Metals in Biology
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Lecture – 04
Metal mediated folding of biopolymers

Hi, welcome back to the 4th class of Metals in Biology; I hope you guys are studying all of you from the books available and some of the references that we have given during our lecture. One of the book that we are following is Principles of Bioinorganic Chemistry by Lippard and Berg, any other bioinorganic chemistry book another exciting one would be the one by Professor Kiam. So, please study from any book; if possible try to study from these two books.

In any case we were in the last class we are trying to discuss metal regulation and gene expression. Now, there are consequences as we are mentioning that the iron metal ion or metal ion concentration at very high level or at a very low level will have its implication in our health. So, for example, there is iron regulatory proteins which controls both ferritin which is a iron storage protein as well as transferrin which we were discussing in the last class translation. So, Iron Regulatory Proteins IRP's in short, they control both the ferritin and transferring translation that is quite interesting, right.

Well, by this way they can control the delivery and storage of the given metal ions such as iron as I was saying for iron regulatory protein. Regulation of toxic metal ions are essentially very important, body ensures that no toxic metal ion even if it is internalized stays in the body for long. So, there would be a process by which toxic metal ion will be excreted or will get rid off those such as such toxic metal ions such as mercury will be will be thrown out of the body. So, that sort of regulation is also present.

Well, zinc finger protein overall controls the transcription process as I was mentioning IRP's controls the ferritin and transfer in translation. We will see also in the subs in this class that Ca^{2+} which is a secondary messenger and found at the sentinel of at the synapse the will also be important in in controlling different metal ion concentration.

Today we are going to first discuss the regulation of iron levels in cell; regulation also has to do with the storage. As I mentioned the storage protein for iron is ferritin, the

protein name is ferritin. It can store a huge number of iron centers, well iron storage protein has 24 subunits and each of them are having 175 amino acid approximately. It has cubic symmetry. Apoferritin means minus the iron center of the ferritin where no iron center is bound, apoferritin can house 1000 iron atoms in the central core.

A ferroxidase center usually loads the iron into the apoferritin. So, there is a protein ferroxidase which is involved in loading the iron into the apoferritin so, that ferritin can store the iron center. So, this is something like a relay mechanism. So, peroxidase catches the iron and then it delivers to the ferritin. So, the transferrin the uptake protein that we have discussed separately, ferroxidase is the transfer protein transferring keeps and releases to ferroxidase and ferroxidase is loads it into the ferritin.

Now, metallo regulation as we were talking in bacteria it occurs at the transcription level. So, DNA to MRNA formation at transcription level in bacteria these metal regulations are occurring. In mammals, the synthesis of apoferritin and of the transferrin receptors are regulated at the level of translation; remind you not at the level of transcription. During MRNA to protein formation that is the translation step in mammals the synthesis of apoferritin and of the transferritin transferrin receptors are regulated.

Now, as we know these iron ferritin subunits are going to have a quite complex structure; all these exact structure although some crystal structure are known, but it is you know nailing down the thousand iron loaded irons a ferritin center is not very easy. Of course, if apoferritin structure are known which is which is having a threefold channel and also fourfold channel overall there are many subunit as we have discussed and it can load the iron center in the ferritin. Now, let us look at briefly the ferroxidase centers which is known the crystal structure is known.

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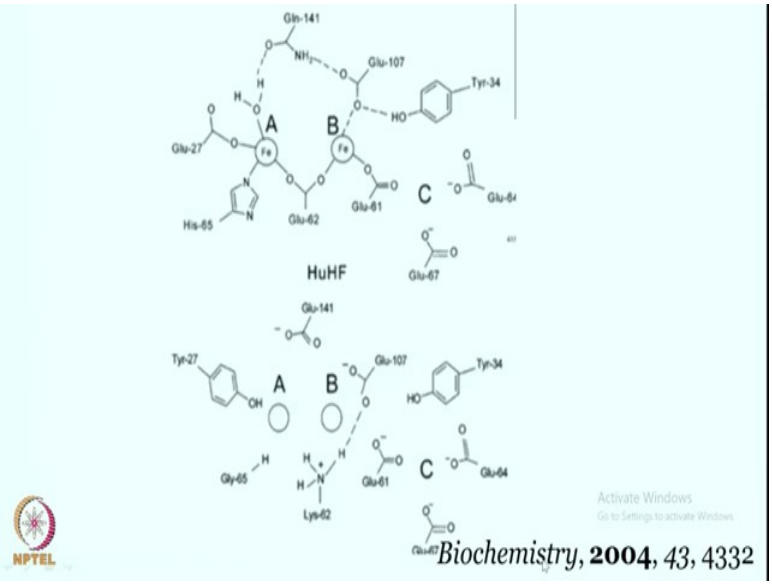

Let us look at the apoferroxidase where you see over here this is the apoferroxidase structure. These the side A and B this is taken from this book, A and B can load or can house two different iron center in the inside. This is the iron loaded form on the top as you can see the glutamate is bridging between these two iron center, this is ferroxidase; remind you ferroxidase loads iron center into the ferritin which is a storage protein.

Now, this is capable of binding iron center this ferroxidase; you see one histidine one glutamate, another bridged glutamate water molecule, another glutamate over there; these are hydrogen bonded with tyrosine. And, even these water molecule as you may know will also be hydrogen bonded; over all its a huge network essentially it tells you that in ferroxidase two iron center can be incorporated and how their structure is going to look like this gives you a clear idea.

Of course ferroxidase is not ferritin, ferretin is the storage protein ferroxidase is the one which is helping in transporting in a way. Of course, transporter is essentially the transferrin, but overall from transferrin ferroxidase is helping in loading the ferretin. Now, ferritin structure at a very simplified iron binding site is not really a good site.

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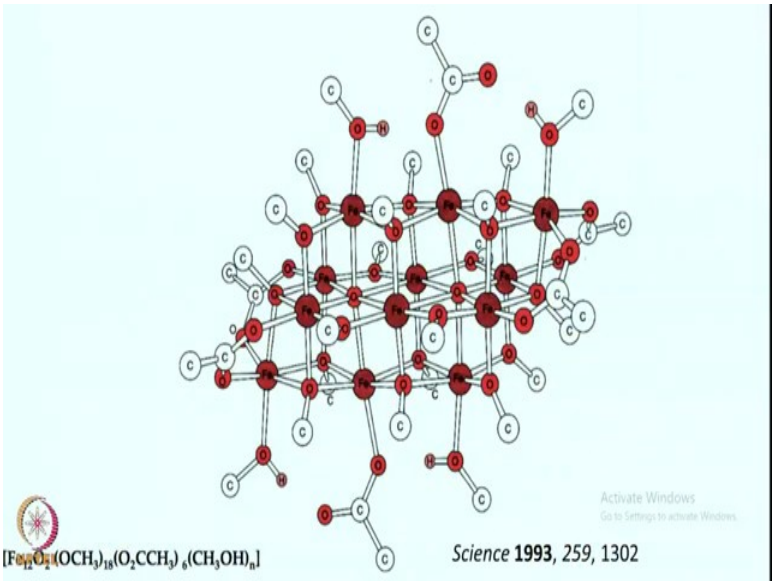

What we have we are we can see for example is the one where we have these you know synthesis attempt, this is a *Science* paper in 1993 which clearly shows that how perhaps multiple iron center can form a nice structure; a mixed valent poly iron oxo cluster; you know as this can be a model of ferretin core and this is somewhat perhaps the ferritin would look like. Of course, this is a model core, model structure which shows that how perhaps ferretin might will look like in the ferritin ok. So, a lot of iron loaded sites are seen in this, see these are mixed valent poly iron oxo clusters.

Well, in the metalloregulation then we have learned that in bacteria a single protein that is For iron Uptake Regulator in abbreviation called FUR, FUR a single protein that is FUR. FUR controls the transcriptions of genes involved in siderophore biosynthesis ok. As we have mentioned that in bacteria the control metalloregulation happens at the transcription level and that is at that level the siderophore which we were discussing in the last class controls its biosynthesis is controlled ok. Siderophore biosynthesis is controlled; therefore, iron uptake is also control at the transcription level. The protein that is involved in this process in bacteria is FUR; for iron uptake regulator.

FUR is a dimer with subunit something like 17 kilodalton, at high iron levels the FUR protein has bound metal and interacts specifically with DNA refreshing the transcription. Of course so, FUR protein the FUR protein has very high levels of iron, this FUR iron

protein has metal bound in them and they will interact with the DNA specifically. And therefore, these you know transcription can be controlled or repressed.

So, overall what is happening is this FUR protein in FUR, FUR protein in bacteria it is loaded with metal, it prevents the DNA to a RNA formation unless it is necessary. So, the high metal ion concentration of FUR will control the transcription or will repress the transcription. In bacteria that is how metalloregulation is happening and that is how the siderophore biosynthesis is getting controlled. If siderophore biosynthesis is getting control, let us say then iron uptake will not be not be uncontrolled all also have control on the iron uptake.

In mammals, expression of ferretin and the transferrin, ferritin is the storage protein, transferrin is the transferrin protein which is which is involved in transporting iron as well as ferroxidase. So, expression of ferretin and the transferrin receptor is regulated at the translation level. So, overall as you can see there is lot of checks and balance which controls the metal ion transport, when the metal ion has to be transported inside the cell that gets controlled by other protein either at that transcription level or at the translation level.

In mammals it is at the translational level where it gets controlled, at bacteria the biosynthesis of different siderophore which is responsible for transporting a metal center; let us say iron their control is happening at the transcription level. Now, we will discuss little more on these topic and we will come back soon. So, we were discussing the metalloregulation, metal it is important to be controlled; also metal plays a vital role in folding of bio polymers. Let us now try to see the metal folding of biopolymers, the role of metal in folding the biopolymers.

So, biopolymers such as proteins as you know better can bind with protein at different sites, their polypeptide proteins right. Now, the peptide has amino acids. So, the amino acid residue can bind with the metal ion and therefore, the structure of the protein can be modulated by a given metal ion. Let us say you have a very high coordination number containing metal ion, it will fold the metal ion protein differently compared to the low coordination one. Let us say you have a metal ion which can coordinate with the different side chain in an tetrahedral fashion or in an octahedral fashion or in a square planar fashion.

Depending on that we can pretty much say that metal ions can fold biopolymers differently, different metals can fold biopolymers differently. Depending on the metal ions its charge, size as well as the coordination number the biopolymers can be folded differently. So, it is essentially what we see that if these metal ions are not the right one or excess of metal ions are present or less of metal ions are present the folding of biopolymers will change. And therefore, the change of this folding pattern can also lead to the agglomeration or precipitation of the biopolymer. And therefore, some of the disease such as you know Alzheimer disease and Parkinson disease can be directly be associated with the adventurous chemistry, simple chemistry, coordination chemistry that this metal ion can play.

Let us look at the few principles for metal folding in case of the biopolymers. Well, metal ions simply can act as template to organize the 3D structure of biomolecules that is what we were discussing now right. It can act as a template for the 3D structure of biomolecules. Now, depending on the necessity metal ion can lose water molecule when binding to proteins. Let us say you have a hexa aqua metal ions of course, no metal ions are completely free it has aqua molecule. If need be only 3 of them or 4 of the water molecule can be chopped off or can be dissociated from the metal centers and the protein can come out.

Selectively any given number of water molecule can be lost from the metal side and then the binding of the protein can therefore, be affected by this loss; depending on the proteins we can have different orientation with respect to the metal ions. Metal ions can retain many water molecule, can lose many water molecule and also can retain many water molecule when binding to nucleic acid. And therefore, these are the processes which will also affect the hydrogen bonding right. Metal ions binding can also facilitate interaction between biopolymers.

Let us say two biopolymers are there they are not interacting with each other at all, a metal ion in between can act as a communicator or some other side reaction other you know unwanted things can also happen. So, two biopolymers multiple biopolymers can be interlinked through the metal center. It is a center of attraction for the biopolymers, biopolymers feels attracted towards the metal ion because metal ion can bind with them right in a coordination atmosphere.

Metal ions with large ionic radii utilize high coordination number for its function right, metal ions with low size or low ionic radii will usually have low coordination number for its function. Well as you can see metal can interact with the polymer biopolymer therefore, it has also the ability can to induce protein misfolding which may be relevant as we mentioned for different human diseases.

So, overall we will also try to discuss let us say at least one thing for sure the calcium binding proteins and their EF hand domains in a moment. And, also we will see if we would like to discuss the metal mediated protein misfolding and the different disease states.

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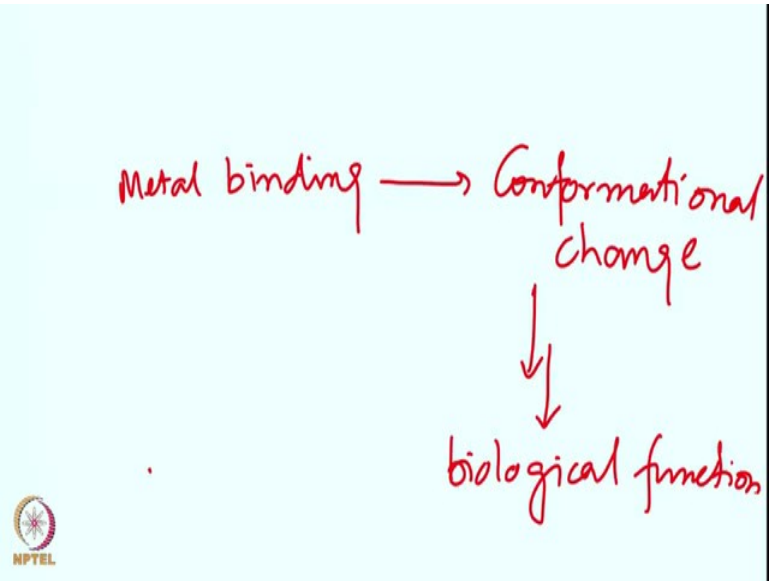

Overall this metal binding is crucial in case of conformational changes that can happen in the protein metal binding and this can lead to the biological function. Well, we will be definitely discussing this in detail; let me tell you that protein folding is quite important to understand we must see how do peptide are folding and proteins are folding.

Now, imagine that we have a situation where only one bed is there and one person is sleeping. Then therefore, the person can orient himself or herself the way he or she want this is a very relaxed situation. But, on the same bed if 10 people has to sleep, now they will feel like disturbed and therefore, the orientation of their sleeping mode will be completely different. So, in a crowded atmosphere versus a non-crowded atmosphere the protein will fold differently. But, more importantly when we have a metal ions

incorporated into the crowded atmosphere of the protein which is usually the case in a crowded atmosphere versus a non-crowded atmosphere; these metal ions will also play key role in folding the protein.

So, inside the crowded environment of the cell this metal ion can play a very crucial role in folding the protein. This folded protein can give rise to the new confirmation to the peptides and protein. As I mentioned metal ions can mediate protein folding which can have physiological and pathological consequences.

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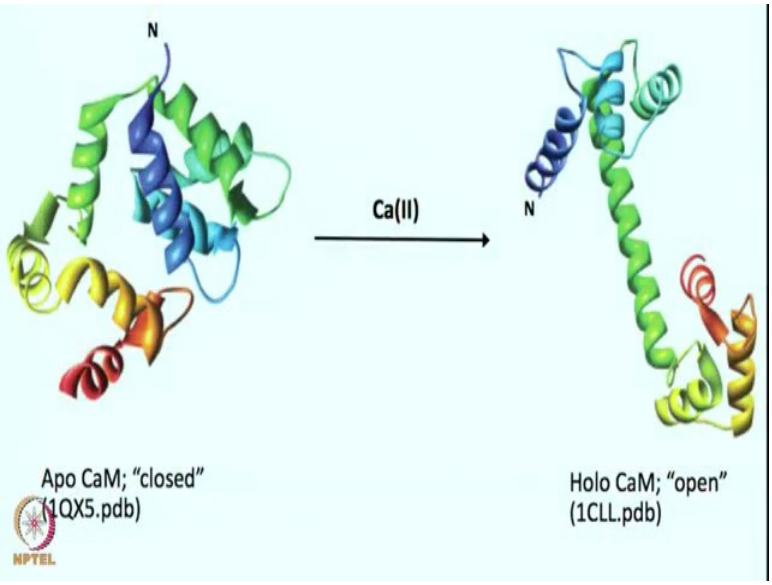

Let us look at one interesting example of the calcium binding with a protein while which is known as calmodulin or calcium binding protein. So, this is the calcium binding protein, this is the apostate of CaM or calcium modulating protein and this is the hollow CaM. This calmodulin which is calcium modulating protein can consist of usually consist of 149 amino acid monomer and it is expressed in all eukaryotic cell. And, overall if you look at overall up to 1 percent of total protein mass binds 4 calcium ions which is quite exciting; 1 percent of all protein mass that we have all the protein that we have 1 percent of that binds with 4 calcium ion. The binding constant is quite good and the dissociation constant is of course, of course, not that high. right. So, this overall calcium binding tendency to the CaM or calcium modulating protein is going to give rise to the very order confirmation. Let us say without calcium it was completely

disorganized, with calcium binding as you can see it gets organized in a particular fashion. right.

So, calcium to plus on the apostate of calcium modulating protein can gives rise to a open hollow CaM structure. This is a closed structure, this is not useful for lot of function. But, in presence of calcium we have a very highly structured situation which is essentially can do quite exciting chemistry and the biological function. Now, we can try to look at this domain separately and try to see what is there in this organized structure. Where is the calcium? Calcium 2 calcium are here, 2 calcium are there.

Now, these are EF hand domain and these are having many crystal structure and you know first crystal structure was obtained with parvalbumin. Now, this helix loop again helix, this structure is quite interesting and quite unique for the calcium binding and the calcium this CaM protein right. This helix loop and the helix structure is quite interesting and we can if we can zoom into that we find that there are 9 residue into it and EF hand domains almost always occurs in pairs.

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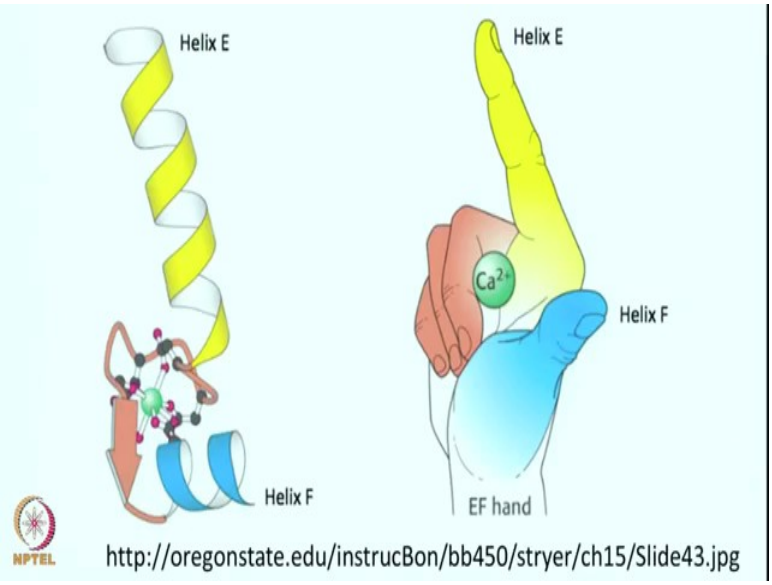

Let us look at some of the more closer structure of it. So, this is helix E right and this is helix F, that was in the yellow and the blue fragment and as you have seen the calcium is binding over here. So, the helix loop helix structure, this helix loop helix structure are quite essentially a very organized structure that is controlled by the calcium binding.

This EF hand domain almost always occurs in pairs. As you can clearly see the calcium is bound in this loop, you can see in the in the in the right hand motive this is the helix E, this is helix F and the calcium binding happening over there. If you zoom on to this side more carefully the calcium binding with the protein backbone can be seen very explicitly.

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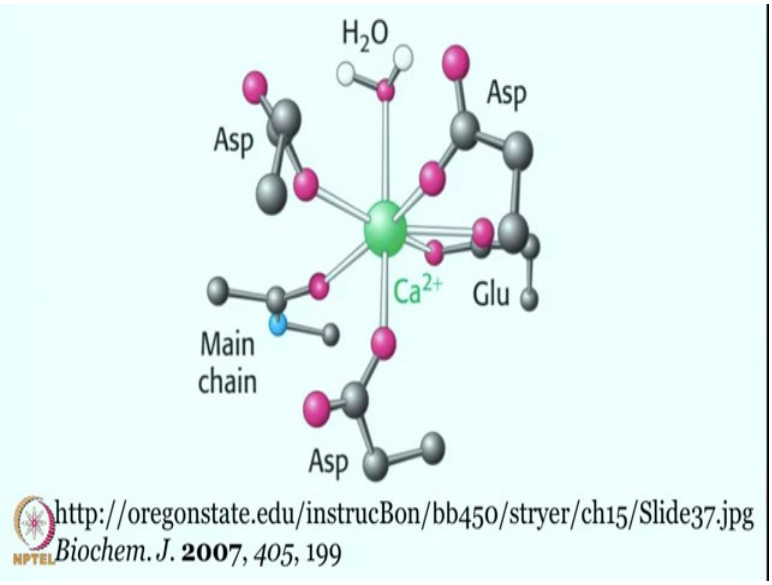

The primary coordination sphere of calcium modulating protein CaM is looking like this, it has 7 coordinated calcium site. So, this calcium is 7 coordinated. We have aspartate, we have main chain and we have another aspartate. We have aspartate this is protein main chain, this is glutamate, this is water molecule water, aspartate, main chain aspartate, glutamate and aspartate. Overall this gives rise to a situation where the primary coordination sphere of calcium is calcium is 7 coordinated. And, this is the crystal structure very clearly showing in how in calmodulin we have a clear calcium binding.

Well, there are many factors that influence calcium coordination to EF hand domain; one of them is certainly the cooperativity and another interesting issue is the cellular magnesium that also controls the calcium coordination to EF hand domain. After binding with calcium this CaM protein now is ready to bind with different cellular targets and can promote exciting chemistry.

Today we will stop here and in the next class we will show how calcium bound protein that is CaM calcium modulating protein, when it is bound with calcium can affect a

series of events. And, that is why perhaps it is called that is a messenger protein; it sends the message to do a particular activity or it activates a particular protein by binding with calcium in the CaM, right.

Of course KD values of EF hand domains of calcium ion vary usually from 10^{-9} to 10^{-4} molar. And, there were many clear correlation between calcium affinity and variation in EF hand domains, let us not discuss too much into that. But, overall what we have seen that the calcium in particular in the last part of this class can bind with the protein and these are found a lot in our body.

Overall 1 percent of the protein mass binds with 4 part 4 calcium center that is quite phenomenon. And, how it is bound or how it is binding is quite exciting, as you have seen that EF hand domain motive where calcium is really bound tightly in the helix loop helix fashion and the coordination environment is quite crystal clear have had 7 coordination with the calcium. The effect of this calcium binding to CaM and how it triggers a series of events will be discussed in that next class. Please keep studying, we will come back soon.

Thank you very much.