Inorganic Chemistry of Life Principles & Properties
Prof. C. P. Rao
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture – 26
Role of Iron in life –Transport & Storage systems

Welcome you all to the next class on the Inorganic Chemistry of Life Principles and Perspectives. And in the previous class we have been talking about the iron transport protein transferrin and the transferrin the functioning etcetera, apo transferrin to the addition of the iron ions either in the form of the iron 2 plus or in the form of the iron 3 plus is in the form of a iron 2 plus its gets oxidized iron 3 plus it gets there is no oxidation required.

But either the case the core is formed only in presence of the carbonate therefore, carbonate is an allosteric factor that is that has been very well proven. And there are two centers one is N-terminal, other is the C-terminal.

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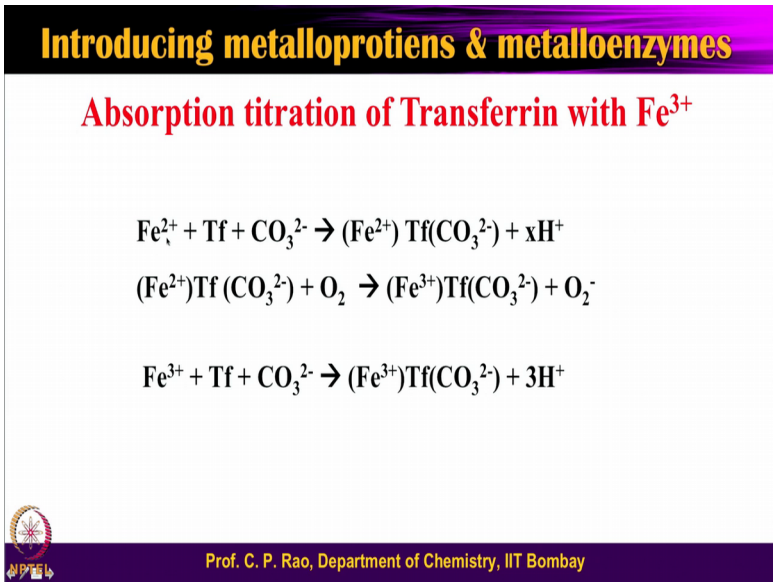

So, let us continue with that and we have seen I have explained to you the iron 2 plus getting in apo transferrin and iron 2 plus then you get a iron 2 plus complex provided you have a carbonate ions then add the oxygen or leave it in the oxygen you will get into or you can have a alternatively the iron 2.

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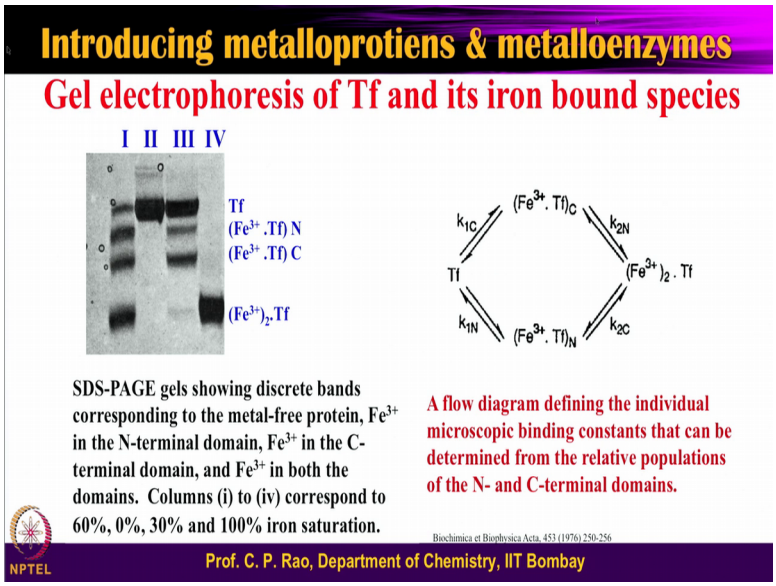

Now, let us look at a curious experiment where I take apo transferrin and add a different extents of the irons and then try to look at that, ok. So, these are used for the 60 percent of the iron 0 means no iron 30 percent of the iron, 100 percent of the irons saturation. See that this is the one where 60 percent iron is added, this is where you have a 0 percent iron which is apo transferrin. So, the apo transferrin shows in the gel electrophoresis a band over there and then this is at the 30 percent of the iron is added.

Here it is the 100 percent of the iron is added. So, when a 100 percent of the iron is added the entire the apo protein is converted to the entire metal of protein, and both the centers are iron and this is in the oxidized form so iron 3, ok. So, this is apo and this is di iron 100 percent di iron, but when you add 60 obviously, you do not have sufficient amount to form both the centers to the same extent.

Therefore, here you do not see any bands in between, but here you start seeing some bands in between because two different levels of saturation of the or say different levels of filling of C-terminal and N-terminal. So, you get that one. So, smaller quantity of a di and then some of the C-terminal some of the N-terminal, so this one is the N-terminal, this one is the C-terminal.

Now, on the other hand if you go to the 30 percent only you never get a di both the centers present either certain fraction which is the N-terminal certain fraction is on C-terminal, but hardly anything where both the things are there. So, now, you can get see it

is a very nice titration taking a apo transferrin adding different levels of a iron and looking at the species.

So, the species are 4 types here you have one is free transferrin that is called a apo transferrin other is only one iron with the transferrin and shown as the N; that means, not nitrogen N-terminal, the other one is shown as a iron 3 with the transferrin shown as C means C-terminal, and iron 3 twice with the transferrin.

So, this is no free, no N, no C, both N and C. So, as a result of this from the intensities, so if you do is this kind of a titration and maybe 10 percent, 20 percent, 30 percent, 40 percent, 50 percent, 60 percent, 70, 80, 90, 100 you get large number of these bands, these the electro forces from the intensities of that you can calculate the a rate rates from one to the other.

So, the from free transfer into C bound and the both the one or from free transfer into N bound and to the both this can be worked out. So, it is not a very essential for this particular course, but just this idea that you can see.

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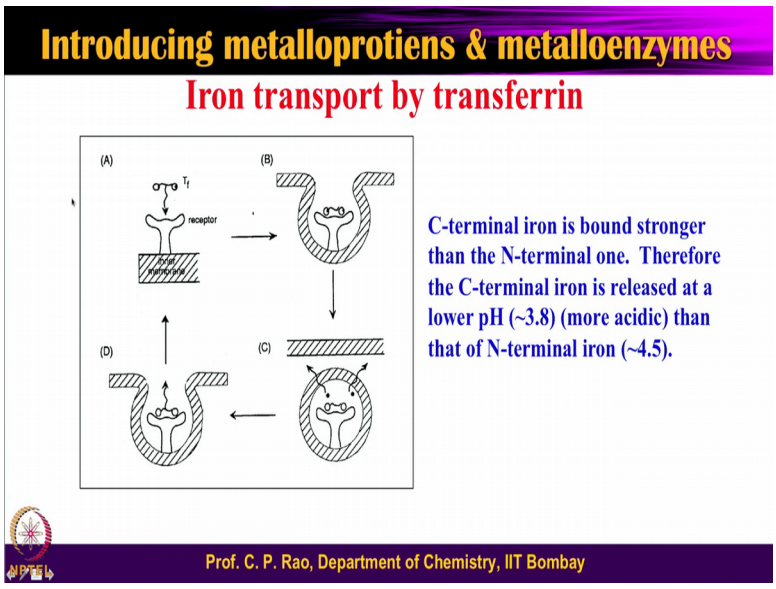

Iron transport by transferrin, ok, so now this is the what is written here is a is a transferring, one is the N-terminal of this C-terminal and the irons are there and this is the cell membrane under the cell membrane you have a receptors. So, there is the receptors will accept this one. You know why? Because this protein has got a lot of the if

you see closely here there are certain regions where you have a carbohydrate attached to that, ok.

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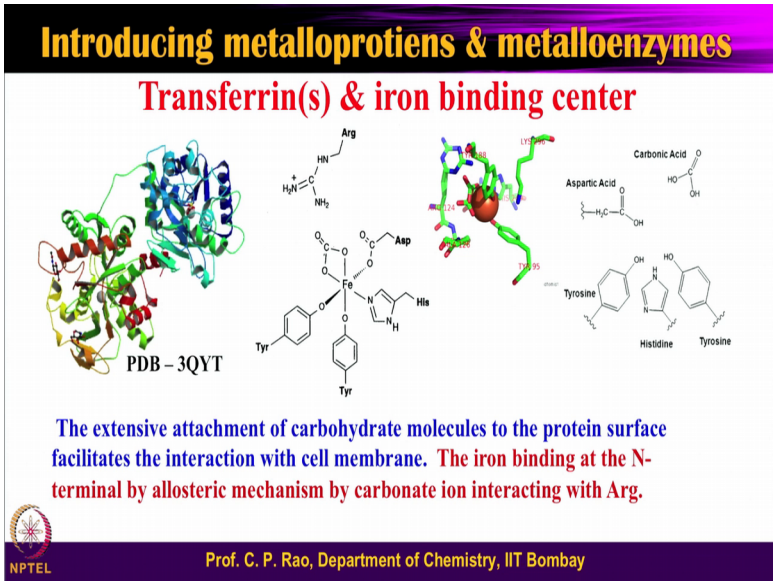

So, therefore, that kind of a carbohydrate post translational modification with a carbohydrate will bring its affinity to bind to the cell. So, that is what we are trying to explain here. So, since you have the transferrin protein with the carbohydrate terminal which will be accepted which will be recognized the receptor then you have a receptor complex.

So, transferrin loaded transferrin let us take this as a loaded transferrin to ions and that will interact with the receptor and the receptor once it is interacted to induce the kind of a vesicular formation into this and this converted into the vesiculate this; that means, taken inside the cell.

So, from outside the cell it has taken inside the cell. In inside the cell this in a inside a list. And you know that as I said that the C-terminal is bound stronger relatively stronger or compared to N-terminal therefore, C-terminal comes out at the great acidity.

So, and this is coming from the because there is a proton pump which will add protons and as a result of that the pH will change as a result of the change in the pH the irons are released and the N-terminal will be released at 4.5 pH a whereas, C-terminal is rarely used around 3.8. As you can see as a much more stronger at the C-terminal this.

So, the iron loaded transferrin the making a complex with receptor and then internalization and then a proton release and then release of the irons and then again the the transferrin is taken out transferrin is not engulfed trans apo transferrin comes out and then this is the kind of a cycle that it goes through,. So, therefore, we have looked at the iron, iron transport and all these things by the transferrin.

So, now, let us look at the iron as I said is required a lot in the body is almost for a body of the 70 kilograms about 4 grams of a iron is required. So, therefore, a lot of iron supplements are there and the iron is also stored when you have a some x some little excess is there and then the iron is taken out of the storage too.

Therefore, it is a right juncture where we can try to look at the iron storage protein. So, in the iron storage protein then we will look at the that is the ferritin how the ferritin is store in the irons. Let us look at this in this particular class now. So, that we can finish the oxygen and a iron transport and storage aspects of these ones.

Now, let us look at in this slide. First look at not on this side on this side you see a huge structure of the ferritin, ferritin is a very huge structure.

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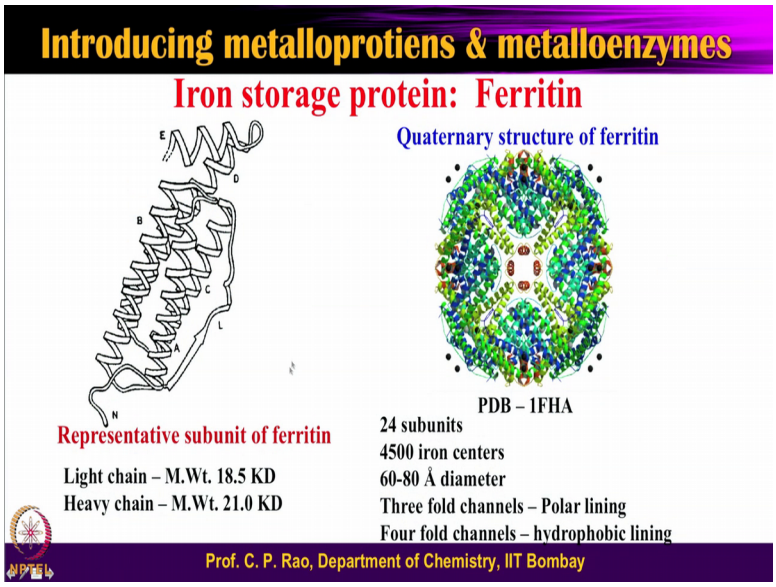

Now, you look at one of these is like this one of these chains here you see that is like this, and it has a different chains like light chain and heavy chain light chain is around 18 and a half kilo Dalton, heavy chain is a around 21 kilo Dalton. And such kind of a

subunits 24 subunits are joined together to form this, so it forms a kind of a very spherically symmetric kind of a ball shaped structure in the ferritin. So, each one of these is a kind of a rod, these rods joined in together forming a spherical structure and it leaves certain regions of a openings and so you can see this it is looks like a four fold kind of thing and there is something which is like a three fold and it is here three fold.

So, it is here three fold so that means, that arrangement of all these rods to form a kind of a spherical ball we will leave with some kind of a why it is kind of a channels some kind of a regions which will connect interior and that we called as a channel. So, the channels which are having a three fold kind of symmetry and the channels which are having a four fold kind of a symmetry. So, and the kind of a damn it is around 60 to 80 angstroms, so 6 to 8 nanometer therefore, these are all nanoscopic bodies. That you can look at a individual one nowadays there is a lot of nano studies or the ferritin is going on in the in the literature in the researchers.

And this as I said 24 subunits on this and it has a capacity to catch up and store 4500 iron, ions, so non iron neutral iron ions. And if you look at a little bit more detail of course, that will come in the later stage, but the three fold axis have a polar a lining and four fold axis has a hydrophobic lining that we will see just in a while.

Now, which we were talking about the hydrophilic channel we were also talking about the three fold channels or hydrophilic in the ferritin. See that this is the one, and where is it form? That is here. And there are some lining residues are there and that can be seen over there.

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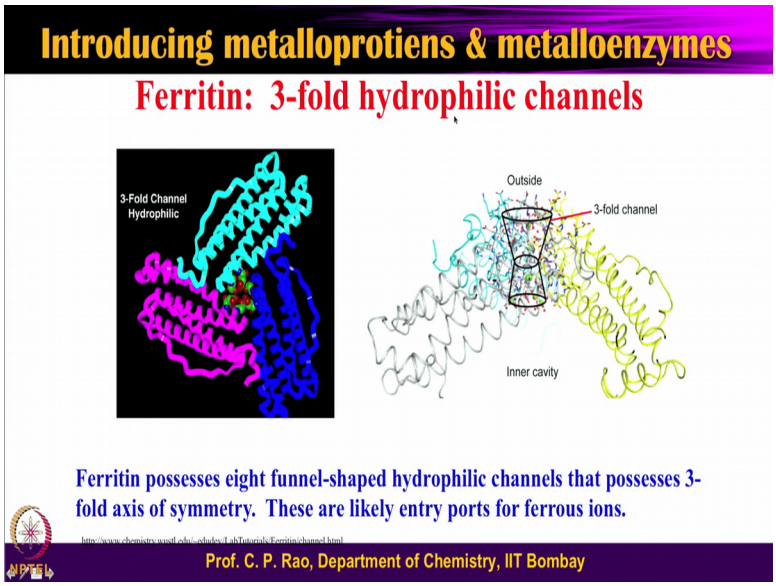

So, it is like a funnel type of structure therefore, some residues are protruding into this and those residues which are protrude in the polar in nature therefore, this particular channel is polar.

So, mostly the kind of a groups like carboxylic groups and some polar amine groups these kind of things that you will have and that forms this thing. And so the ferritin processes 8 funnel shaped hydrophilic channels that processes three fold symmetry. So, these are likely the entry points are entry points for the ferrous ions.

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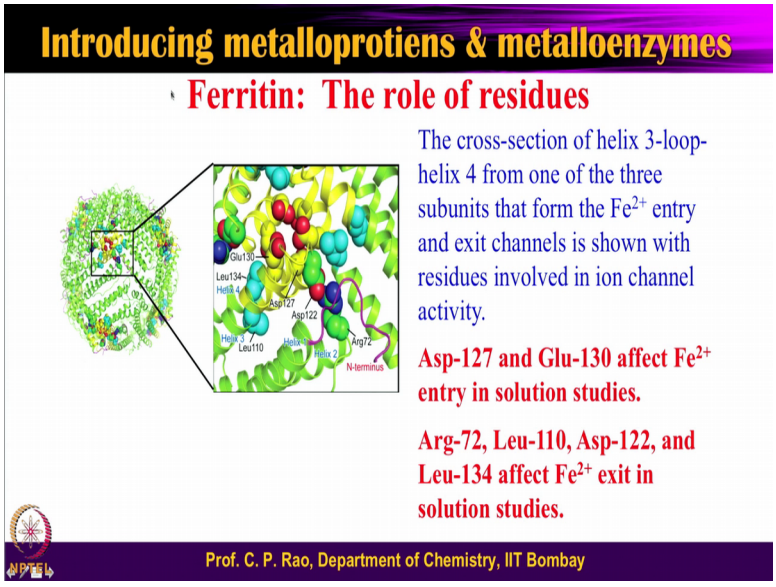

Now, look at a little more cross section of this one. So, you take this one they make a cross section. So, if you look at the cross section very nicely you can see that there are certain red ball, there are other kinds or yellow things etcetera etcetera. So, the iron 2 plus. So, as there is something like if you look at aspartic 127 glutamic 130 these are the ones which are present at the iron entry, they are participating in a iron entry point, then arginine 72, leusine 110, aspartic 122, leusine 134, you can see that leusine 134, aspartic 122 all of these are involved in the iron exit.

So, some ions so that means, there is a passage through which iron goes in there is a slightly away that passes where the iron goes out. So, going in and going out. So, there are some residues that you can find and this is a kind of a funnel or tube like structures. But this tube like structure is not a hollow there are the moieties that are side chains which are protruding and in this case you have a polar kind of a groups.

So, therefore, three fold channels are hydrophilic and three fold channels are useful in providing iron in and iron out. So, iron in and iron out both iron in and iron out are in a iron 2 plus form, but when iron sits inside the ferritin core it is not in 2 plus it is in 3 plus explaining in a while.

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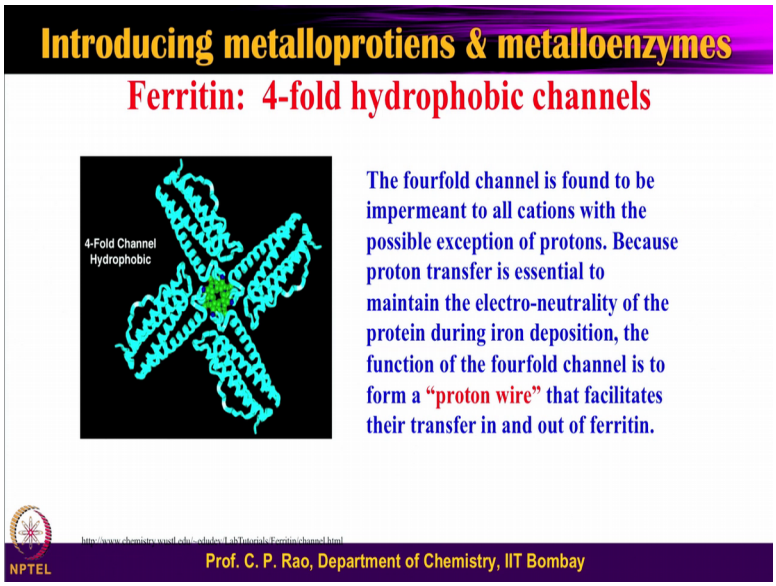

Now, we have just looked at the three fold let us look at the four fold. So, what I said initially? The three fold axis have got the polar lining and the four fold axis have got a kind of a four fold channels will have a hydrophobic kind of thing so that means, there is

some purpose. So, purpose for the hydrophilic is the iron flow, the iron 2 plus getting in iron 2 plus getting out. So, iron 2 plus can have an interaction with the polar groups and therefore, that is where the polar.

Now, there is and as something else is also required the four fold does not allow ions to flow through, but it allows only the protons to flow through, ok. And even that also to the conduit conducting mechanism conduce. So, there is a conduit is formed in this particular four fold channel and as a result of that that you have. So, this will allow only and it will also allow there are certain reducing molecules have to get in enter in order to reduce give the electrons to the iron 3 plus which is stored inside and convert the iron 2 3 plus to iron 2 plus before it comes out.

So, therefore, the role of the four fold hydrophobic channels is that one is the proton a wire formation second is allowing certain kind of a molecules which can give the electrons, so reducing equivalence which we will study in after one or two slides later, ok. Now, we have studied what is the size of this there is a huge symmetry 24 units are there, about 60 to 80 angstrom and there are four fold channels three fold channels and three fold channels allows the hydrophilic part of an iron 2 plus go and getting and coming out four fold channels allows the protons and then certain equivalents of reducing equivalents to get the I mean.

But now, let us look at a how does this iron goes in as a iron 2 plus and forms the core of the iron 3 plus and how does it come out.

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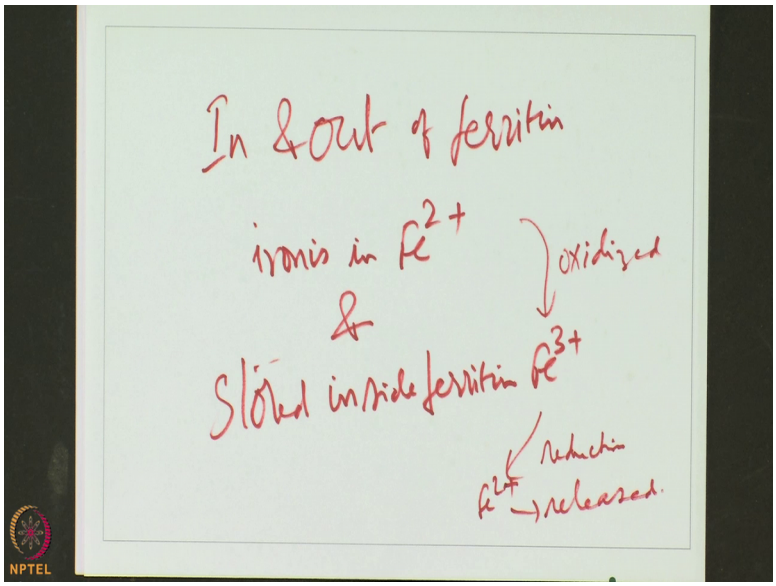

So, let us look at this. As I said that that you have the going in, in and out of ferritin the iron is in iron 2 plus and stored from ferritin is Fe 3 plus. So, this is very essential please keep in mind. So, initially it goes as a iron 2 plus some reducing equivalence will add the electron they take out the electrons from this it will oxidized by the oxygen presence. And then goes to iron 3 plus when it is taking out reducing equivalents will give a electron and iron 3 plus will become iron 2 plus.

So, gets an iron 2 plus and then gets oxidized and then for release a reduction to iron 2 plus and this is released, ok. So, this is a important aspect that one needs to be looking at this one, ok. Let us look at the formation of these ones, ok.

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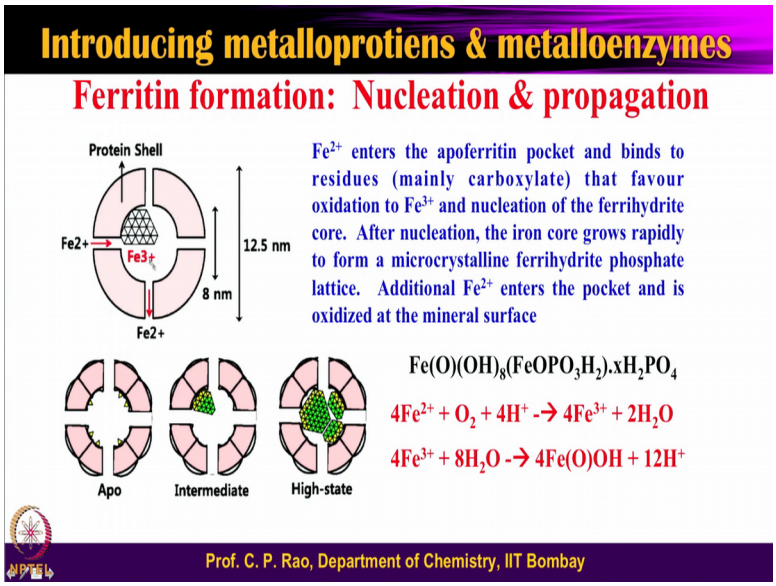

Here you can see that this whole thing is a cross section the sphere that I talked to you about these are an a showing through some channels. So, you can see some channels where the iron 2 plus is coming and you can see the honey bee nest kind of thing is formed here.

So, the whole thing that you have on this, and then you have a core of 60 to 80 angstrom therefore, and the total that is where it can hold something like 4500. And I have already mentioned to you that the in the iron 2 plus goes through the polar channel where carboxylate groups and these things are there. So, once they go in, they oxidized the iron 2 plus and forms the iron 3 plus kind of thing. So, the core keeps growing and also oxidation takes place, and this core can start from a different directions and then fill the whole thing.

So, it starts from here like a honey bee a nest the how it is build if you watch that you will find. So, you have, so it will go the starting apo then some initial stage or intermediate stage and then some or later stage etcetera. So, what is it iron? Iron goes as a iron 2 plus and gets oxidized. So, oxidation and then becomes very hydrate. So, Fe O OH 8, Fe OPO 3 H 2 because these bridges the irons because the iron 3 is which form are always bridged iron 2s are not so bridged at all.

So, the moment you have a oxo hydroxo or phosphate or bridged the iron 3s will be connected and that is what we mean by making the nest into these ones. So, initially iron

2 and presence the oxygen goes in the iron 3. So, and this iron 3 gets hydrolyzed and give iron Fe O OH. So, this is nothing unusual it is.

So, when it has to take out the iron 3 has to become again iron 2. Is this understandable? So, inside this ball there are the through this ball you have the entry pores which you call channels through which iron ions go the iron ions go and bind to the surface and when the carboxylates are there and there the oxidation takes place in this and then it becomes a carbon ion 3 as it becomes iron 3, it also hydrolyzes and therefore, the Fe O Fe bonds will form Fe OH, Fe bonds will form Fe with the bridge with the phosphates. So, the formulas are shown over there.

Now, let us look at the same thing with the some little bit of a spectroscopy I am giving. I told you several times in the in this particular course that the iron can be very well understood from Mossbauer spectroscopy where there is a iron 2 plus iron 3 plus whether it is 2 plus with the high spin or 2 plus with low spin, or whether it is a 3 plus with the high spin 3 plus with a low spin. So, all this can be very well understood.

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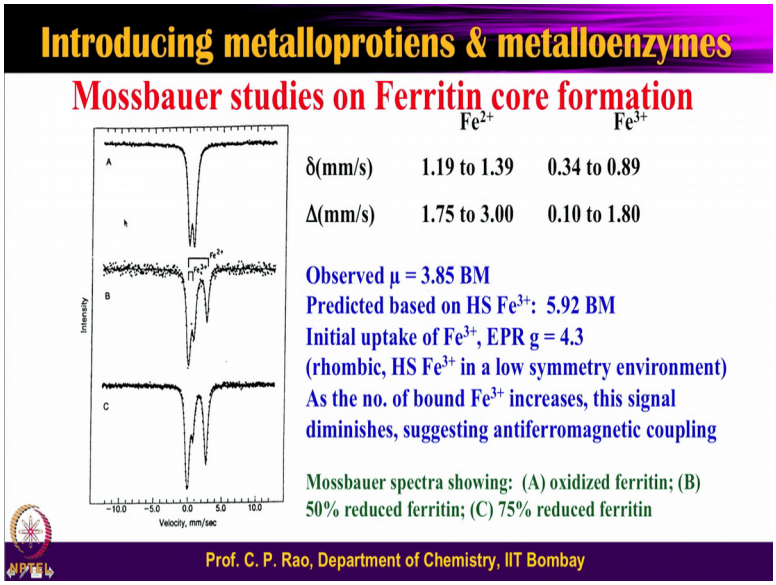

So, therefore, the same thing what we are talking about in the previous slide that is the iron they completely oxidized and 50 percent reduced 75 percent reduced. You can see this is basically a 100 percent oxidized it gives a only one doublet it is from the iron 3 and you start seeing a second doublet over here, this one and this one together is second doublet and this is from the iron 2 and that iron 2 part will go up as you increase the

reduction. So that means, the reducing equivalence which go inside these ball or ferritin will start reducing the iron 3 plus to become iron 2 plus. So, this demonstrates the Mossbauer, demonstrates that the iron 3 plus is indeed reduced to iron 2 plus.

So, these are the parameters for iron 2 plus iron 3 plus that you can make out or compare. So, what is the, so if the ions are not bridged together one would expect the high spin iron 3 you have a 5.92 bore magneto. But what do you find the observed for mole is 3.85 which means the ions and the ions are connected and if they are connected like a anti ferromagnetic coupling, but not 100 percent quenching they support quenching. So, 4.9 becomes 3.9 almost, so that is where.

And this can be identified the iron whether it is a high speed or not you can look at the EPR also, and you can look at this quarter polar coupling will talk to you about the symmetry and then EPR also will talk to you about the symmetry. So, using EPR and a magnetic susceptibility and the Mossbauer spectroscopy you can find out the iron, whether the iron is completely in the iron 3 plus state or iron 3 plus has been come iron 2 plus.

When it will, when will it become iron 2 plus when it is releasing it needs the reducing equivalents and the reducing equivalents will make that part of it, ok. How will the entry? I have already explained you the iron 2 plus goes through this channels and form exit.

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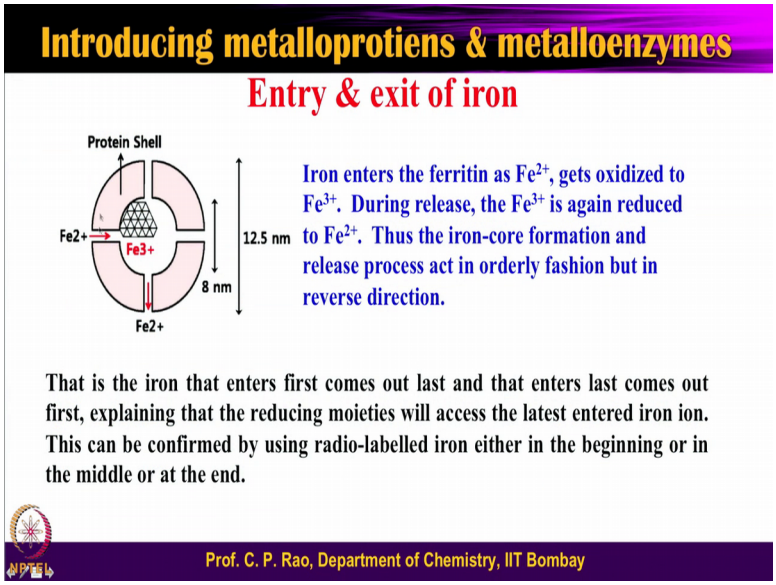

So, how does it exit? And when it goes inside is no more iron 2 plus is a iron 3 plus. So, to release it has to first reduce. So, so the iron that is entered inside the core is formed the iron converted into oxidized to iron 3 plus and formed a ferry hydrate phosphate kind of a bridge complex, and this iron 3 cannot move cannot be released. Why? Why iron 2 can be released, and 3 plus cannot be released? Look back the studies that I talked to you about the inert and labile iron 2 plus is at least 10 power 5 to 6 times labile then iron 3 plus.

So, labile means the exchange rates of the coordination the coordinated ligand is very fast with the iron 2 plus not so fast or very slow with the iron 3 plus and that is the reason why. So, I have given you the answer to why iron 2 it should convert into iron 2 plus before it comes out because its labile it can be transported, and that is where a thing you have. So, therefore, you have some reducing moieties. I will show in the next slide the reducing moieties and these reducing moieties can be utilized.

So, you can find out what level it is entered, what level it is released by using the radioactive labile. So, initially if we use the radioactive labeling that will go and bind to the periphery and then if we use the later it will be in the centre, and when the releasing it comes exactly in the reverse manner.

They, the iron that entered into this ball towards the end will come out first the iron that entered in the initial stage will come out later. So, it is exactly reverses. This can be easily identified by feeding the radio labeled iron at different stages and that is have been done it is not an important aspect of it.

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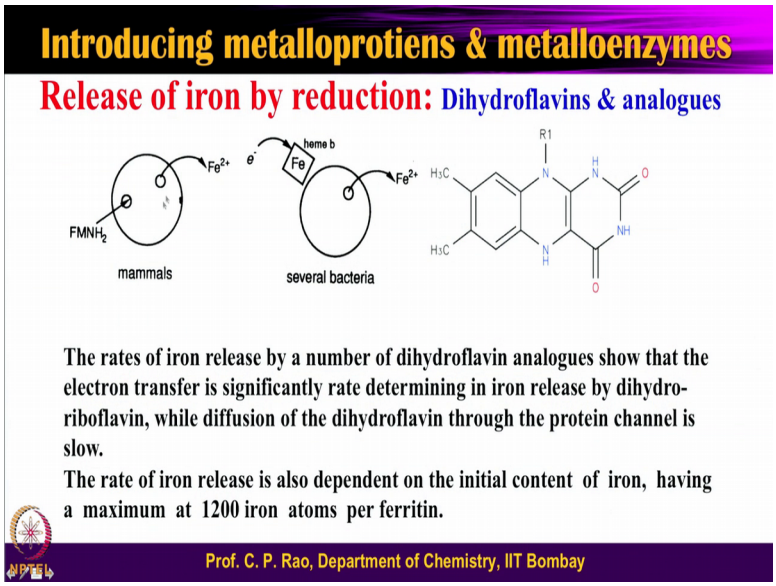

So, now how does iron is released, released in a mammals and in bacteria? There are some heme proteins etcetera or even non heme kind of a analogue. So, this and these analogues this is a flavin kind of analogue analogues, these analogues will get into or these systems will get into the get into the into the ferritin core and reduce the iron release the electron and reduce the iron 3 plus to the iron 2 plus kind of a situation and then that iron 2 plus will come out.

I have already talked to you the 3, three fold the channels have got compartmentalized portion for iron 2 plus getting inside iron 2 plus getting outside. So, both getting inside getting outside both are possible, and both of these are possible the into the three fold the axis of this.

Now, from the four fold a axis you can expect this reducing dihydroflavins all the kinds of analogues to get into this particular thing. So, they can easily get into the this particular these particular species can get into the ferritin core and they release the electron. So, the electron is added to the iron which is present in the core part of this. So, please remember that the iron rewritten here, is different from the iron that we are talking about.

So, this is a protein heme b protein which is which undergoes between iron 3 plus and iron 2 plus. It will add an electron to iron 3 plus in the core and make into iron 2 plus, and thereby the heme will get oxidized. So, heme gets oxidized and the iron present in

the core in this honey bee nest core will get reduced, ok. So, that is what you called as a reducing agent, ok. So, the rate of iron released by a number of dihydroflavin analogues show that the electron transfer is significantly the rate determining in the release process.

So, first of all electron transfer and also this is also in turn related to how much of this dihydroriboflavin will flow inside, there is also another kind of a control. So, first the reducing species has to enter inside through the four fold axis, that is one rate controlling the other one is the electron transfer from that particular species to the iron 3 plus, these are in fact, a slope. So, diffusion or the hydroflavin through the protein channel is also is slow. So, therefore, these are the things which control the release part of that.

So, when you say release there is some kind of a signal comes from where that signal will activate these molecules and pushes these molecules inside and then when they go inside they reduce the iron 3 plus into iron 2 plus and then comes out this, ok. And that is the kind of thing needed. So, the rate of iron release is also dependent on the initial content of how much iron is a suppose you take only 10 percent iron is being built in 50 percent of the iron is being built inside or 100 percent iron is being built in.

So, it will be dependent upon these. So, maximum rate is when you have at least one-fourth is totally around 14, 4500 atoms or irons about 1200. So, one-fourth of the ferritin is filled then its good it gives a good rate to release of this kind of thing. So, now, you understand the iron ions are stored as iron 3 plus in ferritin when the body has sufficient amount of iron ions, and the iron ions are required to different organs of the body and that will be basically transported by the transferrin and when you have a excess it will be stored when you have a necessary it is being withdrawn.

So, when it is stored it goes the transferrin brings and releases as a iron 2 plus and this iron 2 plus go through via the three fold channels and through the polar channels and goes inside. Once it goes inside it will get oxidized to ferric and then bridges with the oxo hydroxo a phosphate kind of a bridging and then forms on a score and the course gets in develop and the whole core can get filled.

Now, when you when you require the iron what happens is there are some cellular signals which will generate these dihydroflavin kind of a molecules or a heme kind of a molecules and pushes into this core of the protein ferritin into the four fold axis, of a four fold channels. And therefore, you have these currents getting and then give the electron

and reduce the species of the iron 3 plus to give the iron 2 plus and the once it becomes iron 2 plus it will come out too.

So, I have explained the transport of iron by iron transferrin and the transferrin having two sets of a centers the C-terminal set and the N-terminal set C-terminal is being bound strongly N-terminal is not bound. So, strongly less less strongly relatively both have got similar kind of core and the core is iron core is formed only in presence of carbonate, and this can be internalized into the cells because the protein has a carboxyl a carbohydrate as the protein exterior and that carbohydrate will push.

And coming to the ferritin part of it ferritin is a huge structures is a is a globular kind of a structure and with the protein having the huge molecular weight and there are 24 subunits forming this one, having four fold and three fold channels and allowing a ions and non see hydrophobic hydrophilic kind of channels.

So, therefore, it goes in as a iron 2 go after going in it converts into iron 3, and bridges with the oxo bridges with the hydroxo bridges with phosphato open forms. And when you require the iron then reducing equivalents have to get in and then add the electron and when it adds the electron the reducing species will get oxidized and the iron 3 plus inside will get reduced. So, therefore, the iron 2 plus will mobilize and comes out.

I hope you now clear the oxygen transport, the iron transport, the iron storage of all this. And next we will get into the electron transport phenomena which is very essential in the biological inorganic chemistry or inorganic chemistry of life.

Thank you very much.