Metals in Biology
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Lecture – 02
Choice, uptake and assembly of metal ions in cells

Hello, welcome everyone to the second class of metals in biology. Today we will discuss choice, uptake, and assembly of metal ions in cells.

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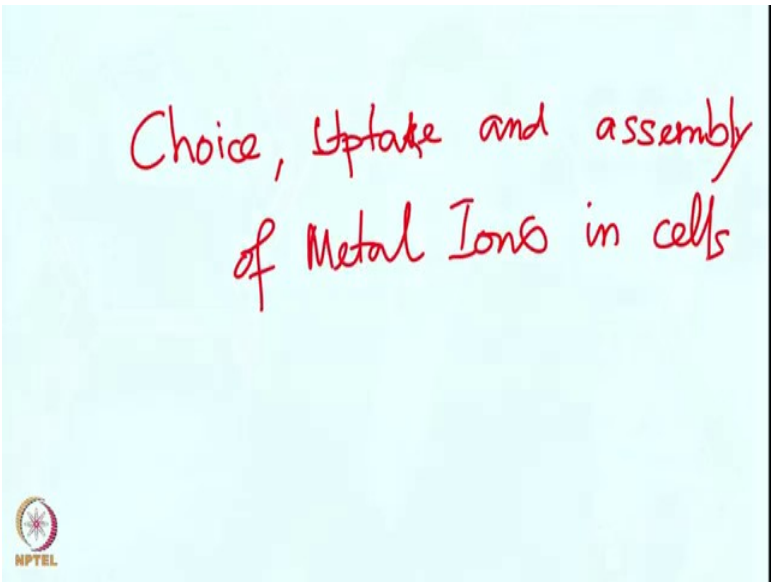

So, we will discuss choice, uptake, and assembly of metal ions in cell. Well let us first discuss the principle of this topic and that choice, uptake, and assembly of metal ions in cell relatively abundant metal ions are used both in geosphere and biosphere different metals are present in different concentration or different ratio.

Therefore, these metal ions are going to be used accordingly in our class, right. over all different metals are concentrated over the years both on earth crust and sea water. For example, some of them are used in very high amount in biological process although they are not present in high quantity either on the earth crust or in seawater.

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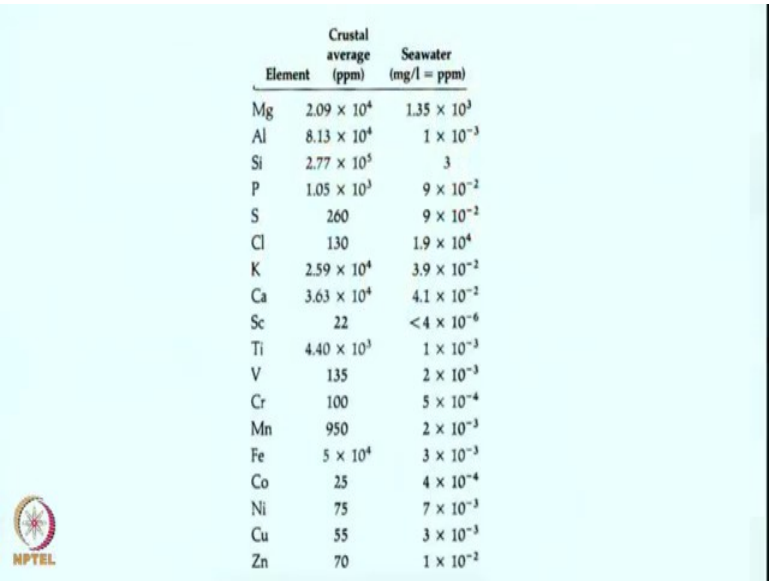

Element	Crustal average (ppm)	Seawater (mg/l = ppm)
Mg	2.09×10^4	1.35×10^3
Al	8.13×10^4	1×10^{-3}
Si	2.77×10^5	3
P	1.05×10^3	9×10^{-2}
S	260	9×10^{-2}
Cl	130	1.9×10^4
K	2.59×10^4	3.9×10^{-2}
Ca	3.63×10^4	4.1×10^{-2}
Sc	22	$<4 \times 10^{-6}$
Ti	4.40×10^3	1×10^{-3}
V	135	2×10^{-3}
Cr	100	5×10^{-4}
Mn	950	2×10^{-3}
Fe	5×10^4	3×10^{-3}
Co	25	4×10^{-4}
Ni	75	7×10^{-3}
Cu	55	3×10^{-3}
Zn	70	1×10^{-2}

For instance chloride as you can see the presence of chloride is very little on earth crust whereas, in seawater this is present in large amount. Therefore, in species where we have sea water based species those are the one which perhaps will have more chloride in them then those are living on earth. On the other hand, depending on the amount of particular metal at a given site maybe those metal ions are accumulated in our body predominantly. Here is this list which shows that how much of what metal ions are present in a given context of the earth crust versus seawater.

So, one of the things we must understand therefore, then the relative abundance of metal ions can control the presence of metal ions in the biological system. So, as you know iron for example, is one of the metal ions which are present in biological system even inside us in human body quite a lot of amount for example, iron is there in hemoglobin in our blood right. Another principle which guides this choice, uptake and assembly of metal ions in cell is the lability, how much labile is the metal ion because it will determine how easily those metal ions are available for a particular purpose can they be delivered, can they be uptake at a particular center.

So, usually in biological system labile metals are often used that does not mean that the other metals which are not too much labile or not abundant in the earth crust or in seawater will not be used. There are ways where nature has evolved over the ages to concentrate a particular metal ions through ATP driven process. And therefore, although

that particular metal ions concentration may be very less still it can be accumulated in our body or in different biological systems.

So, lower abundant metal ions still can be concentrated by ATP driven processes. Entry to cell is controlled by of course, specific channels and pumps that we are kind of familiar with we will discuss in one of the class. So, how it is being up taken by the cells will be determined by the what type of channels or what type of pumps we do have in particular organism.

Another thing is of course, there could be a self assembly of these metal ions which can also help in accumulating a particular metal ions or particular type of metal ions in a particular site. Not only natural abundance the availability and the lability is or labile nature of the metal ions are important. Another important thing is giving the direction from the biological to the biological system from biosphere or geosphere. This is the role which is usually played by the metallochaperone. There are many different metallochaperones in our body which plays a crucial role of delivering the metal ion at a particular center where it is required.

Today we will try to discuss how the iron uptake is happening into the bacterial and mammalian cell. Let us try to discuss uptake of iron into bacterial and mammalian cells, we will take one example is one case for bacteria another case of mammalian cells and we will try to see if we are able to do this reaction quite efficiently.

So, iron uptake in the cell for we want to get iron inside the cell is that going to be a simple process well of course, there are going to be challenges. Challenges is associated with the fact that at pH 7 iron is insoluble quite insoluble and this is where metallochaperone or so called you know, ligand for the metal center will be required and that is where we will discuss.

So, therefore, how to mobilize iron in the biosphere knowing very well that at pH 7 iron is insoluble is going to be quite critical. As you know iron is the second most abundant metal after aluminum and more importantly it is both the $Fe^{2+}$and Fe^{3+} oxidation state that render it functionally useful. So, $Fe^{2+}$and Fe^{3+} are very efficient centers by which a lot of chemistry can be done and iron is need also present in large quantities second only to aluminum on earth crust.

So, therefore, a lot of things going on good for iron the as I mentioned the bad things with respect to iron is at pH 7 iron is insoluble. Therefore, we need some ligand or some method by which iron still can be mobilized in the biosphere. The role of mobilizing iron in biological system, let us say in bacteria is done by siderophore. So, we will discuss one of the siderophore example. In human it is the transferrin that is involved in mobilizing the iron.

Well if you look at lot of these biological studies which try to understand how iron is mobilized are not going to be that easy, because to understand this we need to have a detailed studies including the X ray crystallography. So, in absence of X ray crystallography, it becomes little bit difficult in some of the cases although some other spectroscopic techniques can be used for understanding how let us say particularly metal ions is being after what is the coordination environment around the iron center. What it is understood that in ferric citrate is you know, there is a siderophore which were ferric citrate binding site of the outer membrane transporter FecA is crystallized.

But before this ferric citrate binding site was discovered crystallographically. All of the inorganic chemist and the biologist knew that there is a ligand citric acid which binds with iron that is known, but how citric acid is binding with iron in the outer membrane transporter FecA that was not really known. Well before the enzyme crystal structure is known or the biological crystal structures are known, it was the inorganic chemist who tried to take simple ferric acid and reacted with iron center.

So, the reaction is essentially done with iron nitrate in presence of the you know citrate what inorganic chemist were able to show is two types of iron metal complexes are forming and this has been reported first in 1994. So, let us look at the two iron complexes that forms when citric acid is reacted with iron shot.

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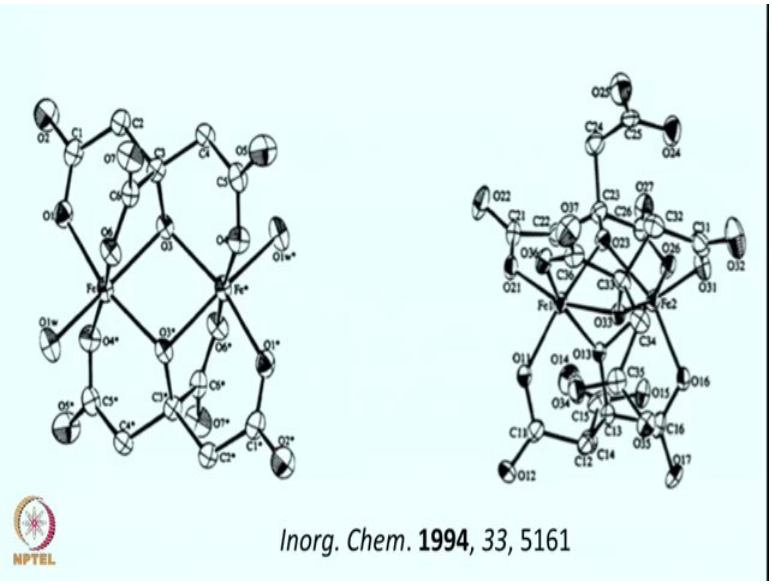

So, here are the two iron centers or two crystal structure each of the crystal structure has multiple iron. In this case two iron centers are there. This is the citric acid or citrate. So, this is the carbon center it has a carboxylate over there and O^- and this is another $(CH_2CO)^{2-}$ another $(CH_2CO)^{2-}$ overall this is one of the citrate and there is another citrate that binds the iron center. Here they are bridged, these two iron centers are bridged by this alkoxy anion. This alkoxide is essentially bridging them.

Now, these two iron centers is this the way what ferric citrate is responsible for iron transporting or a making the bioavailability of iron through these mode of binding. Of course, there is another mode of binding as you can see over here; one can refer to this paper if we want to learn little more.

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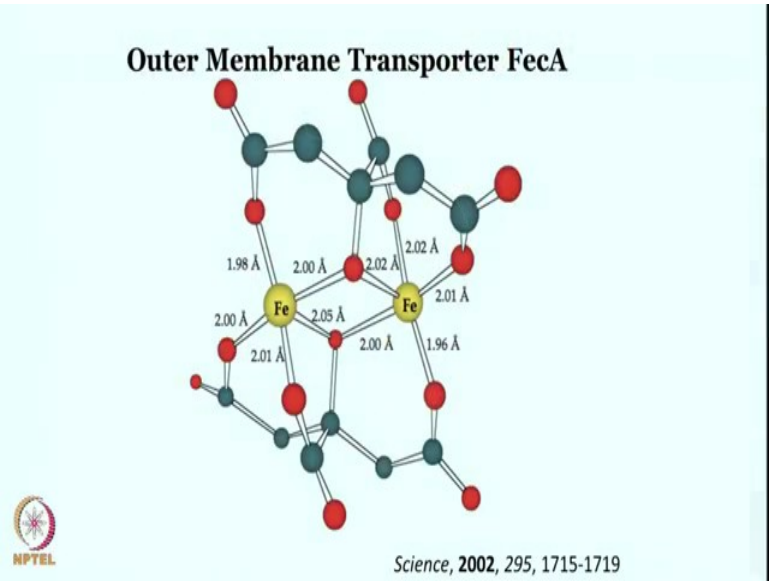

Now, more importantly what crystallographically in outer membrane transporter FecA, it is found that it is the first structure where 2 iron center as we were showing is bridged between these bridgeded by the citrate where the carboxylates as well as the alkoxides are bridging or putting these two iron center together. From this what we can just simply think is the citrate is binding the two iron centers in a way that is shown over here both in inorganic chemistrywise doing the reaction in laboratory we got the idea how citrate can bind with iron. Over here in FecA outer membrane transporter FecA we also find that exactly same structure that is what inorganic chemists are saying.

So, overall therefore, we can conclude that the inorganic chemistry studies of binding citric acid of iron is quite important turn out to be quite in informative and that turn also in latter on turn out to be true where it is found that two iron center exactly the way citrate is binding is similar to that in FecA. Well, so this essentially gives an idea that how perhaps irons is going to be going to be mobilized into the bacterial siderophore or by the bacterial siderophore that is how we perhaps get the iron in intake in inside the bacteria.

So, I hope what I am trying to tell you I hope you understand that this ligand such as citric acid is quite important in mobilizing the otherwise insoluble iron center to make it feasible or to make it available in let us say bacteria. Of course, similar mechanism can

be thought of for higher species. We let us then look at another system another siderophore enterobactin, so this is a bacterial siderophore once again.

Now, here just like previous case where citric acid was playing the key role in dictating the coordination complexes. Here also we will find that a great ligand which will be responsible in bacterial siderophore to sequester the metal ions, so that they become soluble and they becomes available inside the bacteria.

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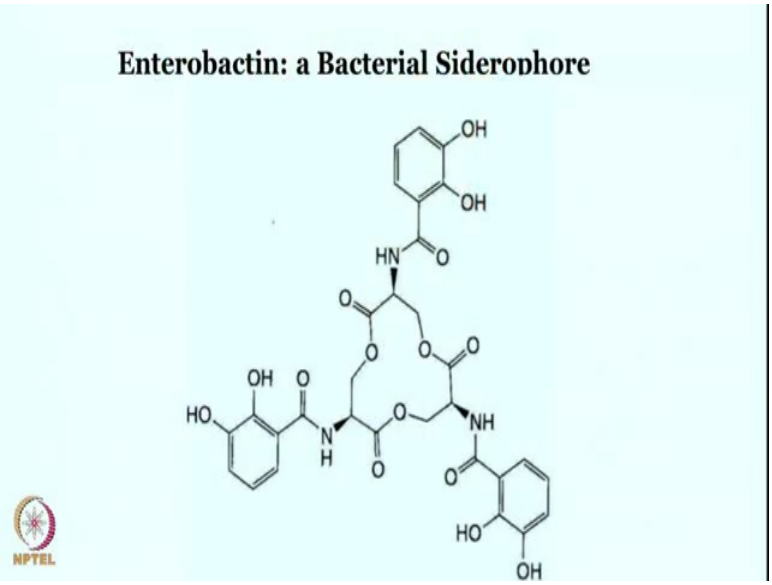

This is the picture or this is the chemdraw of an enterobactin a bacterial siderophore, as you can see this is a gigantic organic molecule. This is nothing but the ligand for the metal center this whole thing is ligand. As you may we will see that is are the possible binding site for the metal center. Now, what was not definitely known is how this possibly binds with the metal center, this is a nice cyclictriserine lactone structure which is enterobactin is bacterial siderophore.

Now, how this is coordinating with the metal center and making let us say iron available for bacteria that was not known. So, this is the ligand which will complex with form complex with iron center and make it internalize or internalize it in the bacteria. In this particular case quite interestingly this iron binding with this enterobactin is quite high.

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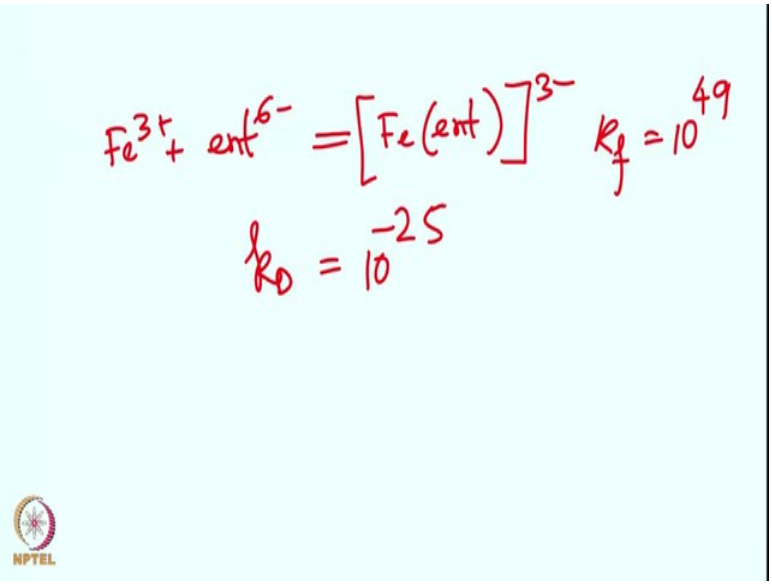

So, for example, Fe^{3+} can bind with these a metals ions quite effectively, Fe^{3+} this enterobactin which I am mentioning as $(ent)^{6-}$ you have seen the catechol unit. All these catechol unit can be deprotonated two each of them are giving 2^- 3 of those centers are there which can give you $(enterobactin)^{6-}$.

So, Fe^{3+} and $(ent)^{6-}$ can react to give $Fe(ent)^{3-}$, because enterobactin itself is 6- iron is 3^+ . Overall this formation or complex formation is possible and that was giving a formation constant of 10 to the power 49 at pH 7 dissociation constant is pretty low at pH 7 dissociation constant is pretty low so, the stability of these complexes are quite high, since the 6 catechol groups have to be deprotonated the means 6 phenolic unit has to be deprotonated $(ent)^{6-}$, and only the delta isomer of this complex is found in nature. A specific cell membrane receptor exists for ferric enterobactin, release in the cell can occur by hydrolysis of the lactone and reduction to Fe^{2+} and or by lowering the pH.

So, this ligand enterobactin that you have seen the gigantic ligand enterobactin with responsible for binding the iron center through this phenol units, but how exactly it is binding that was not really known at the beginning, what was done for this case since we do not know really how it is binding with iron. We tried to get or the researchers tried to get the crystal structure of iron bound complex with this enterobactin or this bacterial siderophore for long there was no crystal structure available.

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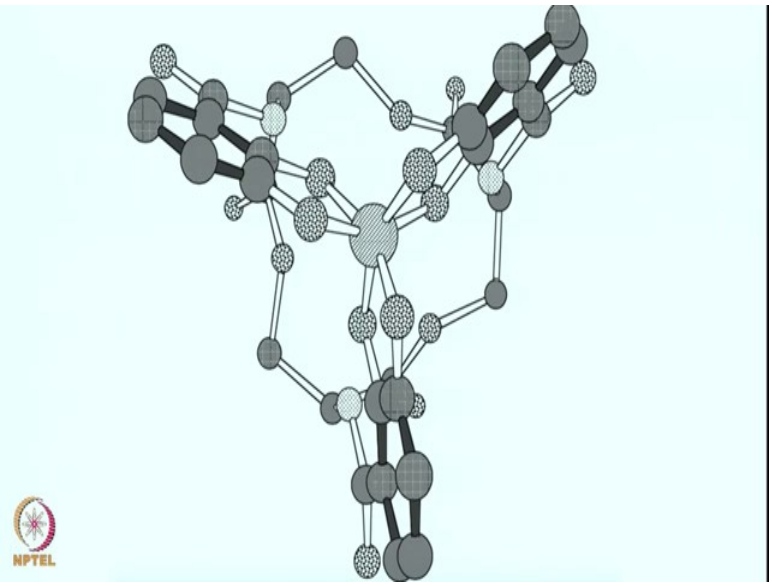

Finally, there was this vanadium crystal structure which clearly shows that we have a case where this diphenol or the catechol units are binding with the metal center in a octahedral fashion. As you have seen this is the vanadium center, these are the two of those units where this catechol units two of the phenolate, two of the phenolate another two phenolate is coordinating with the vanadium center.

So, what we are trying to tell that we are binding Fe^{3+} with $(enterobactin)^{6-}$ all those phenol unit in a octahedral fashion to give $[Fe(ent)]^{3-}$ species which is a quite phenomenal compound in terms of binding with iron. These iron complexes are having very high formation constant something 10 to the power 49 which is indicating that this can essentially bind the iron center very strongly and then can internalize these iron centers in the Fe^{3+} form to get into the bacteria. Of course, a specific cell membrane receptor exists for ferric enterobactin which helps in recognizing these complex to get it inside the bacteria. Of course, one other thing that comes into mind then how is it that these are going to be relieved into the cell.

Before that let me tell you once again that likely although iron crystal structure is not known likely iron is going to be bound in a fashion that we are seeing over here wherein this iron center is going to be hopefully ligated by this catechol units in an octahedral geometry and release in the cells the release of the metal ions in this case, this is a vanadium crystal if vanadium is not there of course, this is the iron this iron catechol

compound or enterobactin compound can be released by hydrolysis of the lactam side chain or lactam backbone at which it is it is residing.

If you look at for the hydrolysis we would need this lactam sidechain to degrade. So, one can think of hydrolyzing over here over there and over there. So these hydrolysis can also help in internalizing the iron inside the inside the bacteria. So, what we have learned so far essentially is 2 system, one is the citrate another is this enterobactin.

Citrate forms a dinuclear iron complex, enterobactin forms a mononuclear iron complex, but with very high binding constant and that is how perhaps from outside lets say from the biosphere or geosphere we are getting the iron and putting it inside the bacteria, these complexation clearly these complexations are going to be quite crucial in incorporating the metal center such as iron inside a particular bacteria.

Now, it is also true the how each and every metal ions that we find in our body or in other biological systems are not completely understood. It is therefore, going to be a constant understanding, constant efforts in terms of research to find out the factors that is responsible for bringing the metal ions inside our body or inside our biological system. Now, another important things in this case is let us try to see how essentially iron is getting sequestered and bring we are getting it in our body.

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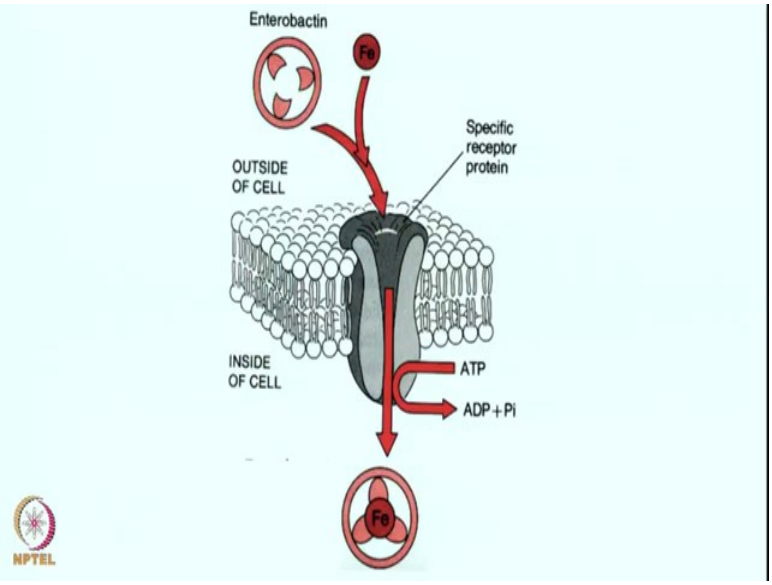

Let us look at this picture where we have enterobactin each of the enterobactin catechol unit is represented over here as is shown. So, one catechol unit, another catechol unit, another catechol unit, this is in enterobactin iron comes and resides inside and there is a specific receptor protein which is guiding this iron loaded enterobactin to get in from the outside of cell to the inside of cell and overall a ATP driven process is responsible for internalizing such iron center in our body ok.

Then let us see what we have learned so far in terms of choice uptake and assembly of metal ions in cell. As we have mentioned at the very beginning of this class that the relatively abundant metal ions will be used relatively relatively abundant metal ions that is present in the geosphere and biosphere will play a crucial role in our biological system. The metal ions which are present in high quantity will be the one which also likely to be found in the biological system, something like iron for example, is present in high quantity. And therefore, it is not a not it is not a wonder why iron is found so much inside our body.

Another important issue that we try to discuss is the labile nature of the metal ions will also dictate how or which metal ions are getting accumulated more the labile metal ions are usually found in our biological system. That again does mean that low abundance metal ions will not be really found in biological system, there is a way to concentrate these low abundance metal ions despite they are present in small quantity in the biosphere and geosphere still under a special circumstances we can find the low abundant metal ions in biological system.

Their control in terms of getting into the bacteria or you know humans body or in other biological system will be controlled by the channels and pumps. We also learned that in bacteria and mammalian cell there will be different mechanism by which perhaps this iron center or other metal ions are going to be accumulated. Now, in the first part of this class we have seen that despite iron being the second most abundant metal center there are quite a few challenges such as there in solubility at pH 7 is a big hurdle in getting them in the biological system.

Although they have Fe^{2+} and Fe^{3+} redox states that are functionally useful, despite that the despite the problem mainly that is the insolubility problem. We can still have them internalize them and mobilize the iron center from biosphere to let us say bacteria and

even in human by different uptake solution such as siderophore and entero and we were discussing one of them and that deals with the ferric citrate complexation with iron, right.

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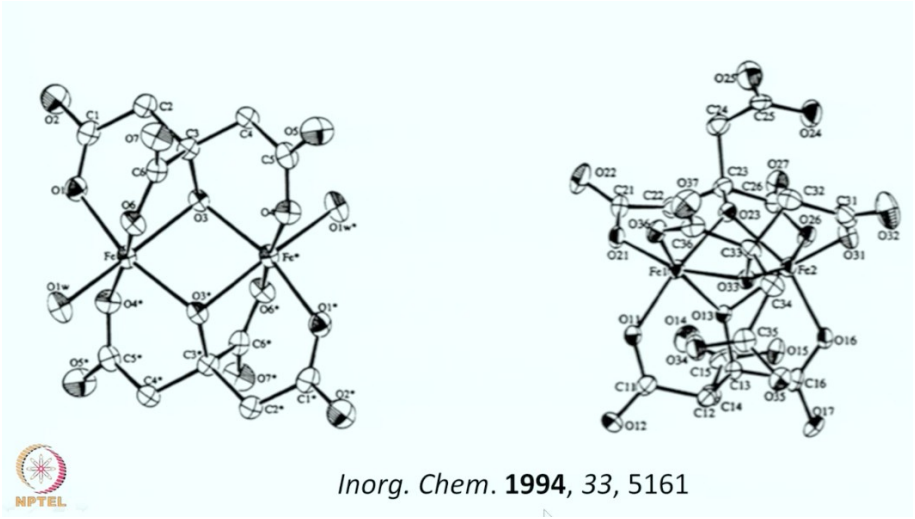

So, citrate it is capable of forming complex with iron, right, these iron complexes are inorganic compound simple inorganic compound. This is the citrate at the top and this citrate is capable of binding two iron center and two of the iron citrates are there. But this is the compound which is discovered by inorganic chemist long back later on in 2002 it is came to the knowledge that we have the same structure that is found in the iron citric acid or citrate complexation.

It is the two iron center binding by this citrate in a in a one is to one fashion that gives the right compound for this outer membrane transporter FecA. We also learned that there is enterobactin which is a bacterial siderophore and responsible for taking up iron into the body of the bacteria. For example, and this is the catechol unit which can bind two of them, can bind two of this center, can bind two of this center, can bind of course, this is a lactam center these are the sites where possibly the hydro hydrolysis can occur.

So, 6 binding site 1 2 1 2 3 4 5 6 overall will bind with iron and perhaps the structure iron structure be something like this. This is a vanadium crystal structure hopefully iron structure will be similar and that is how it will bind this 1 2 3 4 5 6 phenoxide will bind with it and the whole the lactam ring might will get hydrolyzed to release the iron inside the bacteria.

So, this is the whole ligand big ligand that will help iron internalize inside the inside the bacteria and during hydrolysis it can be released inside the cell. And we as we have discussed these iron 3 enterobactin complexes are very very you know very very stable formation constant is 10 to the power 49 and they can form a very good complex at even at pH 7.

Now, this ensures that even at pH 7 where iron is really insoluble still one can make sure that by binding with this enterobactin which is nothing but a cyclic triserine lactone can help mobilize the iron inside the bacteria. So, this is the enterobactin just a schematic diagram, this is iron center which can binds with this enterobactin and in the whole process overall iron can come inside the cell from outside the cell. There is a specific receptor which alerts this iron binding with the enterobactin and that it becomes active overall a ATP driven process makes it possible to bring the iron inside the bacteria and then the hydrolysis of this core will give the release or release the iron center into the bacteria.

And therefore, I hope we briefly were able to tell you that despite having a metal center which is insoluble at pH 7 still the ligand such as that we have seen with citrate and enterobactin the complexation was the key for making them feasible or internalized inside the cell. In the next class we will discuss more about these processes and how these metal ions are getting mobilized into mammals.

For example, in our body that we will discuss in the next class thank you keep studying once again the book to follow would be the Lippard & Berg for the principles of bioinorganic chemistry. There are many other books available including let's say the bioinorganic chemistry by professor Kim that is a fantastic book as well please feel free to read any available book with you. And overall I hope you will be able to understand how nature perhaps have designed to make the metal ions inside our body and make them also reactive, what are the principles governed behind the behind the choice and uptake of the metal center, thank you, åkeep watching, keep studying.

Thank you very much.