand that you determine the structure.

And you find out the corresponding site; whether you have a di-iron site is present or not and the protein structure. So, interestingly if you go for the entire protein structure what you find? Not only the location of the iron site, which is helpful. Because the particular type of x-ray diffraction technique is useful, which is heavy atom method we call. So, heavy atom method can determine

or can locate the electron density very quickly of the iron first. Then the corresponding electron density for the carbon, corresponding electron density for the oxygen, corresponding electron density of the nitrogen can found out. So, the protein structure slowly we can find out, like we find out a very complicated ligand around the metal ion, in our metal ion complexes.

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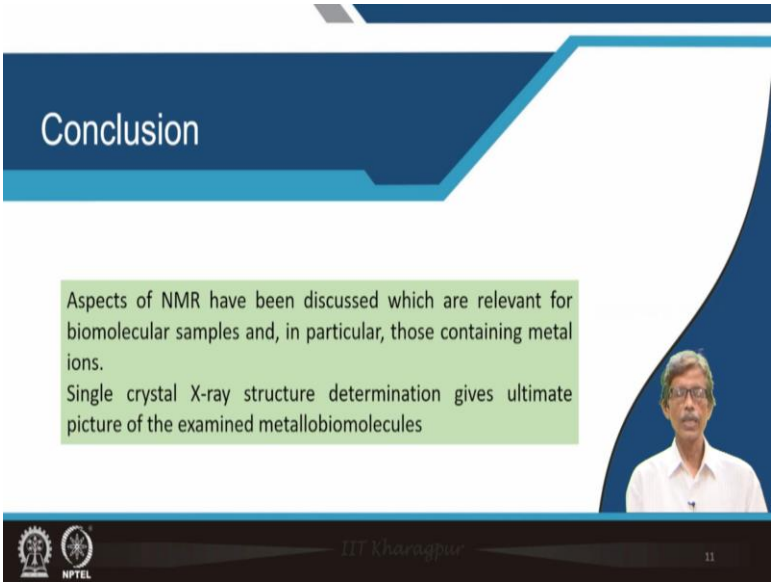

So, in conclusion what we have seen two techniques we have studied; one is your NMR technique and which is very useful for your bimolecular samples. Particularly those which containing the metal ion; because we are interested in bio coordination chemistry, or bioinorganic chemistry, or biological inorganic chemistry. So, that is why metal ions should be there, whether it is paramagnetic or not; that does not matter. But, it should have a metal ion for our studies. Then the single crystal x-ray structure, again the metal ion containing protein structures. The location of the metal ions like Fe2 positions as well as the entire protein structures.

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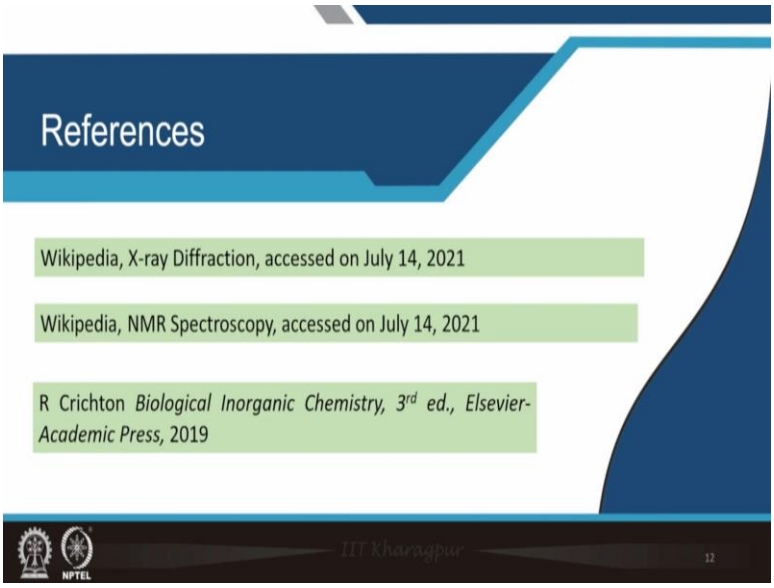

So, these we can find again through the references like X-ray Diffraction page from Wikipedia. And NMR Spectroscopy page also from the Wikipedia; so these are the very basic things you should follow first, then the book. Thank you very much.