Metals in Biology
Prof. Debabrata Maiti
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture – 05
Study of binding mode of Calcium and Zinc in proteins

Hello, welcome back to today's discussion of Metals in Biology. In the last class, we were discussing calcium modulating protein right cam right calcium binding protein; that means, calmodulin. This calmodulin, the calcium modulated protein has 149 amino acid monomer and this is expressed in almost every eukaryotic cells and up to one person of total protein mass binds 4 calcium ions that is quite a lot.

So, protein mass we have 1 percent of it, up to 1 percent of it is bound with calcium and this calcium binding gives rise to the dramatic conformational changes. These conformational changes allows the transmission of calcium signal. So, the moment calcium is bound with the protein, that orientation change of protein structure will gives rise to the signal for another things to happen inside the cell okay or outside the cell. So, that is what we are we are going to going to discuss today.

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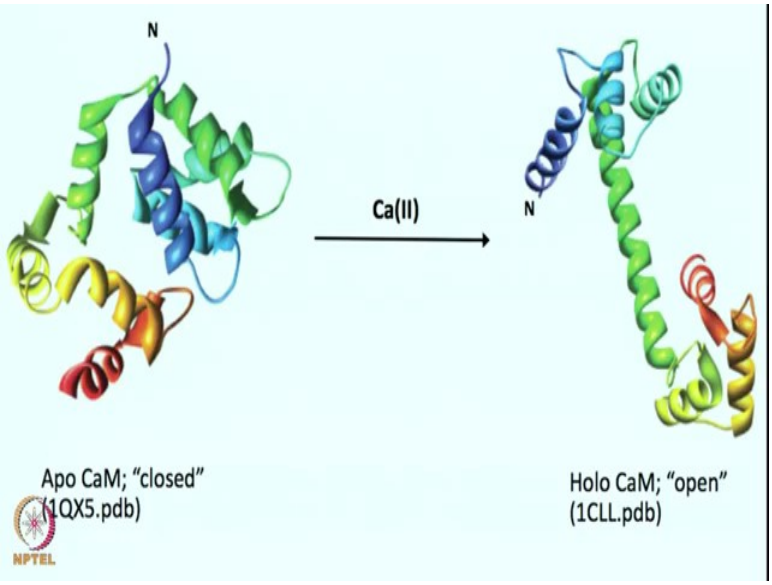

So, this is the closed structure of Apo CaM; that means, minus calcium when it binds with calcium as we have seen it gets really beautiful organized structure as you can see over here right. So, completely disorganized structure Apo CaM becomes organized

upon binding with calcium. If you zoom at this region carefully, we find that there is indeed quite exciting way of binding of calcium; we have seen that also.

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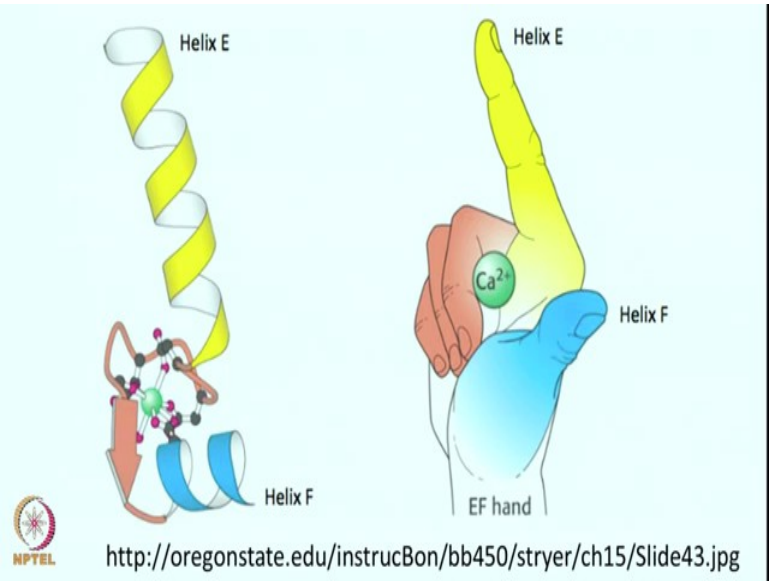

So, this is the alpha helix, this is the loop and this is helix aF. So, helix E look and helix F. So, helix E helix F and the loop as if on the right hand, this calcium is bound as in shown over here. So, this is called EF hand domain protein. This EF hand domain identified in the crystal structure first time for parvalbumin and this helix loop this helix loop helix structure is quite exciting for a number of reason and that this calcium binding turn loop of this gives rise to the turn loop of nearly nine residue, and this always often occurs or always almost always occur in pairs. right.

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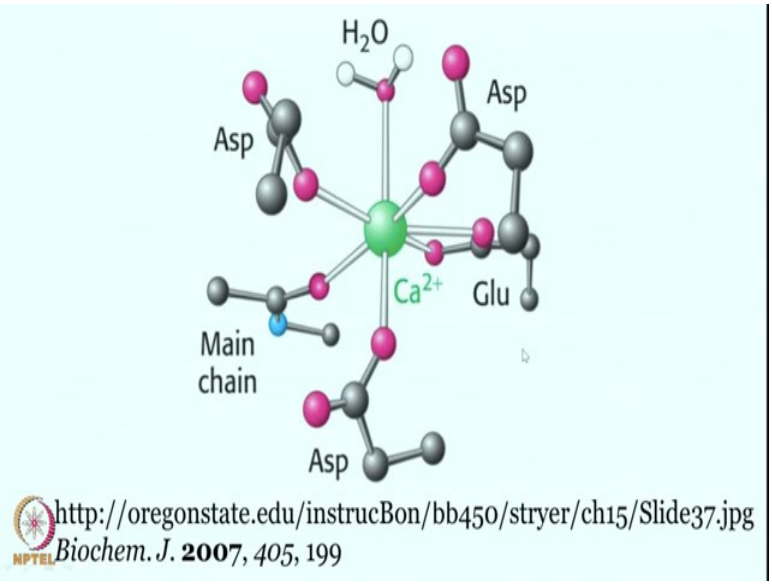

And if you zoom further, we can see that we have a calcium site that is where we were looking at more carefully and it is seven coordinated as you may be able to see primary coordination sphere of calcium is having seven coordination; aspartate, main chain, aspartate, aspartate, glutamate and water molecule is bound over there in this in this beautiful calcium binding mode of this calmodulin structure.

Now the there are many factors that we discussed that influence Ca(II) coordination to EF hand domain, one of the thing is cooperativity well once one calcium binds another calcium binding occurs very quickly, because it can orient the other structure or other site rather easily. Type of cooperativity what we have seen in hemoglobin cases, it is similar to that where one of the binding all of the metal binding leads to the further increase in the binding or the binding constant becomes very high and the and the binding affinity goes up.

So, another in important factor that influence this calcium binding is the cellular magnesium ok. Now this this sort of binding is quite exciting for a number of reason, because it these calcium binding hands physical consequence. Once this calcium is binding with the protein, there is a signal that goes out right and these structural changes are also quite interesting and therefore, calcium overall is acting as a second messenger. It is used the calcium is used to transmit a signal in a cell.

So, this is showing saying that let us say calcium concentration has gone up, once it is binding with the protein though. So, this signifies a number of things, it may activate cellular components for example, enzyme some other enzyme can be activated and other cellular action may be triggered during this cascading signal process.

Of course most EF hand domain proteins are structured in the apostate binding of calcium from the apostate to binding of calcium as we were saying. So, apostate to the binding of calcium results in a change of configuration as you can clearly see over here, change of conformation is happening these are huge changes of confirmation and the therefore, this message or the change of conformation allows the calcium binding protein to transmit the message of an increased calcium concentration. All to transmit this signal, CaM will then this cam will then binds to various target protein after calcium coordination.

So, essentially in the nutshell what is happening is there is a protein in the apostate; it is it is not too much doing anything at the moment. The moment calcium concentration goes up or calcium binds with it, then it gets a particular organized structure. This cal CaM or calcium bound CaM. Now the hollow CaM will be able to target different proteins and they it will be able to bind with them. These overall binding will gives rise to the many consequence in inside the cell.

For example all of course, it can activate a number of number of physiological function, we will see briefly some of its activity.

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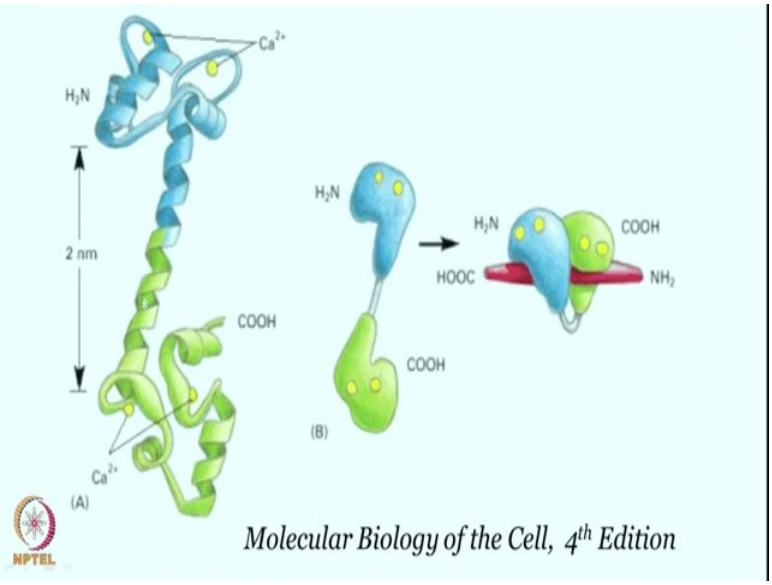

For instance, before getting into that let me say that this flexible alpha helix region. This allows cam this overall and you can see 2 calcium over here, another 2 calcium over there, this flexible alpha helix allows hollow cam to clamp down on target ok. And usually these targets are nearly 20 residue cationic and amplify the regions of protein, and these sort of these short of alpha helix accommodate different other protein as you can see over here, it is this protein side chain. It clamps down and these clamping will have will trigger a number of physiological consequences; for example, it can activate cam kinase II let us see that.

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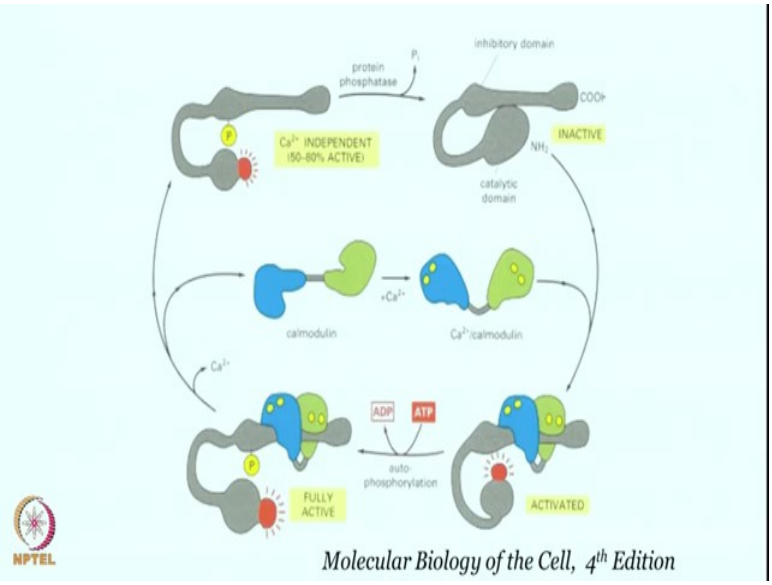

So, this is there is there is inactive form of the enzyme, this activation of cam kinase II pathway by calcium. So, this is the calmodulin as you can see, 2 of the calcium here 2 of the calcium here can bind as it is shown by these yellow dots and upon binding to this to this calmodulin, this calcium loaded calmodulin now with ready. The organized structure upon calcium binding is ready to clamp down on these inactive form of the cam kinase.

So, this is how it clamped down as you can see, it clamping down all on these inactive form of the of the cam kinase II resulted in activation of this enzyme. Once it is activated, this auto phosphorylation takes place. So, ATP is converted to ADP, this auto phosphorylation leads to the fully activated form of CaM kinase. So, without this calcium binding or calcium bound calmodulin this was in active form.

Once calcium is bound, it becomes active and therefore, this is now now very very activated and this is converting ATP to ADP; it is now in fully bound state. So, calcium to signal recognized and transmitted by CaM; and CaM binding changes the conformation of CaM to kinase and therefore, auto phosphorylation is occurring. Well and as you can see subsequently calcium can go out, calmodulin can be released and again this cycle can go on. Of course, there is sometime the memory trace of prior calcium pulse also can lead to this, overall CaM kinase II is present in the nervous system and concentrated at synapse and these are involved in learning and memory.

So, you can see that calcium binding to calmodulin can have also effect in learning and memory of a given person right. So, the proper concentration of calcium or maintaining proper concentration of calcium is absolutely critical as you see, without those calcium calmodulin will not be able to bind with CaM kinase II and therefore, activation of CaM kinase II will not happen.

Upon calcium binding or suitable concentration of calcium, binds with calmodulin that can climb down further on the inactive form of the CaM kinase II. This overall activation will lead to the phosphorylation or auto phosphorylation process where this CaM kinase is fully active. Such an active intermediate or active top of the enzyme will have direct impact in learning and memory and therefore, overall calcium can influence the learning process and the memory process. Again this is a CaM kinase II is a very very important enzyme, which is found in nervous system and it is concentrated at the synapses.

So, far this sort of calcium binding can also be incorporated in a number of beautiful experiments.

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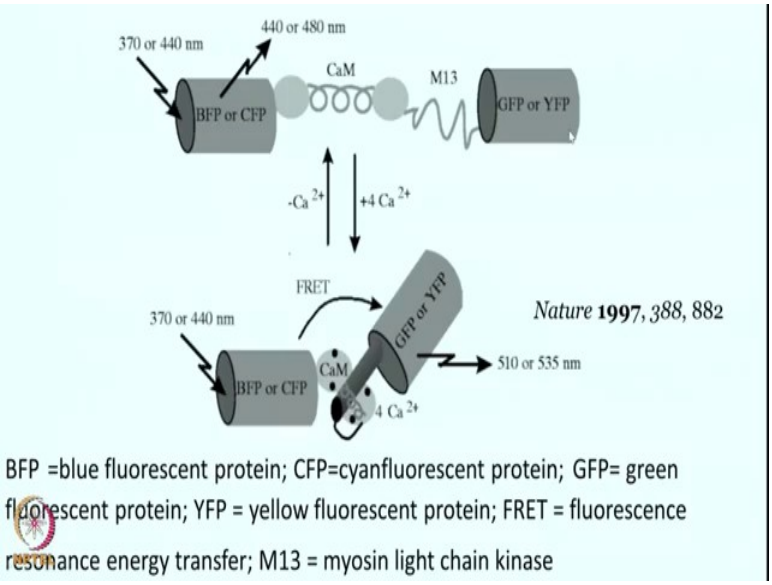

For instance so, this blue fluorescence protein and the green fluorescence protein they are separated from each other and attached with calmodulin ok. So, these two units are separated from each other and attached with calmodulin. This is a research study which is showing clearly that these calmodulin attached these proteins can be brought together while calcium is binding with it. So, this calmodulin is modified of course, and two different proteins are attached with it.

This calcium once it binds, it folds as you have seen how it folds how it gives rise to the very organized structure overall that calcium binding and organization of the calmodulin read gives rise to a situation, where this calmodulin now organized and bringing these two fragment of the blue fluorescent protein and green fluorescent protein together resulting in this fluorescence resonance energy transfer (FRET).

This is quite phenomenal, what essentially we are trying to say is the 2 proteins are separated or apart from each other, they are they are linked by this calmodulin protein. Without calcium they are separated, but when calcium binding occurs this organizes and or reorganization of this calmodulin leads to a situation where these 2 protein which were far from each other they comes very close to each other, and then there is fluoresce fluorescence resonance energy transfer occur which can be monitored spectroscopically.

And this sort of behavior I think it is quite phenomenal, this sort of study to be able to do and demonstrate that how calcium binding can change the geometry and the overall disposition of the different fragment of the protein or different protein is quite phenomenal ok.

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Let us get into a next topic, we will briefly discuss zinc finger domain right. So, that that is going to be the next topic, that is going to be zinc finger domains. well. Zinc finger domains in the zinc finger is required for or zinc is required for specific DNA binding. Now there are there are protein that interacts with DNA in a extended manner, but during this interaction of protein with DNA what is essentially found if zinc is there, that can give rise to the organized structure of protein and therefore, protein will be able to interact with DNA.

Now, there is a specific motifs at which this zinc finger domains are binding. So, tandem sequence binds zinc 2 with sis 2 and his 2 motive. So, if you if you see in a protein backbone, you know at from the n terminal three fourth of it exhibits 9 tandem repeats wherein 2 cysteine and 2 histidine are present.

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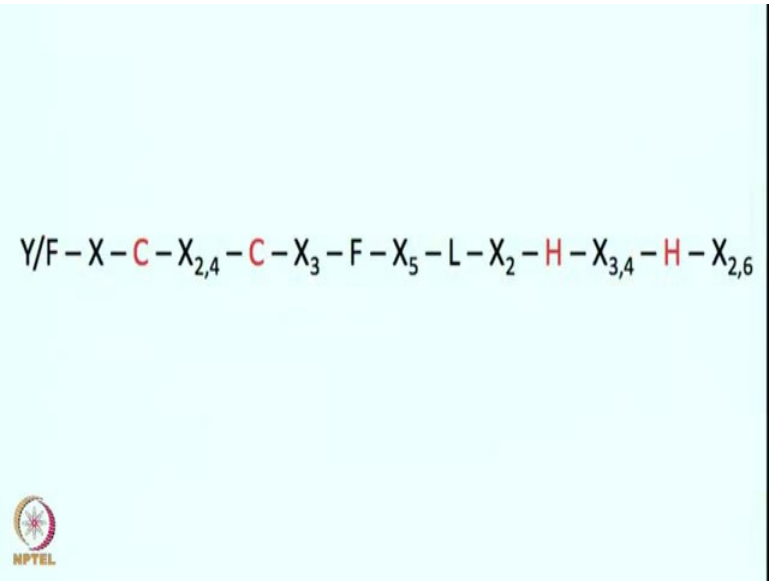

If you see this motif that is present, this 2 cysteine 2 histidine motif which is repeated at the end terminal at the three fourth of it, which is the nine tandem repeats and this is overall can bind zinc effectively 2 cysteine and 2 histidine. Each tandem sequence binds zinc with 2 cysteine 2 and histidine 2 motif.

The motif is of course, found in many other protein and zinc 2 binding with these 2 cysteine 2 histidine you know causes folding of the structure and that can bind them to DNA. So, essentially what we are trying to say is, there is a protein which cannot interact with DNA effectively, but if that protein has a particular sequence as we have seen over here, this sequence will be able to bind with zinc effectively to give rise to the ordered structure.

Some sort of ordering as you have seen in the calcium binding. So, calcium binding gives rise to an ordered structure from a disorder protein backbone right. So, this ordering or the structure orientation gives rise to a situation, where zinc will be bind in a completely organized fashion. These organized binding or organization of the protein upon zinc binding results in interaction of that protein with DNA. right.

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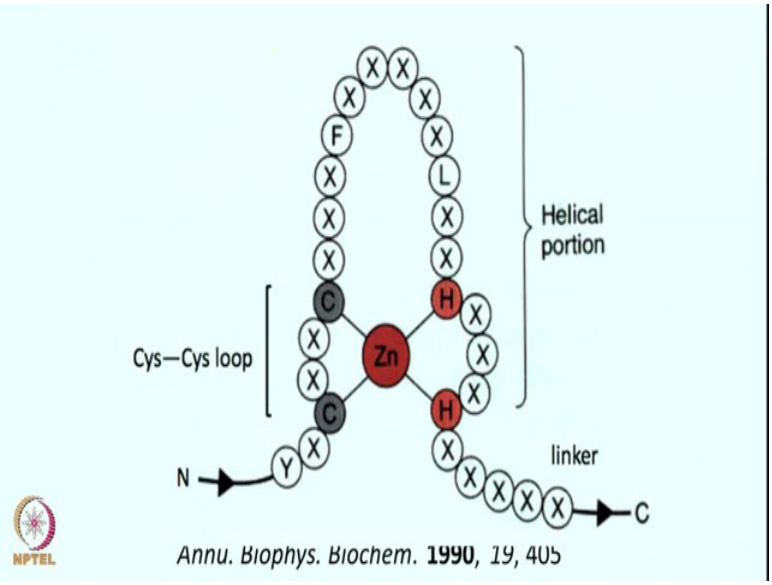

So, let us look at that. So, this is how it looks like as you can see, these are cysteine cysteine, cysteine cysteine loop, this is bound with zinc, cysteine histidine histidine loop. Overall it is also bound with zinc, this is a tetrahedral geometry we will come back to that and this without zinc this is completely disoriented structure right this is the helical portion this is Cys-Cys loop and this is the linker. So, this compound formation has been characterized by various spectroscopic techniques. For example, this protein is taken and zinc is added and then the spectroscopic data are collected. So, in this zinc finger domain which is showing the zinc binding.

The EXAAFS study E X A A F S, EXAAFS study zinc 2 for Zn^{2+} case study shows that this zinc sulfur distance is 2.3 angstrom of course, crystal structure is not really initially known. This zinc cysteine zinc sulfur distance or zinc cysteine distances 2.3 angstrom, this zinc nitrogen zinc histidine distance is 2 angstrom and this is having a tetrahedral geometry.

Of course Zn^{2+} is details system not many spectroscopic studies can be done when zinc is replaced by Co^{2+} which is having 3 d^7, electronic configuration when zinc is replaced by Co^{2+}. It is also found that it is showing the tetrahedral geometry with Co^{2+} and it is coordinated with 2 cysteine and 2 histidine as shown in here. Of course, one can do also 2 D NMR of the double standard beta sheet and alpha helix and this is also corroborating with this fact that this 2 cysteine and histidine is bound.

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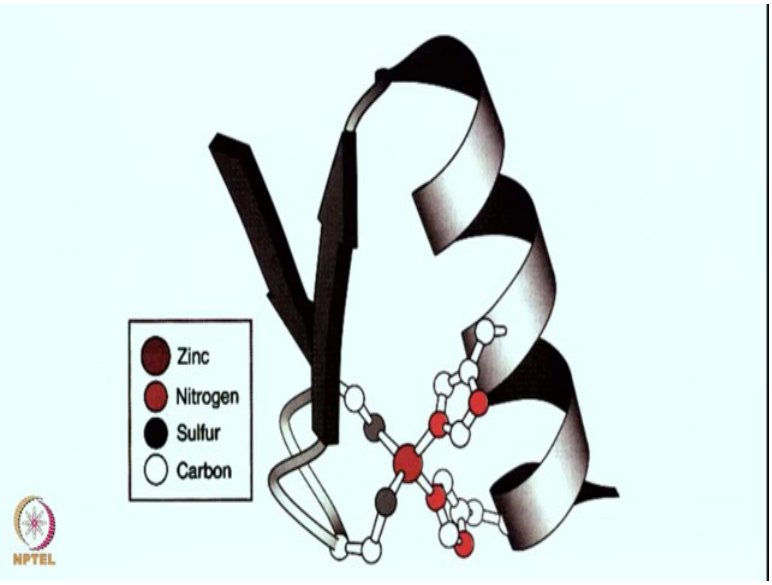

Well let us look at the crystal structure which obtained subsequently this crystal structure clearly shows that 2 cysteine and 2 histidine is bound with zinc, in the zinc finger domain protein. That gives rise to a very beautiful understanding that the zinc finger proteins are going to bind in an order fashion, these 2 cysteine and 2 histidine motive once it is found it will it will bind with it. There are a number of experimental studies has also been done which clearly suggests that no formation of alpha helix in this alpha helix formation occurs in the absence of Zn^{2+}. So, if the Zn^{2+} is not there. So, this alpha helix formation will not be happening and therefore the overall protein cannot interact with DNA.

Quite interestingly this cysteine residue coordinate with zinc first. So the cysteine coordination with zinc occurs first and then the histidine coordination. Of course, the alpha helix formation occurs prior to the zinc histidine bond formation. Essentially telling that these 2 cysteine binding over here occurs with zinc first and then, this alpha helix formation happens and then histidine comes close to the zinc and then it binds. This is quite enormous. I think that is that sort of understanding how the ligation is happening with respect to the metal center is quite amazing.

What we are trying to say is there is a protein backbone, there is a 2 cysteine and 2 histidine motif, the way we have discussed earlier, this sequence is conserved. Now once these 2 cysteine seeds zinc it binds with it, but of course, the alpha helix loop has not

been formed before that. After the system binding to zinc then alpha helix structure forms that we are we are seeing over here.

Now, upon alpha helix structure formation, this histidine comes close to the zinc and then they bind. So, this binding fist then alpha helix formation and then this histidine is coming close to it. ok. Overall the conclusion from many different experimentation and the molecular dynamic studies shows that, the zinc coordination is required for folding of zinc finger through peptides, right. Zinc coordination is absolutely required for perfect folding that would lead to the binding of these proteins with DNA.

The peptide will be unfolded or adopt a completely different fold in the absence of zinc alright ok. So, another question I think we would like to answer then why it is so, specific for zinc and why it is having highest affinity for zinc, this zinc finger domain why not something like Co^{2+} which can have a you know very very good tetrahedral I mean you know dissociates once it is bound, they it could have a very very good orientation with respect to with respect to these 2 cysteine 2 histidine, why it is so that zinc finger domain binds zinc specifically.

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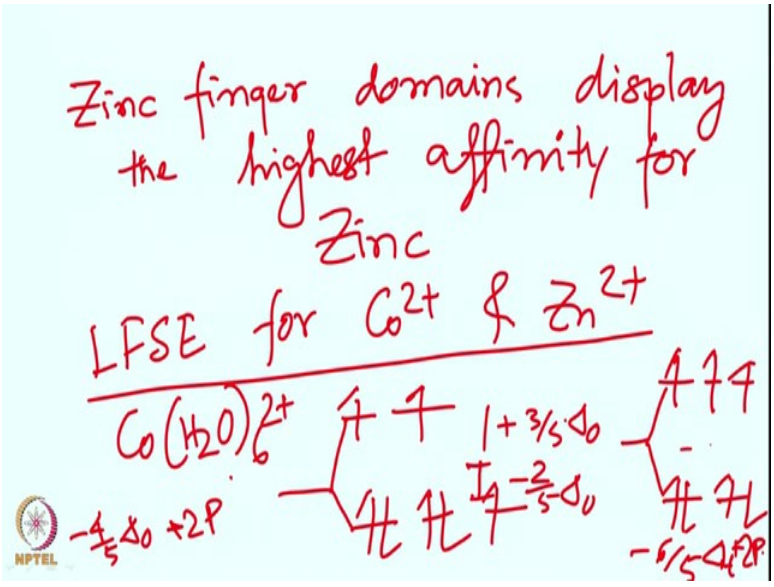

So, the queries we are trying to answer is zinc finger domains, zinc finger domains display the highest affinity for zinc and why is that, right.

Well of course, different metal ions such as zinc, cobalt, nickel, iron is in +2 or +3 oxidation state can bind with these 2 cysteine and 2 histidine motif. That this kD value is quite high in fact, for cobalt. But if we look at LFSE Ligand Field Stabilization Energy we will be perhaps be able to answer these. So, consider ligand field stabilization energy for Co^{2+} and let us say Zn^{2+} coordinates right.

So, if you consider that you will immediately realize that, Co^{2+} let us say cobalt hexa aqua complex. So, of course, no metal ions are free, it has to be deployed it has to be it has to undergo dehydration to interact with the protein backbone. If you look at this, this is in octahedral geometry you will be able to see that it splits the d orbital and this is a d^7 geometry; so, 5 here 6 here 7 here.

So, overall if you calculate the ligand field stabilization energy, it comes downs to -4/5 delta 0 of course, plus 2p pairing energy for both of them right. So, if you if you calculate it properly, then this is of course, going to be +3/5 delta 0. This is going to be -2/5 delta 0 overall it is -4/5 delta 0. If you are calculating it for the for the tetrahedral, cobalt species die cobalt, cobalt die cysteine histidine species, then let us say, let us draw the tetrahedral geometry you can have this overall 7 electrons are oriented in fashion, overall you can have -6/5 you can do the calculation yourself delta t and plus 2p. 2p is small, but this delta t value is very small compared to delta 0. Although this is -6/5 delta t and this is -4/ 5 delta 0, there will be for cobalt there is a loss of 4.5 kcal per mole ligand field stabilization energy while going from octahedral to tetrahedral right. From octahedral to tetrahedral there is a loss of there is a loss of stabilization energy.

For Zn^{2+} there is actually there is no loss and that is because this is Zn^{2+} this is d^{10} system from octahedral to tetrahedral there is no loss of loss of stabilizers and energy therefore, this can happen quite easily for zinc. So, what we have seen so, far is zinc shows tremendous affinity to bind this 2 cysteine and 2 histidine and that is due to the fact that zinc is d^{10} in nature. Some other metal centre such as cobalt, origin or originally they these are hexaaqua cobalt complex 2^+ let us say, they have high ligand field stabilization energy and they have tendency to stay in the octahedral state rather than in the tetrahedral state that is required for the zinc finger domain protein 2 histidine 2 2 2 cysteine to bind right.

That is why this is very specific for zinc and zinc is quite happy over there, and we have seen all over this. But quite interestingly we should also note that this sort of zinc binding calcium binding are are giving rise to a situation where the protein is getting structure and can interact with a target protein or DNA or if even give some sort of signal for us right.

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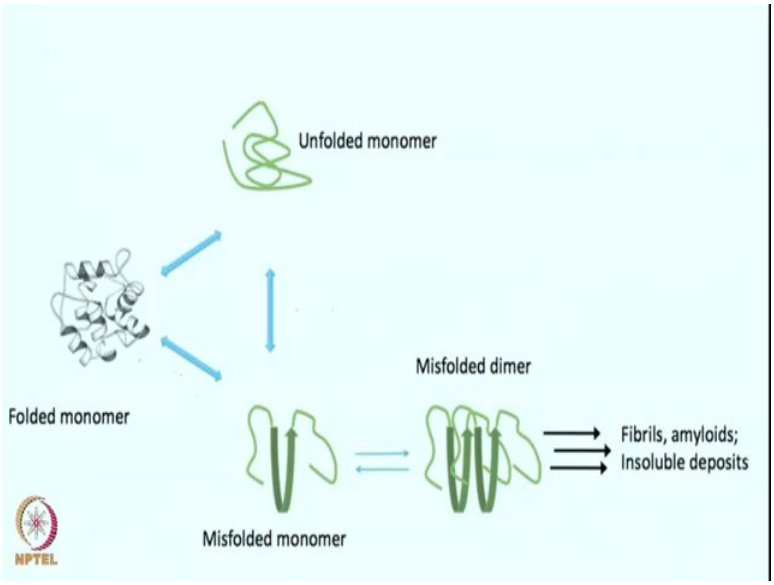

So, let us let us look at very quickly the metal mediated protein misfolding and diseases right. Of course, as you as you can as you know this sort of these sort of things are quite exciting and quite quite difficult to deal with because the understanding of these diseases are not much known right. And these are very difficult disease if anyone anyone have seen any Alzheimer disease patients and Parkinson disease patients you understand that life is very difficult.

And therefore, world wise there is a lot of studies that is that is going on to understand how to provide solution for treatment and how to how to really better understand these diseases. What human diseases actually leads lot of human diseases are due to these folding or rather misfolding of the monomers protein. As you have seen, both the calcium and the zinc can bind with the protein backbone in an organized fashion. But this sort of binding also mean that if they are not happening in an organized fashion the resultant can be the resulting picture can be different in each states such as Alzheimers disease as well as Parkinson disease.

So, as you can see over here this is a folded monomers and this is the unfolded monomers right. These are in always in equilibrium and these are misfolded monomer. This misfolded monomer can then go to the misfolded dimer then that can form fabriles amyloid and insoluble deposits. These processes can be catalyzed by different metal ions right. As you have seen very very recently that these metal ions can bind with these protein in different orientation, there is protein backbone and protein side chain which can bind with these metal centers and gives rise to the misfolding ok. Of course, they can also give rise to the desired folding, but a lot of cases where misfolding is happening can gives rise to the many disease states including Alzheimer and Parkinson's these are really difficult disease to cure at this point.

Better understanding, how to prevent these diseases definitely has to do how better we understand and control the metal binding and the detailed understanding of the mechanism of this misfolding is going to be quite crucial in curing these disease. So, the role of metal ions in these processes of misfolding is oftentimes suggested and then of course, these remain an active area of study not much breakthrough so far has been done. Hopefully in the years to come, we as a scientific group will be able to solve some of these problem for the future generations.

With this, we will see you soon keep studying. See you next time.