Metals in Biology
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Lecture – 03
Control and use of metal ion concentration in biological systems

Hello, welcome back to Metals in Biology. Today we are going to discuss control and use of metal ion concentrations. So, we need to control the metal ion concentration not only we need to ensure metal ions reaches their destination, but if a lot of metal ions are getting accumulated at a given site, that could also be potentially extremely harmful of course, deficiency of metal ions can also lead to many disease states.

So, it is essential therefore, to control and use of metal ion concentration for proper functioning of our biological systems. To maintain a particular metal ion in proper range, the term which is usually we use called homeostasis. Of course, one need to remove excess or unnatural metal ions otherwise, it would lead to the toxification. So, detoxification is essentially an important process which removes excess or unnatural metal ions.

Now, to remove these different metal ions one can think of using extracellular carriers. Of course, there could be passive transport active transport in the form of ion channels and pumps which will we will discuss in subsequent few course classes. Now there are metalloregulation that also are required. So, metalloregulatory protein will control the concentration of metal ions. Once again homeostasis and detoxification are two important subtopic that we must understand. Detoxification becomes essential because otherwise it can metal ions can lead to different disease state. There are as I mentioned there are different ways by which metal ions can be removed from a particular site. Active transport, passive transport; there are metalloregulatory proteins that also control metal ion concentration.

Binding and release of metal ions to receptors are controlled by pH and redox changes. So, for example, you know Fe^{2+} verses Fe^{3+} oxidation state change will control the binding of this particular metal with a particular ligand. For example, some of the sites are Fe^{3+} specific because let us say too many anionic ligands are there. In that particular site, $Fe^{2+}$is not a good fit. We will discuss one such case today, but once again the control

of a metal ion and their usage must be done in a systematic fashion. Redox plays a crucial role to control in redox active metal ion concentration; obviously, a given pH will be also critical for controlling the metal ion concentration.

Well, we will discuss in subsequent slide, the metal ion concentration control and how they are affecting different activities. In the last class, we are essentially discussing this siderophore enterobactin, where we have seen in bacteria how a particular metal ion we were discussing in particular iron centers, how iron centers or iron ions are getting accumulated inside bacteria by citric acid or by enterobactin. Today, we will see one such internalization process by mammals; let's say in humans. So, how iron centers gets accumulated in our body that is what one of the subtopic, we will discuss today.

Now, these that transferrin is the protein that is involved in accumulating iron sites. Transferrin has two subunit; it is a huge glycoprotein, it has 80 kilodalton molecular weight and it binds Fe^{3+}very strongly ok. And what is found that the protein binding site of iron are also capable of binding carbonate $(CO_3)^{2-}$ both Fe^{3+} and carbonate $(CO_3)^{2-}$ are binding to the transferrin synergistically. Of course, perhaps you know the metal containing protein when the metal is removed that is called apoprotein. So, apotransferrin is the one where iron site is not there and also cofactor such as in this case $(CO_3)^{2-}$ is also missing. Now this transferrin has 2 protein domains in each domain, there are two subdomains that clamped down on the iron and carbonate irons once again Fe^{3+} and $(CO_3)^{2-}$ bind synergistically to the apotransferrin. Let us look at the transferrin structure.

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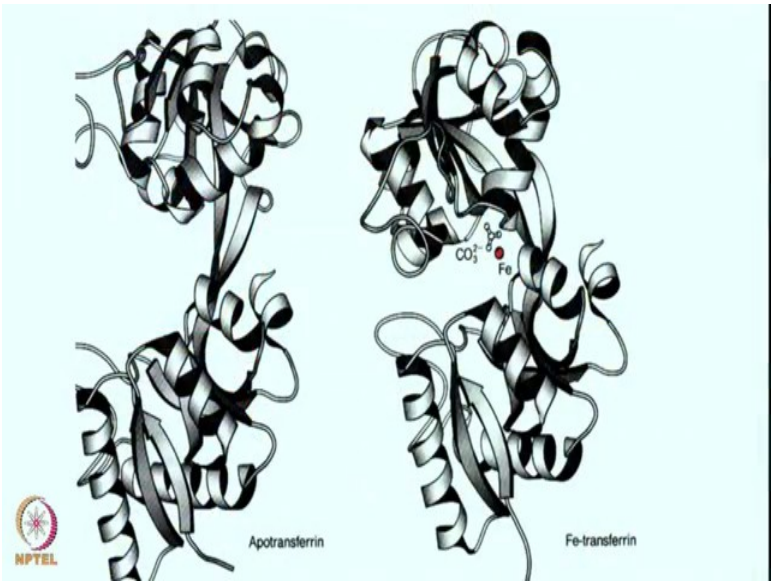

So, on the left, we have the apotransferrin where iron is missing. Now during this process of iron binding; these are the two subdomain, this is one subdomain, this is another subdomain. These two subdomain are linked while iron is binding to this overall apotransferrin to give the iron transferrin protein, then essentially also carbonate comes into picture and they bind synergistically. Both iron and carbonate binds together in this whole protein overall to form a iron transferrin complex.

Now, one thing you must have noticed the difference the distance between these two subunits are decreased significantly during the iron carbonate binding. So, this is what is called the hinge motion. Hinge motion accompanies when iron carbonate binding are happening. So, this is similar to the hinges we know that it clamps down together ok. These two things are clamping down on the iron or carbonate indirectly overall iron and carbonate bringing these two subdomain closer together look at the distance between these two sides and look at the distance between here.

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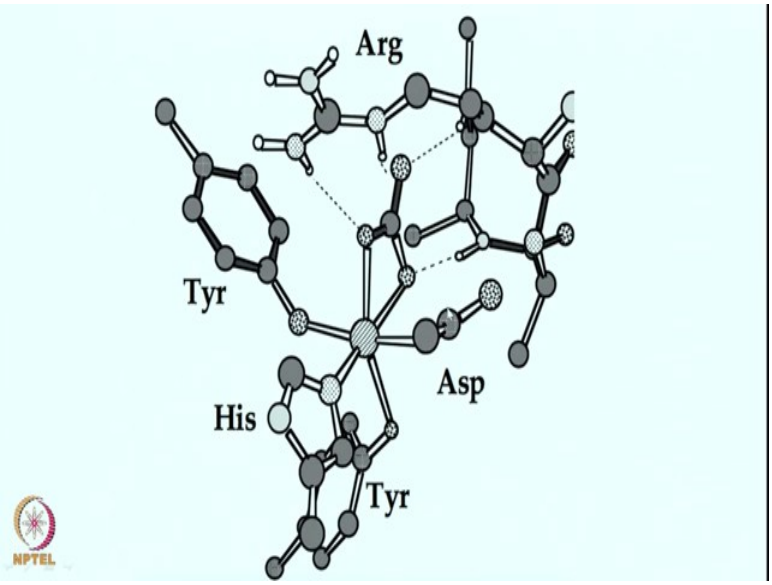

Now, as we mentioned this is a huge glycoprotein we are able to now see how iron and carbonate binds with each other. Now these transferrin active site geometry if we zoom in if we zoom in here, then we will be able to see perhaps the coordination complex inorganic metal complex that is forming when iron and carbonate is binding to the apotransferrin over here.

Let us look at the transferrin active site geometry. The crystal structure we have and that is fantastic to have because that gives you a crystal clear information about the active site. So, we find in the crystal structure that iron site which is over here iron site is binding with 1 histidine, 1 tyrosine and another tyrosine over there. So, 2 tyrosine and 1 histidine is bound with the iron site along with an aspartate over here this is the carbonate $(CO_3)^{2-}$.

So, both iron and carbonate are binding together with 2 tyrosine and 1 histidine and an aspartate bound iron center. So, this iron center is hexa-coordinated. Quite interestingly and of course, this is a crystal structure obtained from lactoferrin; quite interestingly you see that an arginine is present, this is the arginine which is not coordinated directly with the metal center, but this arginine is present overall in this active site. An arginine active site forms a key hydrogen bond with the coordinated carbonate ion. This is the carbonate ion as you can see there is this hydrogen bonding with this carbonate ion. Therefore, overall it helps to affect protein folding around the metal coordination sphere.

So, you have seen the iron center, carbonate, how carbonate is also hydrogen bonded with the arginine. Now these two subdomain that we were talking about in the apotransferrin they bind with this iron and carbonate and both the subdomain comes close to each other as if they are clamping down on iron and carbonate. So, this crystal structure is quite informative in understanding how iron is binding with this apotransferrin to make the iron transferrin and this gives us a overall picture crystal clear and very molecular level detailed picture how these are transporting or helping in internalizing the iron center in human. Of course, these carbonate that we have seen over there is not the only carboxylate ligation present in metalloproteins, there are other carboxylate ligation in metalloproteins and those are quite also interesting.

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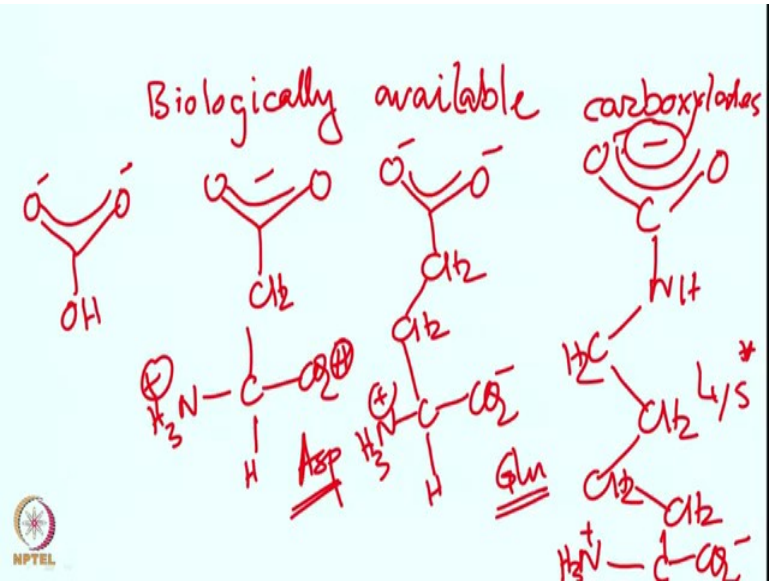

So, let us look at the biologically available carboxylates; biologically available carboxylates. Well one since we have seen carbonate, another easy one would be the bicarbonate right. So, we have bicarbonate of course, another important one could be the aspartate one right. So, of course, I am not drawing the stereochemistry correctly here or $(CO_3)^-$ and NH_3^+ aspartate right. So, this is aspartate and it also has this carboxylate ligation or it can provide carboxylate ligation in metalloproteins. There are other amino acids which are essentially of the similar type such as glutamate; you can also have once again the stereochemistry on the center I am not drawing.

So, this is Glutamate Glu we can also have Lysine carbamate; so, where we have Aspartate glutamate and Lysine carbamate where we can also have the similar binding of this carboxylate ligation in metalloproteins. So, here we will have NH-CH_2 and CH_2CH_2 CH_2 and then of course, carboxylic carboxylate ammonia and H.

So, this is lysine carbamate well carbonate is encountered in transferrin as you have seen this last one is found this carboxylate over here is found in Ureaserubisco and phosphotriesterase right. There are different sites where different this carboxylate ligation can be found. We might will be seeing quite often this aspartate, this glutamate and the sometime when the bicarbonate.

Well, it is also important to note that we are also having in addition to the carbonate binding that we were showing, essentially other anions such as phosphate, arsenate, pyrophosphate, citrate, oxalate are all capable of binding with the metal center. There are different enzymes which can also bind or metallo enzymes that can also bind this sort of anion. Of course, ferric binding protein can also bind these phosphate, arsenate, citrate, oxalates, pyrophosphates ok. Those studies are done quite essentially quite a lot what it can be concluded that not only carbonate various anions can also bind transferrin in bacteria which also have a transferrin receptor.

Well, another thing that becomes very clear that iron must bind as Fe^3; this is Fe^{3+} not Fe^{2+}. So, this has to be in the ferric state. If it is reduced then of course, you need one need a bacteria reductase right thus affording the control of iron binding and uptake in the organism. So, this site for example, is very good in only specifically binding Fe^{3+}, but this will not be a great binding site for Fe^{2+}. Overall therefore, we can have a control of iron binding particularly when Fe^{3+}center right not for Fe^{2+}center and this sort of control binding and very selective binding also helps in uptake in the organisms.

Now, let us look at how human transferrin is actually involved in the iron release. So, how is overall process happening?

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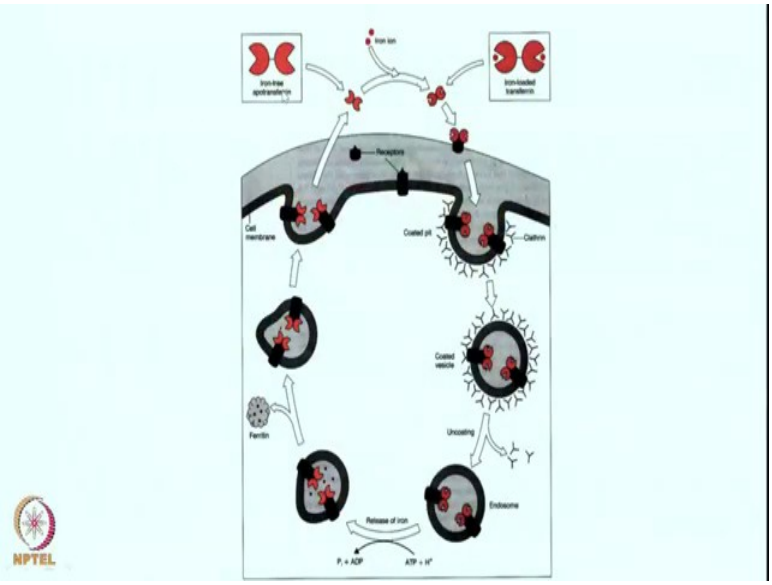

So, the iron free apotransferrin that is the first step that we look at, this is each of the centers can load one of the iron; these are two different subdomain. Now this iron free transferrin which looks like pacman right; now it can pick up iron ion as you can see these are iron loaded transferrin from outside the cell to inside the cell, we are trying to see how iron is getting transported and what are the process involved. So, it starts with iron free apotransferrin once of course, there are receptor on the on the cell membrane and this receptor gets activated when iron ion is bound with the apotransferrin.

Subsequently these receptors bind binds with binds with this iron loaded transferrin and they try to enter the cell, overall a coated pit formation is happening, these are the clathrin which actually protects the protects the vesicle formation or helps in the overall vesicle formation. Once this vesicle is form this is a coated vesicle as you can see from outside there was no such vesicle or protected information or protected apotransferrin was there only after iron loaded transferrin information and getting activated and binding with the receptor one can then can see that this coated pit comes into the picture, coated vesicle essentially it protects these are these are the fat layer right it protects the iron center from getting misplaced. So, that it can reach to its target very reliably. Subsequently once the target is kind of identified or target is reached then uncoating of this you know of this coat that is nothing, but fat layer hawkers.

Now, this uncoating gives rise to a state, where this iron can be released of course, it is going to be a going to be a ATP driven process and release of iron over all happens and then the rupture of the vesicle gives rise to the release of iron inside the cell and here in the ribosome. So, here these irons gets accumulated into the ferritin, we will come back to the ferritin in a moment. As you have seen transferrin is the transporter of iron, ferritin is the one which is responsible for storing iron inside the cell. Once it releases iron, then remaining apo form of the transferrin goes out of the cell membrane and from the cell overall again the catalytic cycle or the iron loading circle starts again. So, this is the human transferrin mechanism how iron is getting released.

Now, this iron release in cells by receptor mediated process is happening of course, as you have seen this is the receptor and this is happening you know in quite a reliable way, it can be done again and again and again without any loss of activity as such. So, these are very effective process and that is the beauty of the biological processes.

So, far what we have seen is how transparent is capable of internalizing or bringing the metal ions in particular iron, that is iron specific iron is getting incorporated inside the cell that is quite interesting. It is a very simple process, but then understanding at a molecular level is quite exciting. As you have seen that there is Fe^{3+} ion not Fe^{2+} and a carbonate that clamps down the 2 subdomain of apotransferrin to bring them together. On top of that you have seen that the coordination site is quite interesting in these cases we have 1 histidine and 2 tyrosine and 1 aspartame bound with the iron center along with carbonate there is arginine is also patting or giving some sort of support through hydrogen bonding.

Just giving the cozy feeling so, that carbonate feels like welcome over there, it binds with iron strongly, but still further the secondary coordination sphere type of interaction through hydrogen bonding, helps quite a lot in overall stabilizing this iron carbonate structure or the geometry in the apotransferrin to give the iron loaded transferring. As you have also seen how these apotransferrins are loaded and then they are getting carried inside the cell. These are schematic diagram overall it helps in releasing the iron at a destination or it can be stored as a ferritin (Refer Time: 23:09) or (Refer Time: 23:10) in ferritin multiple iron centre can be seen stored. We as you will see in this ferritin this sort of ferritin protein we can have even up to thousand iron centers that is quite exciting to note.

And once again, it is not only the carbonate that can bind with iron center or any other given center there are other carboxylate ligation in metalloprotein. We have seen bicarbonate aspartate glutamate and lysine carbamate which are once again found in different active sites.

Iron must bind as Fe^{3+} in this transferrin or apotransferrin not in Fe^{2+} state. So, the oxidation state plays a crucial role in controlling the metal ions and overall uptake in the organism right.

Now, let us try to see the metal regulation of gene expression. As you have seen how metal ions is getting internalized in the cell now we need to also understand that we need to regulate the metal ion concentration inside the cell. Now this metal regulation are governed by many different factor. Of course, without proper regulation of the metal ion concentration as we were discussing briefly that there are many consequences many diseases both higher concentration and lower concentration of metal ions can lead to many different diseases.

Now, the principles behind these metal regulations are quite simple. Metal mediated protein structure changes affect transcription right. So, when metal is binding with protein the structural changes that happens by such metal binding with protein, it affects transcription. Well as you know regulation of metal ion concentrations are going to be dependent both by transcription and translation.

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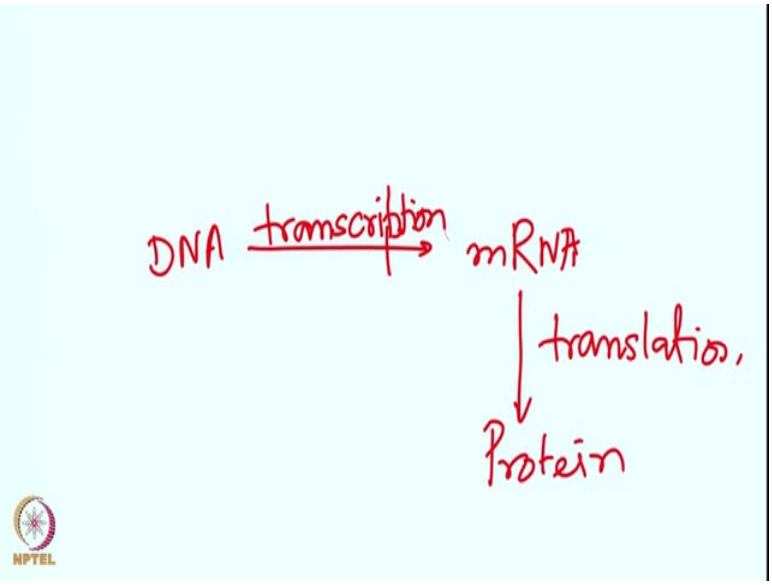

Let us just briefly mention what is translation and transcription as you all know perhaps. So, DNA is being converted to mRNA by transcription and the translation is essentially is the mRNA to protein formation, right.

So, overall so DNA to mRNA formation and mRNA to protein formation these are the transcription and the translation and we will be seeing more of that in the next class. But let me try to tell you that metal regulation is quite an important topic which will determine the importance of metal and any malfunction in the regulation process can lead to the different disease.

Metal mediated protein structure changes can affect transcription. Metal mediated protein structure changes also affect translation. Apo versus halo-metalloproteins bind DNA and RNA completely differently. Of course, metalloregulatory protein is a sensor just like what we have seen in different cases in organic chemistry everywhere metallo regulatory proteins is the sensor.

Metal induced protein structure changes also activate enzymes. So, if there is a metalloenzymes which does not have metal; that means, the apometalloenzyme now once you incorporate the metal, it's going to be active. So, metal induced protein structural changes during the metal internalization in into the apoprotein, we are going to see the structural changes. And therefore, it participate also in activating the enzyme because these are metalloenzymes we are talking about; also metal induced protein structure changes are metal specific.

Let's say there is a iron specific or iron specific enzyme, it is not possible to induce the similar changes with any other metal. So, let me try to tell you once again that metal mediated protein structure changes affect transcription. Metal mediated protein structure changes affect translation, we will discuss this in little more detail, metalloregulatory protein is a sensor, right. It senses what to do it decides, what to do or it tells what to do. Metal induced protein structure changes also activate the enzymes. Well will see some of these processes in the next class, let us keep studying and we will see how metal ions overall are involved in controlling the metal ion concentration as well as how they are getting stored in different places such as ferritin. Till then keep studying. We will get back in the next class.

Thank you very much.