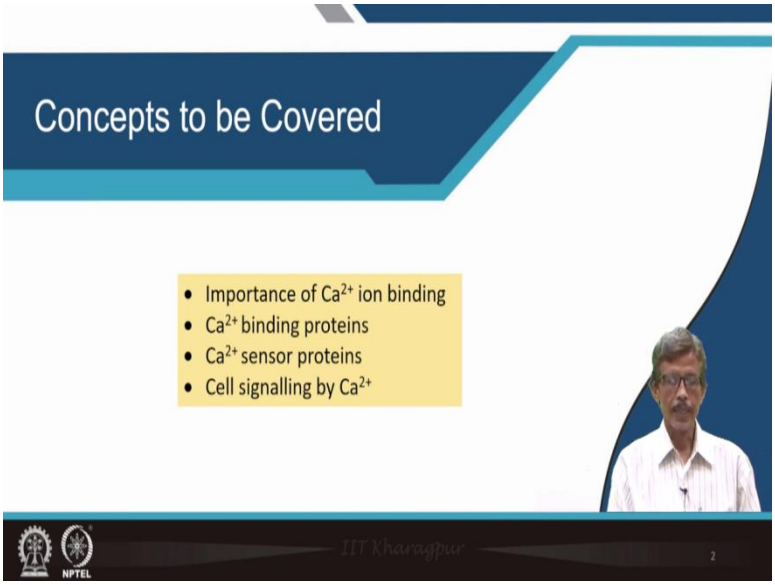
Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology, Kharagpur
Lecture 30
Module 06: Phosphate Metabolism and Cellular Signaling
Cell Signaling by Ca 2+ Binding and Sensing

Hello, good morning everybody. So we were in at the end basically of module 6 and by this time we know how the different phosphate metabolism and the different cellular signaling processes are dependent on the corresponding environment of the available metal ions. So, we will finish with the calcium 2 plus in cell signaling and also sometimes the sensing because these are very two important things from the biological point of view as well as analytical chemistry point of view because if you have something how we can sense the presence of that particular metal line within the cell or within any other environment.

(Refer Slide Time: 1:08)



So we will see now, all the time, we are talking about the importance of calcium to ion binding because the biologists or the biological chemistry people basically always try to say about the binding but in our time in our chemical knowledge of coordination chemistry, we always try to tell it as the coordination so metal ion coordination.

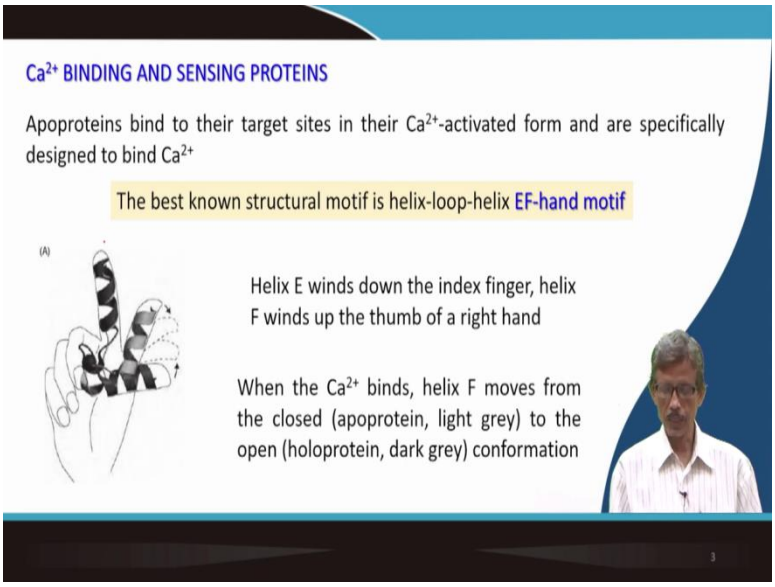
So, this particular calcium two plus will follow basically how the beautiful coordination chemistry is operating over there, then definitely we can have the different appo proteins,

these appo proteins can bind the calcium 2 ions, again by the method of coordination. So, we will have the calcium binding proteins have different structure after metal and binding.

So, you will have the hollow protein and the protein corresponding appo protein. So, when you have the metal and binding the confirmations and all other things will be changing. So that is why we get not only the change in the structures, their functions will also be changing and obviously the different reactions and the reaction mechanisms will also change.

So, the sensor proteins is that that those proteins particularly who can censor or which can censor the binding of the calcium 2 plus ion in that particular environment, where you have the available donor points or the donor groups or the donor atoms, then how the signaling can be done through this binding process and what are the different types of signals it can send for our regular activity like that of our muscle contraction or the muscle relaxation.

(Refer Slide Time: 2:48)



So, you can have therefore, the two types of these proteins one is that you can consider that the calcium binding is taking place. That means we will talk in terms of only the coordination of the calcium two ions, then whether we can sense it or not, that means some things would be there like that appear the sensory organ like we have.

So how we can sense that whether you are binding a calcium ion or a magnesium ion? So, if you can have a different sensing property for all these things, different techniques, basically the spectroscopic techniques or spectral photometric techniques or the fluorescence technique are useful nowadays. So, they are giving using some sort of force or something where the calcium binding can be sensed.

If you have the sample from your cell origin or sample from some other sides, they sample from your hard water we all know the presence of calcium in water sample give you the corresponding hardness. So, that hard water basically can also be analyzed if you have a very low concentration down to that off not at the ppm level parts per million level, but it can be ppb level parts per billion level or parts per trillion level.

When the concentration is less, we cannot go for simple flame photometric method or any other simple conventional analytical tool we cannot use we have to go for some sophisticated or high end instruments for those measurements. So, you have the appo proteins where the metal line is not there, they can have their corresponding target sides and they are calcium activated form, what is that calcium activated form?

It is nothing, when you have the calcium binding your corresponding structure and the functions will definitely change. Because if you have the corresponding donor groups, the way I say all the time about your EDTA, EDTA 4 minus so if you EDTA 4 minuses there, so that it can be a linear molecule, and you can have 6 donor groups around all these things. So you can have 1, 2, then in between 2 and another 2 6.

So, you do not know that the typical folding of that particular molecule when the metal ion is not there, but if you go for binding of that particular EDTA molecule to that of your available calcium 2 plus, it will try to take all the necessary positions around calcium, which are in the geometry which is known as the octahedral geometry. So, you can have the binding of two nitrogen and four oxygen donors in such a way as and you can have the wrapping.

So, one tridentate part will come and another tridentate part will come in this particular form. So, if you have one try dented part, so, O O N type of thing, but it is not that is not a meridionale one, it is a facial one. So, this one face and another face of the EDTA molecule will come and give you the corresponding one as the octahedral binding.

So, you see your fingertips, your fingertips are the three donut points from the left and the three other from the right. So, it is coming like this, because it is the binding of the calcium. So, the binding of the calcium is so, important that it will try to give a regular geometry to that of your simple the simplest possible ligand an iron what you can think of is the EDTA ethylene (())(6:14) tetra acetate anion.

So similarly, if you have a huge protein structure and some point basically can be available for binding of that particular calcium ion or calcium two plus ion you see there will be a

structural change your primary structure then secondary structure that said is structured all these things will be chained and that can be triggered by only calcium two plus coordination.

So, one such motif basically the helix loop helix, so, like your tridentate part one tridentate part and the tri data part if you fix these two parts, you will get a hex identity like and like add a for minors, but if you something if you have the protein, so, you have the helical part from this particular fingers and this particular fingers then you can have in between the loop so, you have the helix then loop, loop between these two my these two fingers and then you have another again helix.

So, that is known as EF hand motif because all the time the way I am sewing that what is your exit intent like and the binding of that accidental again around calcium similarly, the hand motif is always very much important to understand something how the things are changing, and how you get all these things, you see that you can have the helix as well as the loop in something very can have the right hand these are not the left ones and our right one. So, if you can have the right one and the right one is like that is that the corresponding one we use all the time the finger tips basically.

So, if you can have the wrapping of these fingers with this particular helical motif of this protein chain or the polypeptide chain, and you can have the other one, so if you can have the movement of these things, so these fingers so the thumb can move basically during that particular coordination or some other type we can call is as the EF hand motif.

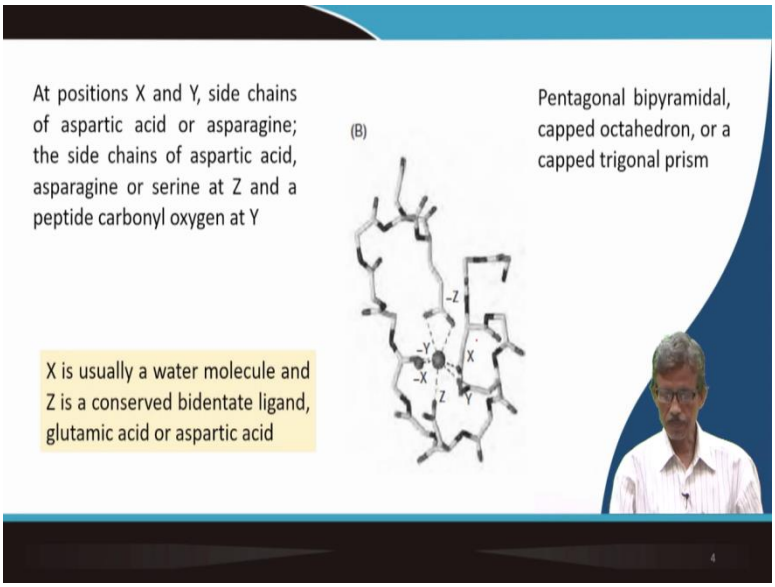
So E is one fragment and F is another fragment so helix E. So most of the time we will be talking about this a thing these are these are typical nomenclature, the helix E and helix F is not that the end terminals are the C Terminus basically and if they are right. So, basically the wind winding process is basically down to the index finger and helix F if it finds that corresponding thumb on the right hand, it will get the corresponding configuration.

Now, if you have this particular helical or is adamant on these two fingers only, then you can have the calcium. So, bring the calcium allowed to bind it and during that particular process basically you're this thumb is a thumb thing that we helix F not E it is in E and it is in F. So, your F moves from a closed or the appo protein which is the light gray in color. So, one is light gray and another is the dark gray.

So, appo protein part when the metal ion is not there. So, how the movement can take place when the metal ion is coming into the picture and try to go for the coordination like your

calcium binding to your EDTA. So, that way, you get a corresponding closed form, but when the calcium binding is there, the opposite thing is happening now we have the open conformation. So it moves from a close to a open conformation.

(Refer Slide Time: 9:33)



So these sorts of confirmations are always there and that changes are there and we can think of the slight movement of this particular donor graphs which are available. So if you now can see from that particular arrangement to another one more simplified one and is basically the stick tie stick arrangement. So stick elements so that is basically loop management and the helical elements of these proteins.

So now you guys if you can just pinpoint the central spear, the central the gray spear the dark gray spear what you are going to find from there that you can have the different positions. So, when you find out the location of the metal on the access structure determination, basically try to point out that particular location of the metal and then the immediate environment. So, what are those immediate environments, the immediate environments, what do you see from our X, Y, Z and in another side you have minus X minus Y and minus Z what is that.

So, if you have a typical octahedral geometry, but it will be very much distorted not a typical octahedral geometry. So, we will have the X axis, so, in one direction it will be X and another direction it will be minus X, if you can the Y direction Y axis the one is plus Y and another is minus Y. Similarly, for Z which is the perpendicular one, so, is the Z plus and Z minus.

So, you immediately you can think of what is happening for a typical octahedral geometry if it is allowed around calcium two plus. So, the positions all these are positions basically not

your nitrogen donor or your oxygen donor or your Sulphur donor, but your x y all these things are coming from the aspartic acid or asparagine and the sidechain of the aspartic acid and asparagine and serine at Z, one is X and there is Y and there is Z and a peptide carbonyl oxygen at Y.

So, these are the arrangement basically if you are able to look at it then level it in terms of the mind as it is you do because all these are the extra structure identification. So, extra structural identifications basically gives us that particular information that where you can have this particular environment and then you can think of the corresponding distortions and all other things you see the Z the minus Z at the top.

So, the minus Z at the top it is basically this particular one from the carboxylic acid. So, if these two oxygens donors are available around this calcium center, so, it is basically binding So, that particular one for the bigger calcium two plus it is showing a bidentate coordination.

So, Z if it is there from the asparagine or the size that I know anything, so, if it is for going for the corresponding bidentate coordination. So, your coordination number can be increased from six to seven and also X is a sphere, sphere like things, sphere is there giving us X. X can be a mono dented water molecule. And Z is a concert bidentate ligand either a glutamic acid or an aspartic acid.

So, it is also interesting to know in that way that if you can have all other points available, but if you have the open face some faces a little bit open is not a very close and compact arrangement of the octahedral geometry. But if you some place is open so, if you have all trigonal faces occupied run if you have a octahedral geometry you have eight numbers of those diagonal faces you nicely draw octahedron and on the top and the squat pyramidal party we will have the fourth and at the bottom also you have four so, four plus four eight diagonal faces.

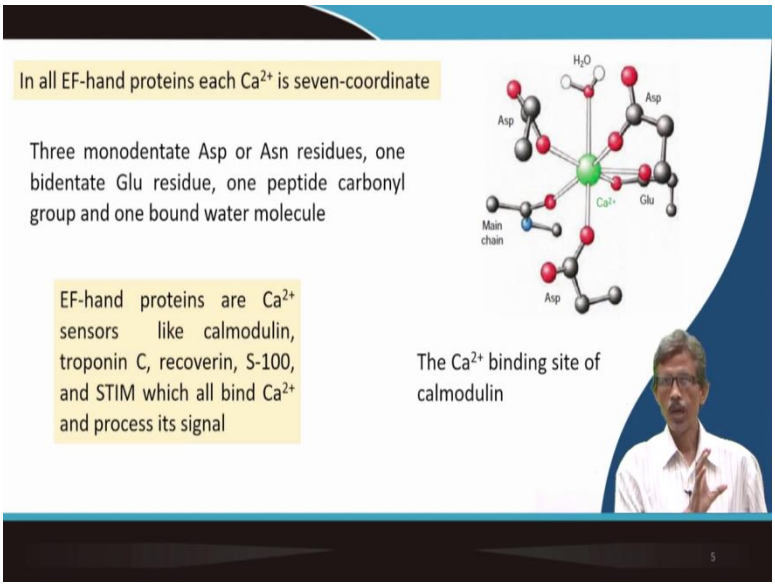
So, on that particular phase, if you just open up that particular phase and the bigger one, then what can happen you can have a entry off a water molecule. So, water molecule can have the access to interact with the calcium two plus center and it can go and bind to that particular calcium center how and then what is happening already you have the six coordinates inside.

So one extra coordinates inside is coming and that basically giving the corresponding increase in your coordination number from six to seven and we can consider it as a gapping of one of the phase. So that can be a gapped octahedron. So, what are the other possibilities

the other possibilities are well known for a coordination number of seven if we consider the coordination number is seven, you have a corresponding pentagon at the base and the two positions at the top and the bottom side is pentagonal by pyramid and the cop captech octahedron I told you just now and also the cap trigonal prism, you can have a trigonal prism which is opposite to that of your octahedron is like this and this is the corresponding prison. So, this is the prison management.

So, one of the phase and you have the diagonal phase as well as the rectangular phase also. So, one of the phase up this gap will go for a coordination number of seven.

(Refer Slide Time: 14:22)



So, within this EF hand motif for this calcium two plus it is seven coordinated. So, you see that if you can have a seven coordinate one, so only one part. So, immediately if you look at very quickly, you will be able to, this is octaheron, almost octahedron is not so on the right hand side only. So this part only so the glutamate part and that is ASB part. So this particular part basically because what is the at the top, so this particular part is a little bit crowded.

So the crowding is there because these crowding is happening because you have the opening up of the thing where it is opened up your water and is able to enter from the top and you can have a capping and has been not in terms of the extra coordination from the water molecule. But from the other point you have to critically look at when you determine the whole extra structure, we find we have the access structure in your hand and on that excess structure basically, you can find out that okay is not that water molecule which is capping one part of that particular trigonal face, but the other part can also be conceived as a kept part.

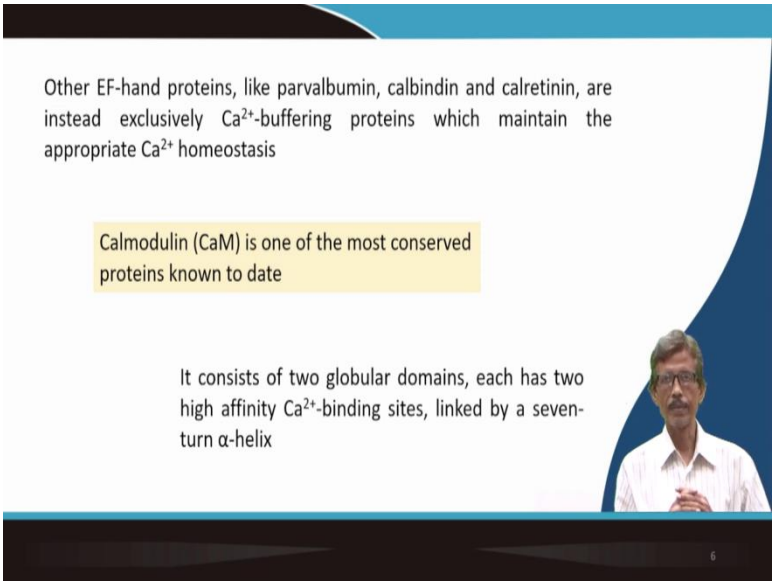
So, it is the binding site therefore, on calmodulin. So, calcium binding the protein some calcium binding protein, this cal is which is can be considered as the modulating thing, so, calmodulin, so you will be able to remember it also nicely. So, these are the corresponding arrangement of the amino acid residues.

So, ASB ASN and bidentate glutamate ju L and one peptidyl carbon and so, these two are very interesting coordination one you see at the bottom the main chain, you will see if you look at on the lower left of the main chain, your CO that means the carbonyl oxygen your, the oxygen is at the tip of the carbonyl function this is carbon and this is oxygen. So, the tip of the thing is that your oxygen.

So, that oxygen is coordinating and interacting with your calcium two plus center and you have the water molecule at the top. So, the positioning of these two monolithic groups are important, giving you the final arrangement. So, that EF hand proteins are calcium two plus sensors like many examples are there one example we are talking over here is calmodulin then you can have that troponin c you can have that recovering is 100 and STIM.

So, these are all examples particularly the trade names or the names basically the biological names do not bother about remembering all these names, but you can know also, you should know also that there are many examples like that of your calmodulin.

(Refer Slide Time: 17:10)



So, in this protein hand, what you can have like parvalbumin, calbindin and calretinin. So, these are instead of exclusively calcium buffering proteins, which maintain the appropriate calcium two plus homeostasis. So, there are other examples apart from that what we are

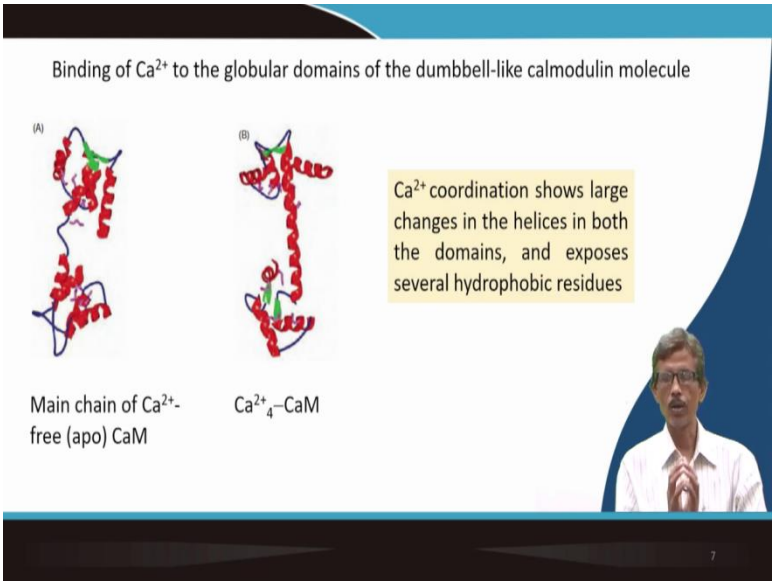
listing over here. So, these the second list basically, so, all these basically are basically not that of your thing, but it is basically a buffering system, what is buffer, what is buffer solution, the textbook definition of the buffer we all know and we all talk in terms of the hydrogen ion or the proton, whether we are able to change the corresponding pH of the medium by simple addition of one or two drops or three drops of acid or base.

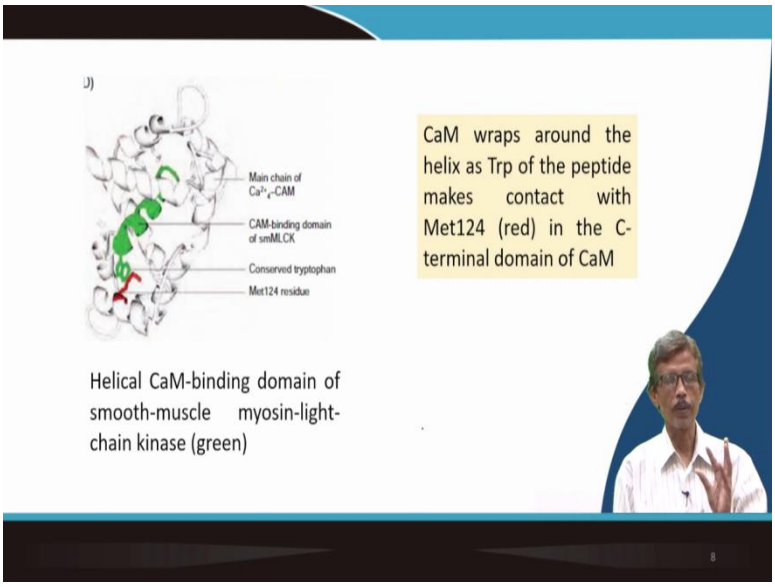
So, it will resist the change in the corresponding pH. Similarly, the calcium buffering system would be also like that, that whenever you have a buffered system, that means you can it can replenish the available calcium or the depletion of calcium if you add some extra calcium or if you take out that calcium.

So, the buffered system is always important and which is directly related to the calcium homeostasis that means the stable concentration or the fixed concentration of the calcium ion in our system. So, that will be very important thing and this terminology is also important in terms of your all the metal ions in the offering condition as well as that corresponding homeostasis whether you are talking in terms of your corresponding availability of the calcium ion or the iron ion or the copper ion or the zinc ion.

So, calmodulin abbreviated as Ca capital M is one of the most conserved proteins known today. So, that is why it has been studied nicely. It has been studied extensively also for all these cases, what it can have, it can have two globular domains and each has two high affinity calcium binding sites. So, you can have the two domains is not that it is going something which can be considered as a mononuclear calcium compound, but if you can have it is well separated is not like that of your binuclear system, but is only two separate systems but you can have two domains and you can have the calcium binding sites like a seven turn all filing so seven coordination number is seven and also you will easy to remember also that he will alpha helix serving seven times so turn his number is seven.

(Refer Slide Time: 19:40)





So if you are the corresponding chain the protein chain is a globular type. So globular protein recalls a global ad is a particular type of structure or the nature of the protein. And another one is your dumbbell light thing. So globular domain of the dumbbell like calmodulin molecule important, so, the binding is there so, we try to know now that what does it mean basically. So, you can have a global ad domain and you can have the dumbbell like calmodulin molecules.

So, dumbbell means one sphere and another sphere and you have the rod. So, if you have one calcium from one particular domain and another calcium from the another domain and in between we had the thin connection of the rod of the dumbbell. So, that is the thing that

structure gives you the corresponding dumbbell like arrangement and that dumbbell like element is very important to know.

So, these are all cartoon drawing, we have to draw it basically try to practice yourself is there so, that you can draw these in the cartoon thing like your drawing basically, your picture your drawing, but these are structural determined on and three dimensional structure, what do you determine by doing the protein crystallography. So, protein crystal structure determination gives us this final form, where you can find out the calcium position where we can find out the other donor group positions, otherwise, you will not be able to locate the three dimensional elements or the theoretical positions of all these points as well as the metal ions.

So, main chain appo that means, the free calmodulin So, there calmodulin is not there. So, if you go for another enrichment, so, another cartoon diagram or the cartoon drawing, where you can have the helical drawing and all these like if a piece of chalk if you give it to me and I can go to the board and I can draw it nicely, because you put the put the chalk in a different direction and you turn it, so, you can very quickly you can draw this helical arrangement.

So, you can practice it also. So, this is the structure where you can have no calcium two plus which is calcium two plus three calcium two plus V and another one you can have calcium two plus and four sides are binding. So, is one that by nuclear domain or not by nuclear is a two domain part and another two domain part.

So, if you have two plus two domain parts, so, altogether we are looking for binding a for calcium ions or the calcium two plus centers, which is very much similar to that of our hemoglobin. In future we will talk about the hemoglobin where we know that the assembly of four myoglobin units are there and the arrangement or the placement of forsage mononuclear arren based myoglobin units can give you the entire quaternary structure of your haemoglobin.

So, due to this coordination, you can have huge changes in the analysis, number of more helixes are there. So, those are aliases in both the domain. So, we considered that we have one domain and we have another domain. So, the structure of those domains are changing due to the metal ion coordination and while doing so, basically you see something is getting stressed in the second drawing drawing B, A and B you have only A and B you will be able to identify it also and if these two drawings are given to you should be able to tell which one is for the calcium two plus three is a condition I know it needs the calcium two plus bound

condition, you should be able to identify it because that can be our at your questions also and all these things can be nicely like that.

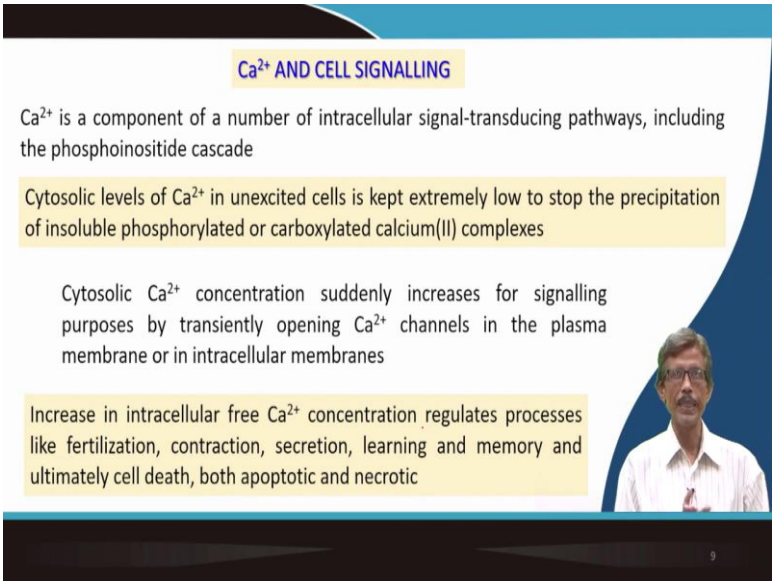
So, if you can do this, so, if you when you are stretching these things, so, one by a double calcium domain and another double calcium domain, so, you can stretch it in that way. While doing so, you are basically exposing the hydrophobic residue because the hydrophobic residue is the backbone of the protein chain which basically repel the water molecules. So, that particular part is coming out.

So, if you consider the corresponding environment or the sphere like element, the hydrophobic part is getting exposed due to calcium coordination. So, that can be another very important understanding or knowledge what you can have that due to calcium coordination you are able to expose the hydrophobic part from this protein chain.

So, how do you can have the mentioned now, the cam binding domain of SM m LC k. So, these are the abbreviated form of the big protein things only, but, we farther we can try to something where you can have the cat medulin binding domain and the smooth muscle mass the light Jen kinase is the green part. So, is a separate one also. Though the separate one basically what it tells us that that you can have a myosin light chain kinase is there so, that kindness can be very close to this particular element.

And we can have a much more complicated element and what calmodulin is doing is basically wrapped around the helix add that tryptophan residue of the peptide chain and making two other new contacts of the cam molecules one is your muthoni and 124 which is red in color. So, this lower part So, whatever environment is showing to you that you can have the entire structure, then farther which to identify from the again from the excess structure, that what extra thing is there, what domain is coming over there in the C terminal domain of CA you can have the corresponding placement of the methadone in function.

(Refer Slide Time: 25:13)



So, once you find out then only you can think of that whether you can have some role to play for that particular understanding of why these particular extra groups are there. So, in the signaling process, what do we know that the calcium two plus in binding in binding so, calcium two plus and cell signaling, so, binding we have seen now, what we can know about we can see at the end basically, that how it can go for the corresponding signaling process.

So, a number of intracellular signal transduction or transduction paths you can have with phosphoinositide cascade. So, this is some kind of biological cascade again due to binding of the calcium to by the carboxylate ends of the insoluble phosphorylated groups. So, if you have the cytosolic levels of calcium two plus of the unexcited cell that means, it is not getting excited it is not sending this signals.

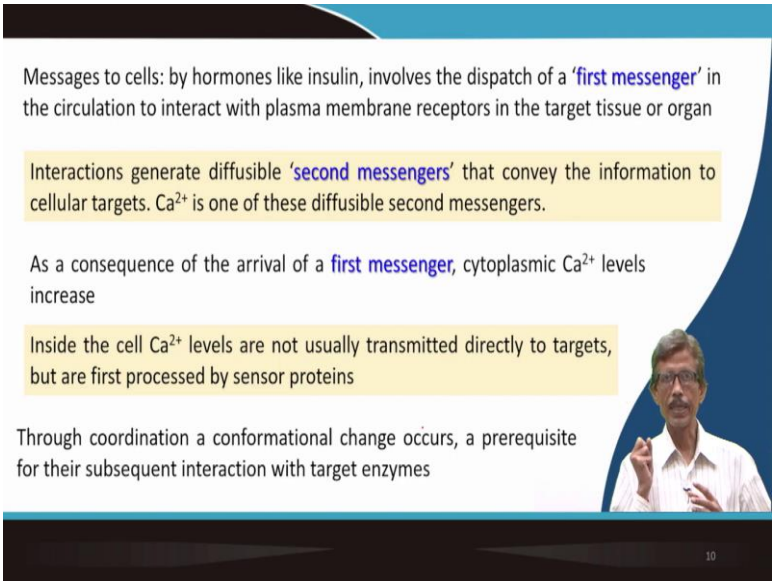
So, it is basically extremely slow and to stop the precipitation of insoluble phosphorylated or carboxylated calcium complex. So, if the concentration is not reaching some point, it will only be in the solution, because for precipitation also we require some optimum concentration like crystallization, when the solution is supersaturated then only you get the crystals.

Similarly, at some point of saturation will also give you the separation of the solid compound from the solution. So, cytosolic calcium two plus concentration when it increases, so, you have the flux or the flux what you can say and when the concentration is changing, you can have the signaling part pass or the signaling processes can start operating for the transient opening up calcium channel that means, you are opening up the sluice gate and calcium is entering and more and more and which basically increasing your intercellular free calcium

two plus concentration which can be useful for many biological processes starting from your fertilization to apoptotic and necrotic cells also the cell death also the learning process and the memory process.

So, why do we need to know about all these changes in very simple thing in terms of your quantitative inorganic chemistry of metal ions, that how the calcium two plus concentration in the cells can do so, many dramatic things and which is that slide is very much related to our health and our wellbeing.

(Refer Slide Time: 27:37)

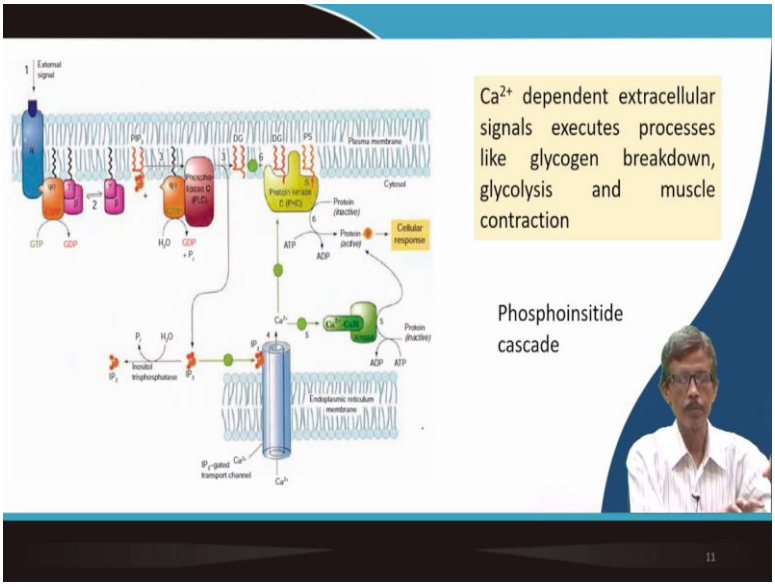


So, when it is sending signals the messages you have to send to the cells. So, you have the first messenger and you can have the second messenger during their circulation process, we can have the many receptor molecules and which can basically target the tissue and finally, the typical organ say your kidney or your lung, then the second or the secondary messengers can also come in and that can also be a cellular target and that is why these particular metal ion is your second messenger and the first messenger you have the cytoplasmic calcium two plus level which is getting increased and inside the cell the calcium levels are not usually transmitted directly to the target.

But our fast processed by the sensor process proteins which basically knowing that you have the calcium and it is trying to bind those calcium there and getting those things as your target enzymes and those target enzymes can bind those meta ion. So, in essence what we are trying to understand is that your calcium binding and so many other transformations are taking place

so many changes are taking place. And that is why it can give you some signals to sound good and useful processes.

(Refer Slide Time: 28:54)

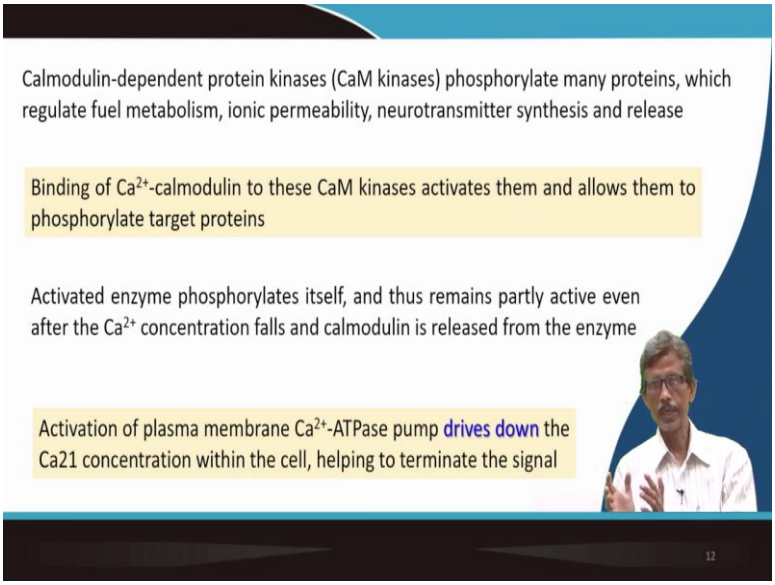


So, if you can have the extra cellular signals and which can execute the processes like very simple one the glycogen breakdown glycogen is our storage from our glucose your the carbohydrate storage is in the form of glycogen. So, in the breakdown process, we know these the glycolysis and it is related to your muscle contraction.

So phosphoinositide cascade, so these are typical technical term phosphoinositide cascade can take place. So is a very complex biological pauses and all these things try to understand all these do lipid by layer you know, so, one lipid by layer at the top and other by layer on the bottom also you have the pump like thing calcium pumps already we have learned all these things, but try to locate where you can have the calcium basically what we are showing over here, one point to the other.

You have the external signal which is coming from there from the top basically it is coming over here and is going basically ultimately for your corresponding calcium so movement of the calcium and some other regular and important molecule like ATPs.

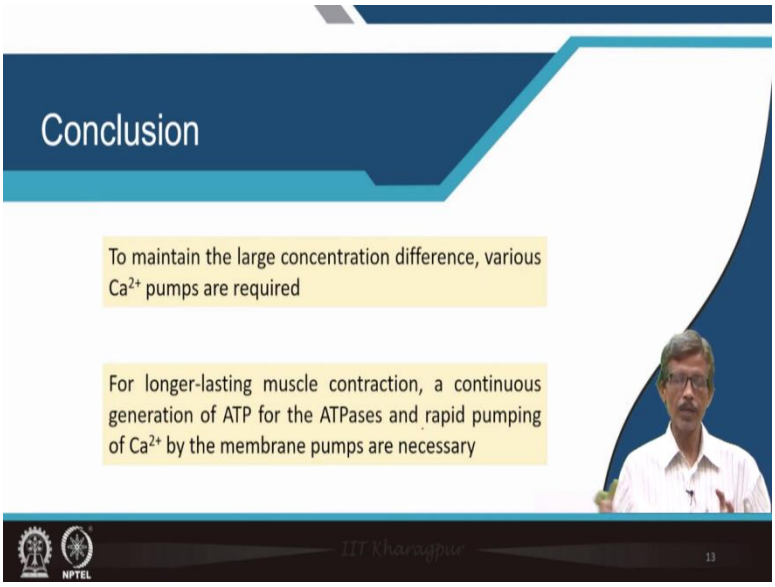
(Refer Slide Time: 30:01)



So this calmodulin dependent protein kinase is that they are which can phosphorylate so many things and the synthesis and the release, then binding of the calcium two plus carboline to these cm kinase basically activates the system. So all these things basically again, I am reminding you all these things, because if you can have like sodium and potassium ATPase, you can have also a calcium two plus ATPs pump, which can drive down not calcium 21 is calcium 2 plus concentration within the cell and helping to terminate the signal.

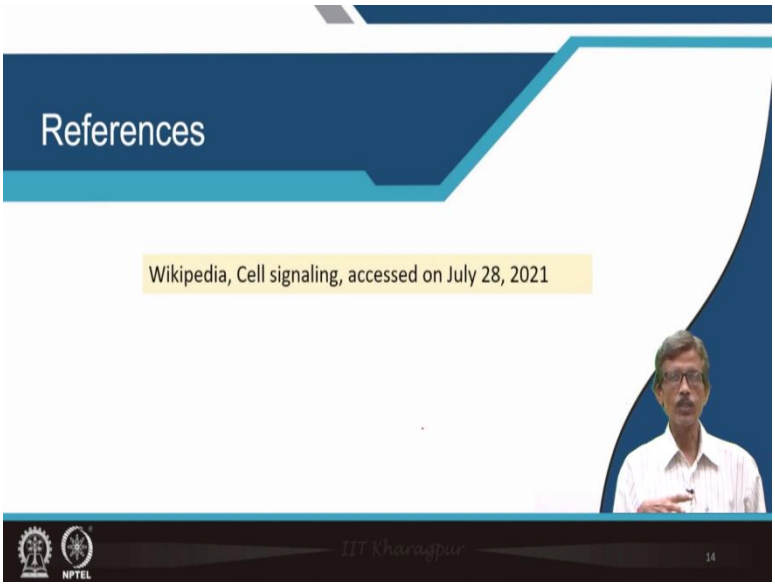
So, you had the signal when the calcium concentration is changing, and you are taking the help of the pump and at that one point, you are basically stopping the signal process.

(Refer Slide Time: 30:43)



So we have learned in that way, how we can maintain a large concentration difference to activate your pumps and when you are required the pump for some biochemical activity. And for longer does lasting muscle contraction, a continuous generation of ATP is required from the ATPases and rapid pumping up calcium two plus by the membrane pumps are necessary. So you have the membrane, you have the membrane pumps, and you just basically pump the calcium two plus ions.

(Refer Slide Time: 31:13)



So the page on cell signaling on Wikipedia is useful and also the book. Thank you very much.