Biological Inorganic Chemistry
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Lecture 17
Transport of metal ions in bacteria and plants

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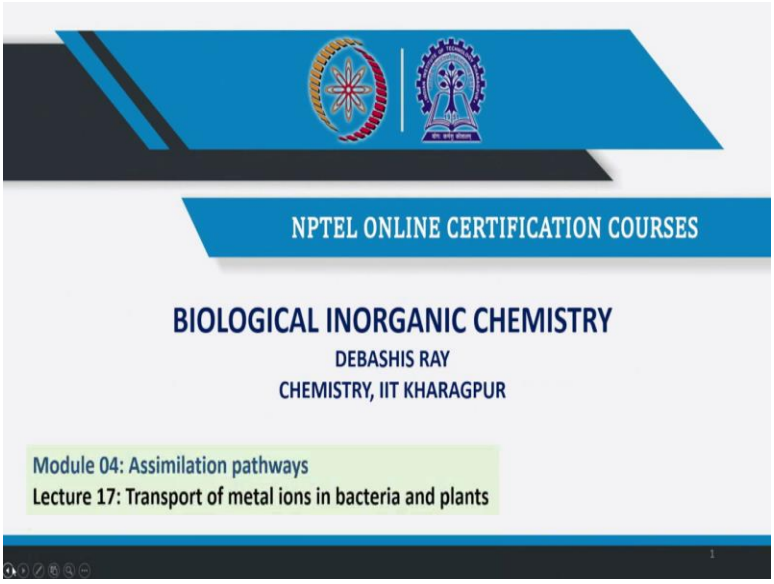

Hello. Good morning, everybody. So, we will start again where we have finished last time regarding your module number 4, where we are talking about the different assimilation pathways as well as their transport and storage. So, in this lecture number 17, where we will be talking about the corresponding thing in terms of the metal ions, there are more three, four metal ions that we will be talking about in bacteria and plants first and the next class we will talk about the fungus and the mammals.

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So, we will try to call her some of the four basic points, how the intercellular metabolism in bacteria is taking place. Then we will talk about the metal ion transport and storage in bacteria. What are these metal ions? We will try to focus our attention mostly, firstly with iron, because the large amount of literature is known with regard to iron first, then copper, zinc and if possible manganese and other metal ions we will talk about with the presence of those metal ions are definitely very less.

Then at one point also we will talk about the transport and storage in plants. What are they? We are also dependent on plants. We take plant materials as our food material. So, we should know also how the different metal ions are being stored in the different varieties of these plants and how the mechanism is going on.

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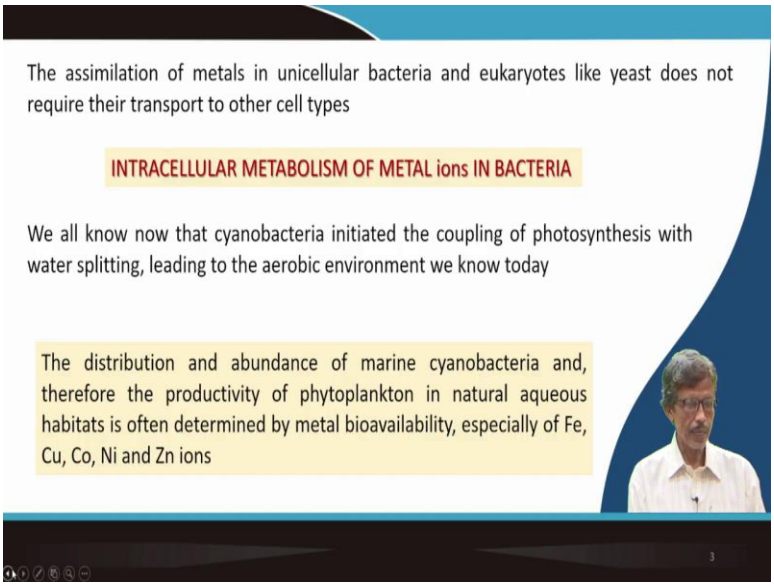

So, during this assimilation process, what we have seen the metals, particularly the metal ions, not the metals, everywhere you whatever we are talking about they are not metals, but they are metal ions. In unicellular bacteria and eukaryotes like yeast, what is the yeast we know all we use the yeast and we will talk a little bit about yeast also, but in case of yeast that does not require any transport mechanism to their cell types. So, where we required the different things that we will also see, so in case of this transfer, in case of this metabolism, in case of this storage of metal ions in the different bacterial cells.

So, if we want to study the different bacterial cells and their typical accumulation, what do you consider that how the different metal ions say the very basic metal ions and which is highly concentrated as well as in our earth crust what we know the iron. So, if we only talk in terms of the iron and how the bacteria is also struggling for iron that we also know already we have discussed a little bit about the siderophores and all these things. Now, if we talk in terms of the cyanobacteria, in our last few classes also we discussed about cyanobacteria for the fixation of carbon dioxide, so the photosystem also with regard to your cyanobacteria.

So, they are involved in the photosynthesis. And when we all know that in the photosystem 2, in the photosystem 1 we know the chlorophyll is playing the actual role for its excited state and transferring your CO_2 with the attachment of water giving you the glucose molecules. But at one point of time, you also have, you have to split the water molecule for evolution of the dioxygen

molecule. So, this water splitting reaction in photosystem 2 leading to the aerobic environment what we know today, because earlier the environment was typically reducing and we do not have the oxygen environment also.

So, few billion years ago, the cyanobacterias came into the picture and they started doing their photosynthesis and that is why our environment has becoming oxidizing as more and more oxygen are getting accumulated in the environment. So, during this process, what we see the distribution of these cyanobacteria in of marine origin, last time we are talking about the marine chemistry so this is also related. If we talk about the cyanobacteria, definitely it will be related to your marine chemistry. So, you have the phytoplankton in natural aqueous in habitats.

So, in natural in habitats, if you have the cyanobacteria and how the cyanobacteria can control the availability, again the bioavailability or not metal, metal ions, specially the ions of iron, ions of copper, ions of cobalt, irons of nickel and the corresponding zinc ions. So, the marine cyanobacteria, how they are important.

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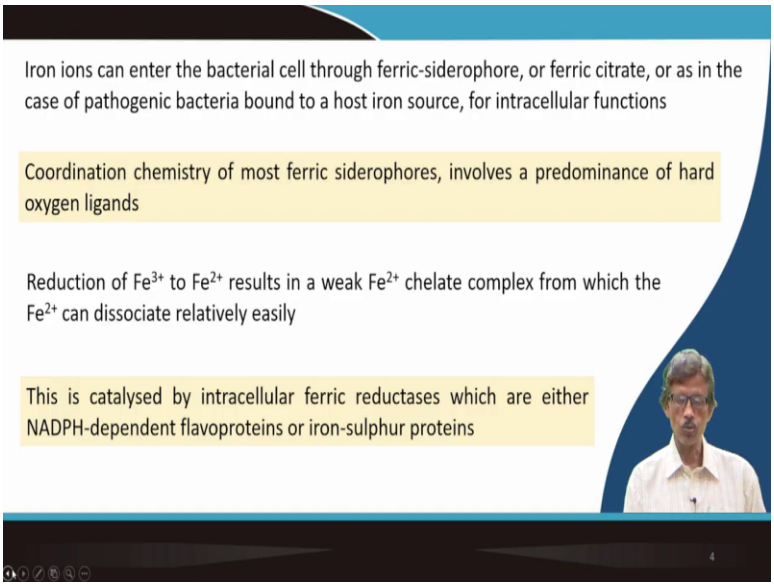

So, if we know consider that okay the iron ions are coming into the picture and they can enter the bacterial cell, then the mechanisms are there and everything we know now that how the cell is engulfing the iron ions, the ferric ion or the ferrous ion. So, in the bacterial cell the growth is again taking place. Already we have discussed a lot about the siderophores. So, now, we know that situation is due to the ferric siderophore.

So, iron in the higher oxidation state that means the trivalent iron which is your ferric state and the siderophores are mostly giving you oxygen environment, the hard environment. The oxygen is a hard one, so the ferric ion is also hard one. So, the binding is much more stronger. So, enterobactin and others are there and ferrioxamine and all these are the categories of siderophores which are useful for binding your ferric ion.

Then, if the simple acetate ions like citrate ions, so acete ions are there, so citrate, if citrate is there, ferric ion will immediately take up that citrate anion to give you the corresponding ferric citrate as the salt in solution definitely. It has the solubility in aqueous medium also. So, this particular one is very much similar to that of your formation of ferric acetate. We all know that if you have the acetate anions are available and iron source is there like iron oxide or hydroxide immediately we can make in the laboratory the corresponding ferric acetate.

So, in the biological medium also when the citrate anions are available, it has a very good affinity to bind or trap your iron sites. So, the iron centers will not remain as your hexa eco species. So, these bacteria are notorious definitely because they are all pathogenic bacteria. So, if we have certain amount of these pathogenic bacteria bound to your host iron source, because we have iron and the mammalian or the human being has also the iron source, but the bacteria will try to gather that particular iron if the binding constant for the siderophores which is going to abstract or trap that particular iron, the Fe3 plus ion, so definitely you will have the corresponding equilibrium constant or equilibrium formation constant of that particular species will be higher.

So, for their intercellular function, because the bacteria is taking that and it will go inside their cell, so for their function they also need iron. So, there will be definitely a competition afterwards also when we talk more about the iron biology. So, we will talk more about this particular behavior, this particular binding and the typical structures categorically. But right now what we can understand from here that if the ferric siderophores are forming and we all know that different groups are coming out like citrate anions so the coordination chemistry will definitely take the seat to carry forward your corresponding iron formation and cell abstraction.

So, hard oxygen donors, as I told you that hard oxygen donors are there, so that will take care of your coordination chemistry part for trapping these iron. Now, if we consider that okay I have to release that particular iron because the transport is dependent on the release of that iron taking up

by some other group, some other protein molecules, so how to go for the reduction. It is a very simple reduction again. We know that if you have a typical reducing agent, you can immediately deduce Fe3 plus to Fe2 plus. So, the reduction can take place and results in a weak Fe2 plus chelate complex from which the Fe2 plus can dissociate relatively easily.

So, the only thing is that if you have the available reducing agent, not only you are going for the reduction, but you can take out that bound iron which was already bound to your siderophores anions, the anionic part of those siderophores. And these are catalyzed, it is a catalytic reaction, by intracellular where it is within the cell, so that intracellular outside the cell, so intracellular ferric reductases are there which are NADPH-dependent flavoproteins. So, flavoproteins are we all know these, mostly these are organic reducing agents or you can have the FeS, the iron-sulfur proteins, the rubredoxin or ferredoxin type of iron-sulfur proteins.

We all know that they are also very good reducing agent. At a specific potential, they can reduce the substrate and that can also be catalytic in nature. And these iron-sulfur proteins can also function as a redox mediator. Now, we know after discussing your cyclic voltammetric classes and all these now we know that the mediators are there. So, these iron-sulfur proteins, if they are directly attaching to this bacterial cell, they can transfer those electrons, but that has been gathered from other reducing agent or some other mediator such that you can reduce the Fe3 plus ion within the siderophore molecule and reduce it to the ferrous ion such that you will have the release of ferrous ion from that particular point.

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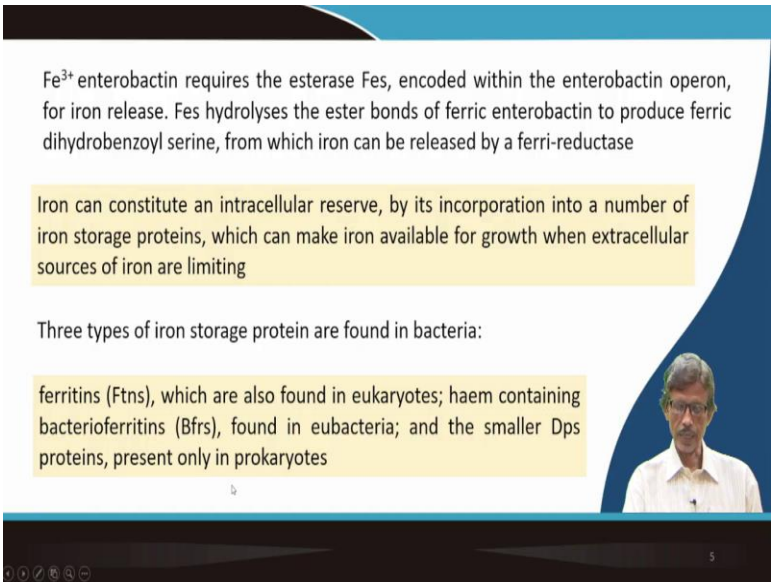

So, one such example, very good example, so bacterial siderophore is your enterobactin, is a very big molecule and this particular one can also trap the Fe3 plus, but it requires the esterase, F esterase, FeS, so this Fe and esterase part, so FeS, encoded with the enterobactin operon. How we produce these things. So, the genetically it can be controlled for its production, but it is required. Where we require? We require these esterases for iron release. So, these iron releasing or iron reduction action to facilitate the FeS hydrolyzes the ester bonds of ferric enterobactin to produce the ferric dihydroxybenzoyl serine. What is that?

So, we know we have ester group. So, you have in the enterobactin structure, afterwards we again discuss it, that is a cyclic triester. So, you know that ester is forming between the hydroxy group and the carboxy function. So, if you have COH function at one end of the amino acid and another and you have the OH function, the alcohol function like your serine so that is why you have the benzoyl serine. So, benzoyl serine or serine, sometime we call it also serine, so these amino acids, so this substituted amino acid, so that particular amino acid if you allow it, the biosynthetic route for the formation of these enterobactin molecule is such that three such substituted serine molecule will come and give you the corresponding cyclic triester.

At some point also I have discussed earlier that if you have the simple glycine and if it attaches another glycine so it will giving you a gly-gly type of peptide, it is a dipeptide. Two units of glycine is giving you a dipeptide. But if you have a three units of glycine, we will get a

tripeptide. So, instead of your amine function, if you have the hydroxy function, this hydroxy function because this serine will have what, this is the amino acid. So, serine amino acid, you read it nicely, after some time we can talk about the structure, but today also we will not discuss about the structure and all these chemistry reactions.

So, apart from your NH2 function and COH function, you will have the CH2OH function. So, you have the extra alcohol function. So, if you use that alcohol function for ester formation, not for amide formation, what will happen? You get something that is a triester type of thing. So, three of these ester bonds will be there and is a cyclic part. So, the cyclic part, but you will have the pendant group. Pendant groups are the amine functions, because we are not utilizing and we are not taking these amine functions into consideration for the amide bond formation, but that amine functions if they are benzoylated, but that is dihydroxybenzoyl function.

So, you will have some pendant hydroxybenzoyl group, hydroxybenzoyl group, another hydroxybenzoyl group. So, you will have three bidentated parts below, above you will have the triester cyclic platform. So, that is available for binding your iron site. So, now this particular iron ion can constitute a intracellular reserve. So, this intracellular that is within the cell you can also have the corresponding stock of these iron ions by its incorporation into a number of iron storage proteins, because the iron storage proteins will also be active again. Without help of these storage proteins you cannot get this particular biomineralization process, so which can make iron available for growth when the extracellular source of iron are limiting.

Why do we require extracellular? Because the extracellular iron requirement for making other molecules, other new molecules, but you have the reserve within the cell. So, you have the intracellular reserve. But if the extracellular concentration is less, there will be some sensing molecules, the channels will also open up. So, those irons which are inside the cell can go out then within that extracellular fluid or extracellular medium to have a iron pool outside the cell.

So, what are those? So, those are utilized for your iron storage. So, they are of three types. So, that is why we see that there are three types of iron storage proteins, which are found in bacteria. So, it is only bacteria. So, do not confuse with all these things. Because we are taking everything together, in our next two classes, because this module we will be finishing with these parts only, in these two classes we will be talking about some of the new term, we will define it the proper assimilation that make the environment of iron, that homeostasis we will talk about, but we are

talking about plant, we are talking about the fungus or fungi, we are talking about the bacteria and we are talking about the human being or the mammals. So, these are the four category of thing which are interdependent to each other.

So, that is why you think about whenever we are talking about the ferritins in bacteria or the ferritin in our body. So, we should be very much careful about, do not confused with all these things. So, we have ferritin also. Bacteria, will also have the ferritins. So, which are also found that is why, the direct statement is that is why that you, it is also available in eukaryotes. And then also we have the bacterioferritins. So, once the name is Bfrs or Frtns is as simple abbreviation as is not so rigid one. So, is the aggravated one is very quickly we can write it.

So, haem containing that means some part of these molecules have the haem thing that means you have the porphyrin unit bound to your iron center. Then eubacteria also it is available and the smaller Dps protein and present, which are present only in prokaryotes. So, starting from your eukaryotes to prokaryotes, you have iron everywhere, only thing that how to know these things, how to understand these things and how the function of these things can be monitored.

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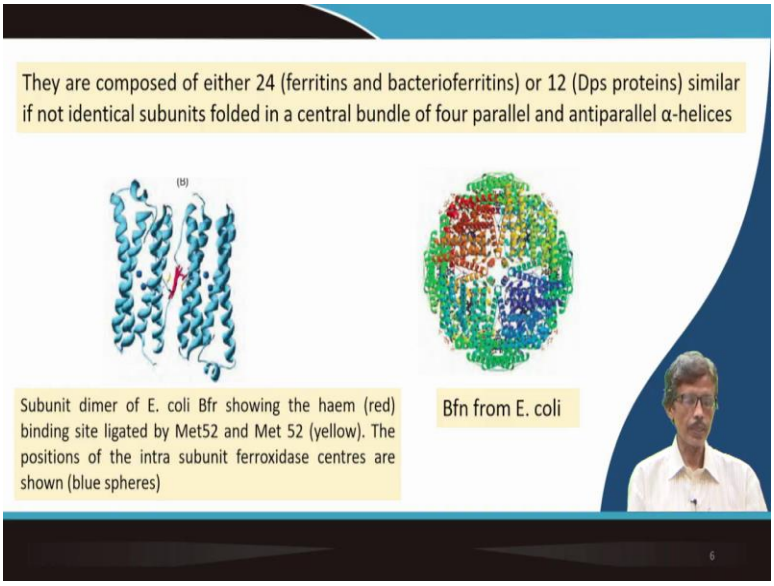

So, these ferritin molecules is well studied for the human ferritin also have 24 subunits, which are present for ferritins and the bacterioferritins. So, both of them have a similar structure. Property-wise also they are of the same type, because they all are using for iron storage mechanism only. But these Dps proteins are different. These Dps proteins have 12 such subunits,

if not identical subunits folded in a central bundle to four parallel and antiparallel alpha helices. We know we have discussed at some point what are your corresponding alpha helix part and the beta sheet part.

Now, the alpha helix part and the beta sheet part will there for the folding. And if you have 24 or 12 such subunits, so the folding is there. Then after folding only the thing having some donor groups at this particular my finger's surface can some donor groups is available over there and that donor group will come. So, this donor group will have the claw that is the chelating part and so that part will come and grab the corresponding metal ion. So, one such thing is the whole unit, the whole unit is a globular part, this is extra structurally found, this is extra structurally determined and the color coding is that to determine something that even if it is a sphere type of arrangement, but it can have some symmetry if you look at some point.

So, if you look at, at this particular point that means this particular central cavity and immediately at the center if you see how many of these groups, protein groups are surrounding this particular central part, whether you have three points or whether you have four points, so it is four. So, you will have four such points are there. So, that means you have a C4 symmetry, which will pass through the center point of the protein globular unit. So, that way is forming and the 24 subunits we can think of also if we consider in coordination chemistry we know what is octahedron which is 8, the number is 8.

Now, if you multiply into 3 it will be 24. So, how the 24 units, say 24 bundles of these proteins, if I give it to you, how you can arrange those in a three dimensional fashion such that you will get it sphere type of arrangement. So, this is nothing but your bacterioferritin from E. coli. So, this is the bacterial source is E. coli. And then the individual this folded thing, the individual folded things, what is there, is a subunit dimer of the same thing, same bacterioferritin E. coli what we have and that particular one showing the haem so it will also have the haem part. This is unlike our ferritin we do not have any haem part over there and binding to ligated methionine sites, methionine 52 and another methionine 52 in yellow.

So, and the thing is that the position of these intra subunit ferrioxidase centers which are blue, so these are the inter oxidase, the inter subunits or the inter subunit ferrioxidase centers are there. So, these two units are within that particular bundle and then the whole entire bundle thing can

be folded and the folding will take place in such a fashion that ultimately we get a sphere type of arrangement.

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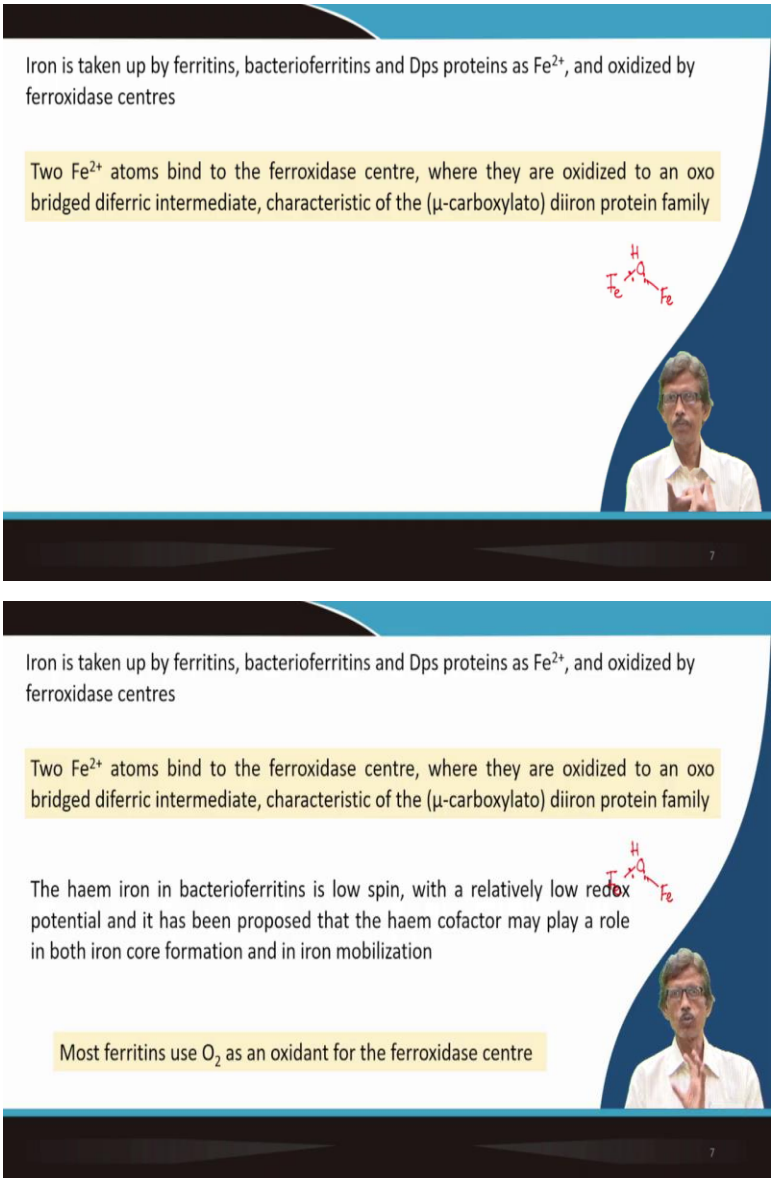

Now, if you have sufficient source of this iron, iron is taken up by these ferritins or the bacterioferritins or the Dps proteins as Fe2 plus. So, initial abstraction or initial taking up mechanism is based on the ferrous ion. Then we all know now that biologically available oxidizing agent is there which is ferroxidase. So, these ferroxidase centers are also nearby. So, at a certain potential value we now know what is this potential value, e0 values, the biological e0

values for these capacity for oxidizing the iron 2 center in all these ferritins to Dps proteins. So, these are functioning as the oxidizing agents.

And when two such iron, two ions are binding to the ferroxidase center they are oxidized to an oxxo bridged diferric intermediate. How it is happening? So, if you have an iron site and that iron site is bound to the ferrioxidase center so what are there apart from these iron site and the binding of the ferroxidase center? So, if you find that the water molecules were there and after deprotonation water has been converted to the hydroxide ion. So, if that diferric unit is there immediately that is forming more and more number of hydroxide anions. So, more and more hydroxide anions are there.

So, if one iron is there and that is bound to Fe one such OH function so that OH function is there that Fe if you have this is forming. So, if it is a terminal, OH is there. But you see this OH will have more number of lone pairs. So, already two of these lone pairs are bound to this particular one. But if you find that the other two are also there, which can also form another bond to other Fe. So, that is why you have a bridging entity. That OH, this bridging OH has three such lone pair of electrons. So, these three such lone pair of electrons if you consider, so two are there on the water molecule all we know, so out of these three lone pair of electrons, now two will be utilized for holding your iron center.

Similarly, we can also have the carboxylate end. So, carboxylate ends from the amino acid residues of the protein chain can also coming into the picture and that can also again, so from this side is Fe, OH Fe unit and the bottom you can have that clip from the acetate units. So, you can fix that di-iron site nicely over there. So, here now the haem iron what is there in the bacterioferritin is in low spin and with a relatively low redox potential and it has been proposed we also know now that the haem cofactor may play a role in both iron core formation and iron mobilization that we are studying in this class.

How the code is formed for the storage mechanism and how the mobilization is also taking place because iron is coming after, one after another is coming to store over there. So, this mobilization is also important for this particular case also. Then the most ferritins also use O2 as the oxidant for the ferroxidase center. So, O2 is the ultimate oxidizing agent, but you have the different mediators such that the redox equivalent from the O2 is being transferred to the other mediators which are available for your oxidation reaction.

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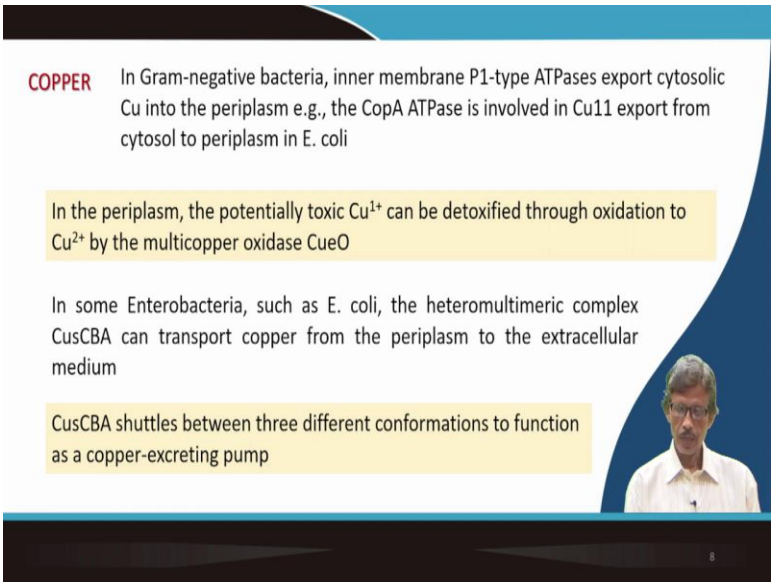

Now, what about copper? So, if we go from iron to copper, we see that the similar type of mechanism is there, but the detailed studies can found or can give us something where we can have this particular thing about some type of bacteria whether you have the Gram-positive bacteria, which is Gram staining, the color staining, Gram-negative or Gram-positive bacteria so that you have the membrane and then a membrane is dependent on ATPases and the cytosolic copper how it is there and how much it is moving to the periplasm. So, the CopA, so these are the abbreviated form of the ATPase which is hydrolyzing your ATP molecule involved in copper is not copper 11 is copper 1 plus that means cuprous ion.

So, for this cuprous ion which is getting exported from the cytosol to the periplasm in E. coli, what we are studying is very easy to understand all these things nicely, because the E. coli is the model substrate, where we can study everything, something we develop on these, something we can, the model substrate. So, the bacterial E. coli is always a very good substrate and on it, we can study all these things, whether you have the iron thing or whether you have the copper thing.

So, in the periplasm, the potentially toxic the copper 1 plus is the toxic one and that can be detoxified through oxidation to copper 2 plus, when you have the multi-copper oxidase, availability of the multi-copper oxidase thing. So, is the oxidizing agent. And in some of these enterobacteria, such as in E. coli, the heteromultimeric complex CusCBA is also available. The CusCBA started between three different confirmations, not the oxidation state, but also it is

functioning for the different confirmations for copper excreting pump, because if you take out that particular copper and the copper removal thing also like your proton pump, so proton is pumping from one side to the other, similarly, copper is getting pumped also from one side to the other. So, these are the corresponding machinery, the biological machinery to move this copper from one side to the other.

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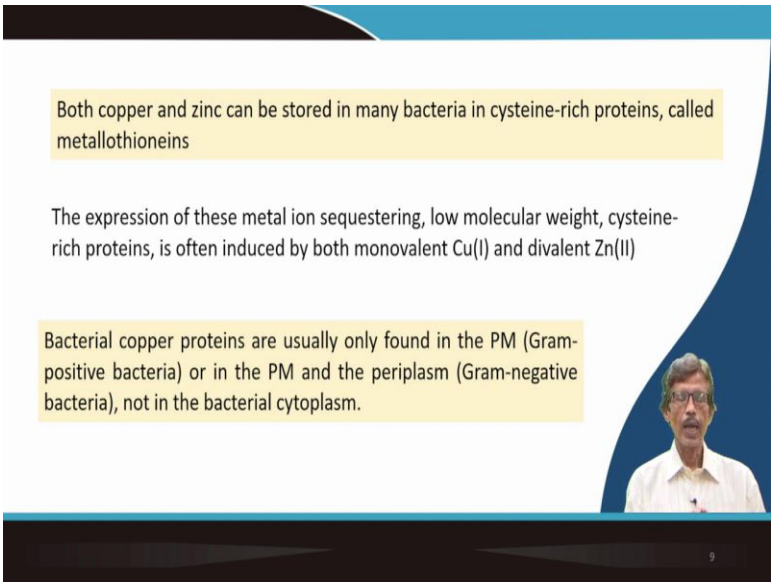

But along with copper, if you have the zinc in this bacterial side, so you have the cysteine that means sulfur-rich proteins we call them as the metallothioneins. Again, we just study it in detail. Do not worry for that. So, this particular part, I will just go a little bit faster way, because we will come back again, do not worry. These are the introduction part that where we can study all these things. So, these cysteine rich proteins that is many sulfur groups are coming and we all know the copper has very good affinity for sulfur and zinc ion also has a very good affinity for sulfur.

So, these bacterial copper proteins are usually found in the Gram-positive bacteria and in the particulate matter on the periplasm and not in the cytoplasm. So, that is important.

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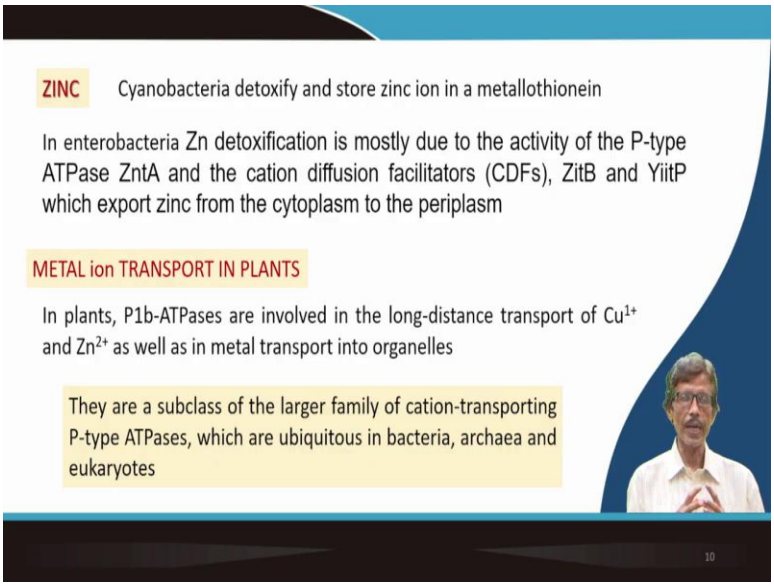

So, also quickly we can see about what we can know about the zinc. And this zinc cyanobacteria detoxify and store zinc in metallothionneins. And these enterobacteria zinc detoxification is also important. And there are several groups that are also important and which can take care of that zinc, the assimilation of the zinc, storage of zinc and its proper utilization.

Then, quickly we also see for the plants, in the plants there are several other groups like ATPases are there, but that can also transport copper 1 plus and zinc 2 plus and then again ATPases are there like bacteria. So, we will get a family of these cation transporting ATPases, P-type ATPases, there are classifications. So, these levels are all for classifications only for archaea and eukaryotes.

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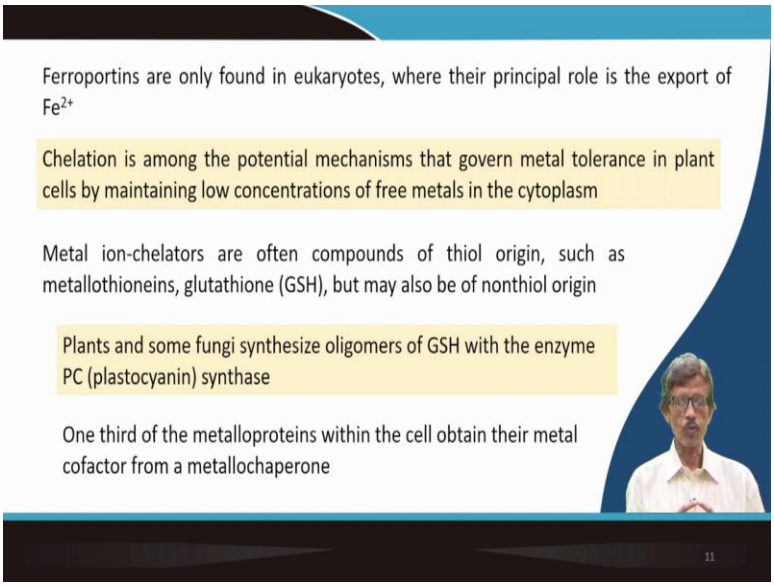

So, these ferroproteins are usually found in eukaryotes where they are finding and we use the chelations. And the metal ion chelators are often found with thiol origins, different thiol origins of the metallothionein and glutathione, is a tripeptide. They also have the corresponding sulfur functions. And plant and some fungi synthesize these oligomers and GSH for the formation of plastocyanin. And overall the one-third of these metalloproteins within the cell what we have, the metal cofactors from metallochaperon. So, metallochaperon are there. So, these are special type of these molecules, of the protein molecules which are useful for these taking care of the copper and the zinc, but they are in the cytosol.

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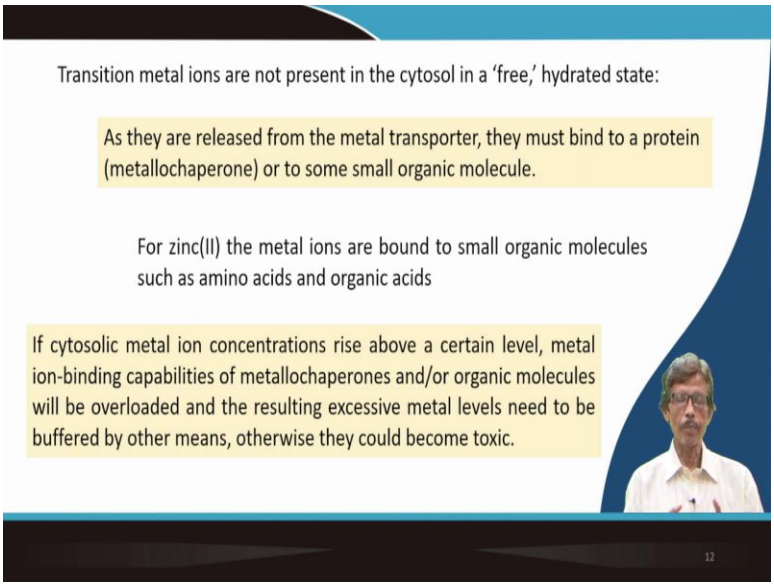

And when they are releasing for using the help of the metal transporter, they must bind to these metallochaperon and to some small organic molecules. And for the zinc, the metal ions are bound to small organic molecules also such as the different amino acids and the organic acids. So, what is ultimately happening in the cytosolic metal ion concentration is controlled by if you can have some mechanism that you can have the excess metal ion levels or you can have the shortage. When you have the excess metal on concentration, it should be toxic to your cell, whether it is a bacterial cell or whether it is a fungal cell.

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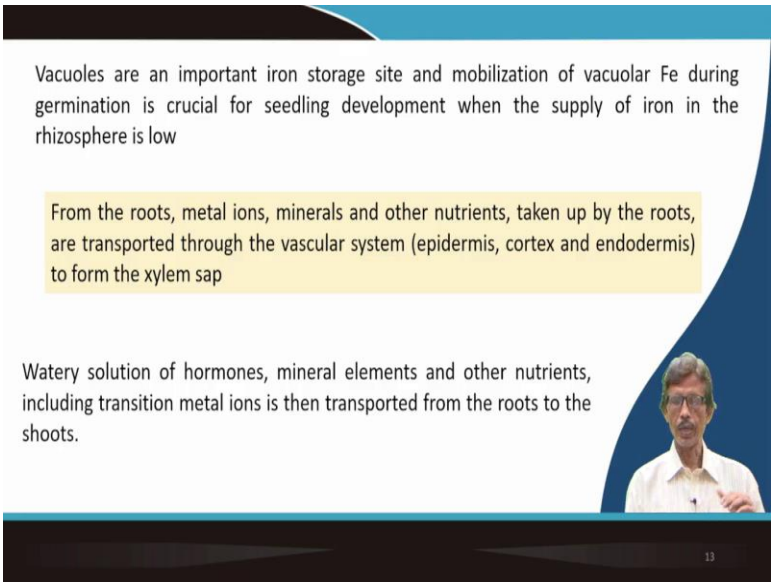

So, you have the vacuoles and the vacuoles are an important iron storage site and within these vacuoles also we can store these metal ions, but we are talking something where with the plant material. So, you have the xylem, you have the phloem. So, it taking up from the root the metal ions, the minerals, other nutrients and then it is going to the vascular system containing epidermis, cortex and endodermis and it is going ultimately to the xylem sap and is all in aqueous solution.

So, this watery medium or watery solution of all these including hormones also they are then transported from the roots to the shoots that we know from our school days that how it is going from the roots to the shoots. So, that upward movement is always there and while doing so it is taking all other metal ions.

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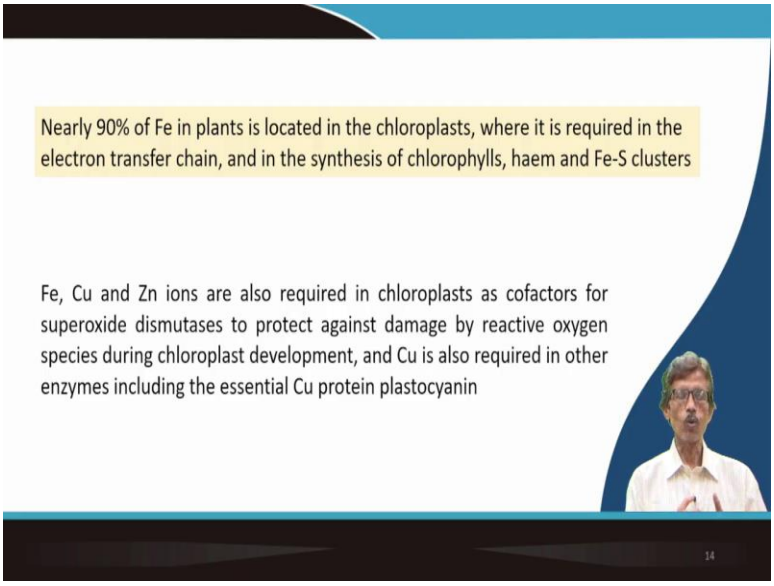

So, naturally 90 percent of this iron in plants is located to the chloroplast where it is storing and require for the electron transfer chain like plastocyanin, electron transfer chain is there in the chlorophyll, it is there in haem and it is there in iron-sulfur proteins. So, for these three metal ions the copper proteins we have in the plastocyanin, which is there and required for other enzyme synthesis so is required for the chloroplast formation, other cofactor formation, superoxide dismutases formation, because that superoxide dismutases is protect all these cells against Ros, the reactive oxygen species.

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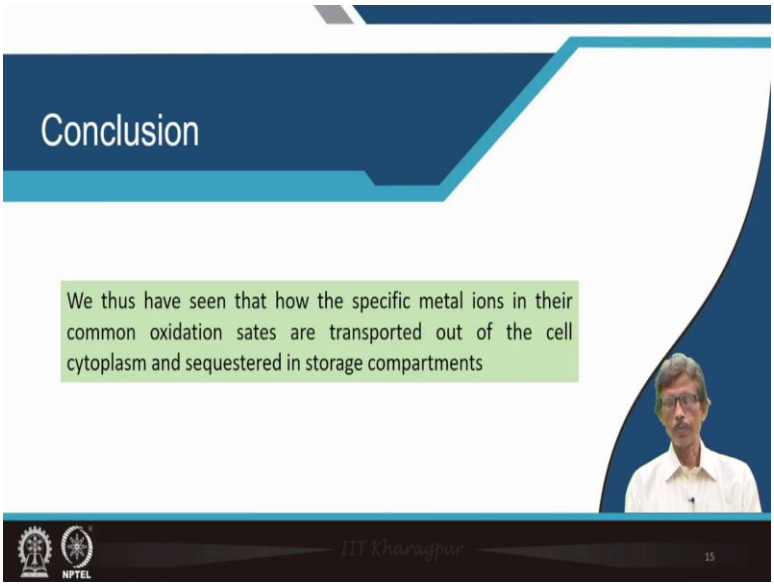

So, in conclusion what we can now summarize this thing is that what we have seen so far during this class that how the specific metal ions in their common oxidation state that means the commonly available oxidation states whether you have plus 1 oxidation state or a plus 2 oxidation state we are transporting, but the reactivity was there different like copper 2 plus is less reactive, but copper 1 plus is more reactive. And then you have the solution. The medium is the cell cytoplasm.

So, instead of your test tube, what do you have learned from your school days in chemistry classes, now you have a corresponding container of the metal ions those are cytoplasm. So, the cytoplasmic solution we are studying not the solution what we are getting from the test tubes. And you can have the different other storage compartments, not the test tube number 1, but you can have also test tube number 2, test tube number 3 and sometimes the bigger thing which can be your reaction flask, which can be your beaker or which can be your conical flask.

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So, again, we have the references. We can consider this particular part from Wikipedia also. We can start with the evolution of metal ions in the biological systems and finally you can go for only one book and all these classes because I am typically following everything from this particular book, such that if you have this book, you can again go through this book and you can understand it nicely. You can have questions on it and you can have your own answers also with it if you go by reading this particular book nicely. Thank you very much.