Inorganic Chemistry of Life Principles & Properties
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Lecture - 20
Functioning of ATPases & nucleases [Na, K]ATPase

Welcome you all to the next lecture on Inorganic Chemistry of Life Principles and Perspectives. In the previous class, I have built all the necessary components required to understand ion transport in ion pumps like sodium potassium, ATPases etcetera using the synthetic molecules as well as the molecules of the natural source. Now, let us go into the case of the ATPase.

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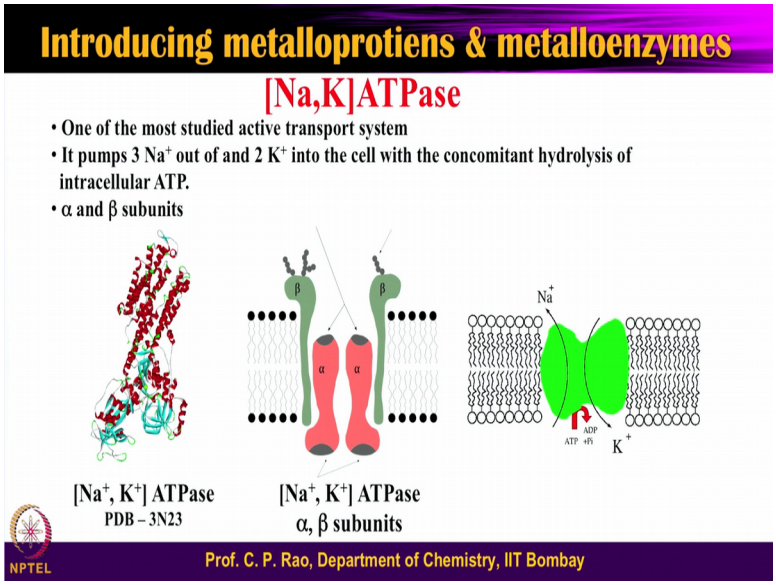

We will study in more detail one of the ATPases that is sodium potassium ATPase. Let us look at this particular slide. So, one of the most studied active transport system is the sodium potassium plus. What it does basically is it pumps 3 sodium ions out of the cell, and 2 potassium ions into the cell. We have already seen the concentrations of the cellular concentrations in all of these.

And this is an energy based process; so, this is an energy based process. So, it will hydrolyze the ATP. In fact, in the body, the most of this energy is consumed mainly for the ATPase pump activities of this sodium potassium or calcium magnesium or calcium proton, these kind of a things.

This protein has several subunits, you can see the protein looks like somewhat like this. A lot of helical alpha helical structures you have and you also have some beta sheet structures here to and the it is a quite long a cylindrical kind of a protein. Because the such kind of a cylindrical kind of a protein you will have a very nice preference to build itself into or to push itself into the membrane, you can see that. So, the alpha subunits here, and the beta subunits here so that is how it is. So, we are getting into this one this subunit here this one and this one.

So, you get more or less this particular membrane is completely filled by that. And the small things that are shown over there are nothing but the glycosylated portions here so that comes since the proteins glycosylation post translational one you do not need to worry about it. So, otherwise you take it as granted this whole protein if you try to insert it and you can see in this form. So, you have the beta kind of thing is as alpha subunit and beta subunit, nothing to do with the alpha helical and beta sheet no. So, it is this is what is inserted into this.

Once you have this, the sodium ions you know going pumping out of it and potassium ions pumping in and the ATPase utilized. And whatever I said in this point here this has been shown over there. So, this point and this particular depiction are one and the same you can expect. And this particular structure which I have shown here is inserted into this. So, you can understand. So, this protein gets into the membrane and then functions. So, because it has to take ions from outside and bring inside; it has to take ions from inside and take it outside and which is shown over there. So, it takes ion from inside and takes it out; and takes ion from outside drops it inside.

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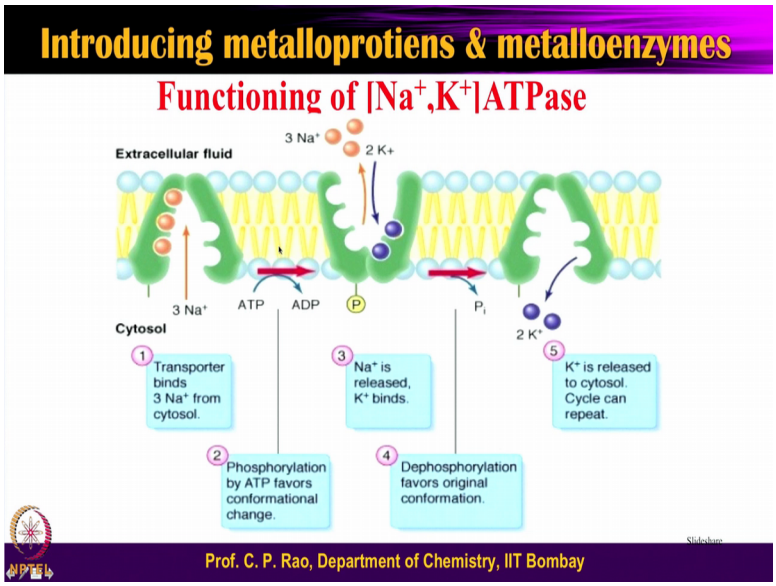

And how does it do? Let us look at in a very gross manner. And let us assume this is the this is the protein, and this is the membrane you have, and you can easily see. And these are some sites for binding these are some additional sites. So, initially the protein the ATPase protein which is which is let us say apo will pick up 3 sodium ions, these 3 are sure over there, and 3 sodium ions are now should be transported out they are taken from inside and it is bound.

At this stage, the ATP goes to ADP means it loses one phosphate and that phosphate is added to this particular protein. So, this is a particular protein, so that means, the protein is phosphorylated. When the protein is phosphorylated its conformation absolutely changes and that is what you shown like a v inverted v, and showed one more inversion we will make into u or v kind of a shape; so, because this is a phosphorylation.

Once it is phosphorylated, the protein conformation change. Then when the protein conformation changes, the sodium ion affinity to the pump is reduced. So, therefore, sodium is released because affinity is reduced. And at this stage there are unfilled sites which are picked up by the potassium the potassium affinity to these sites will increase.

Now, there is the protein which is phosphorylated undergoes a dephosphorylation ok. So, you have a step of phosphorylation, step of depho phosphorylation. Where it dephosphorylates, then it loses the affinity for the potassium and the potassium comes.

So, so one inside, one outside so into the cell and then now the free ATP is ready for the next cycle and that is what we showed over here.

So, the transporter binds to 3 sodium ions from the cytosol, then there is a phosphorylation by ATP and then the sodium plus is released because sodium plus affinity is decreased and then that is goes into the dephosphorylation step and dephosphorylation stage of course, at this stage the potassium is bound and the potassium comes out the same thing is shown over here steps as 1, step 2, step 3, step 4, step 5 kind of thing.

So, now you understand the protein has different sites for sodium binding, potassium binding. Their affinity is vary depending upon the protein conformation. And in this continual conformation, it has an affinity for sodium ions; in this conformation, it has an affinity for potassium ions. The conformation change can be obtained by the phosphorylation and they can be returned back by dephosphorylation. This is the thing that need to understand this.

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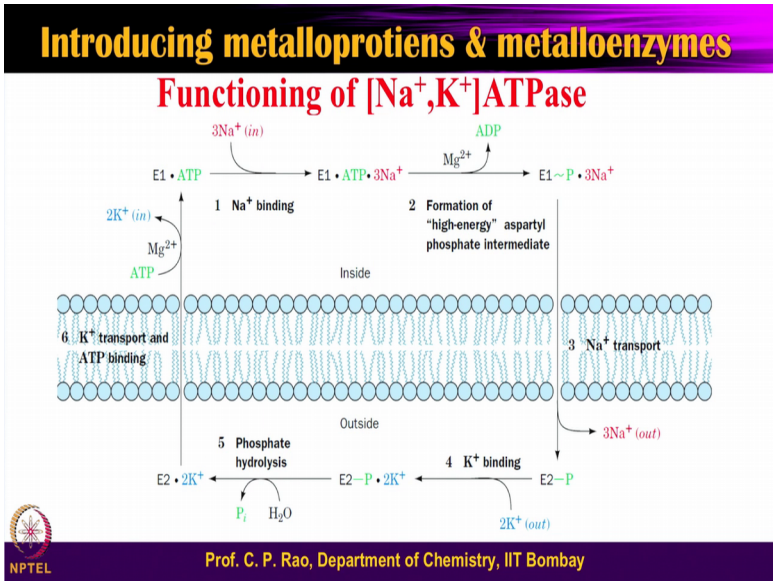

The same thing is explained in a little different way here. We have the E 1 is a one of the one of the conformational state of the protein. And of course, the ATP bind then the it is active. And at that stage the sodium ions are taken in so the sodium bind binding takes in place and so therefore, you have a E 1 ATP sodium. At this stage, the magnesium ions will activate the ATP and then may convert into ADP and attach that one of the phosphate

group to the protein. So, that is the one where it adds the protein. So, when it adds the phosphate group is added to the protein, the protein conformation is;obviously, changed from E 1 to E 2 and at that stage the sodium plus has no affinity sodium plus is released out. So, you can see what is happening outside here and what is happening inside here kind of thing.

Now, at this stage, the potassium ions can bind because the protein has a different conformational state which is E 2 conformational state. At this stage, it has an affinity for potassium ions, and the potassium ions will bind. And once the potassium ions bind, it will trigger with the dephosphorylation. And the when the dephosphorylation takes place, the affinity for potassium is reduced. And this protein is carried over here or this complex is carried over here. And in presence of the magnesium, it will be the dephosphorylation takes taking place and that will result back to the original complex.

So, the affinity for sodium affinity for potassium here, so affinity for sodium and affinity for potassium and this even conformation to E 2 conformation. So, E 1 before phosphorylation, E 2 after phosphorylation; after dephosphorylation, again it will go back to E 1. And this is the kind of a cycle that occurs for the sodium potassium ATPase in these systems. So, I hope you understand that one.

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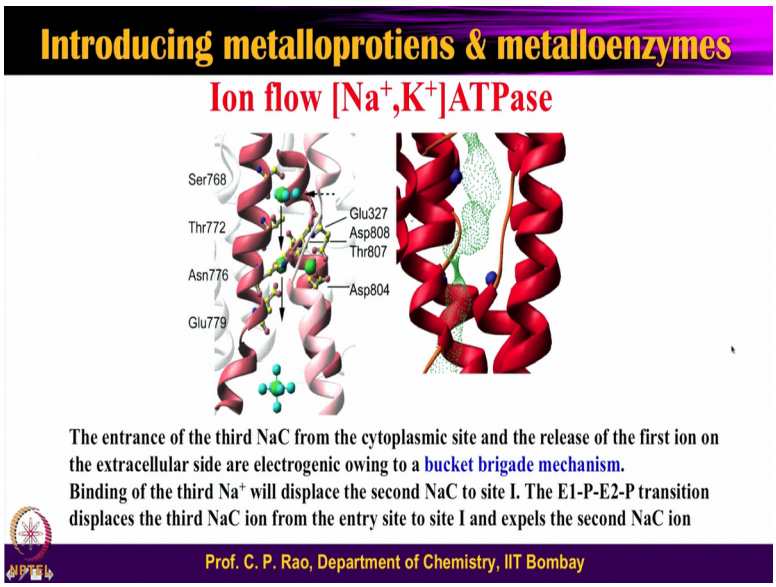

Now, we talked about in the previous two cases, we talked about the 3 ions of sodium and sodium binding and then 3 ions of the sodium released 2 ions of potassium binding 2

ions of the potassium released, these are the aspects that we have looked at already earlier in that; so, the same thing through a conformational state. So, the previous picture, in the previous slide, it is demonstrates the ion binding property; in this slide, we demonstrate the conformational state of this. So, one E 1 conformation and after phosphorylation it; because E 2 configuration; then, dephosphorylation will come back to the E 1 conformation. The two things are coupled together is not that these two things happen at two different stages, they all happen together.

Now, let us look at one little more information in this context which explains the sodium transport or sodium movement sodium ions movement in this you see that. So, one of the protein part inside the inside this membrane, you have the two alpha helices coming closer you can see that the they have the sites of the binding and another site of the binding and this.

So, therefore, as the conformational chain initiates the one of these ion over here will drop down here. The ion which is present over here will drop down here in the presence of water because the water will de ciliates from here from this and then make it. So, you have a smooth flow of ions going from one center to the other to the other into the bulk into the ins releases the ion is being released you see that. So, finally, you have a release of the ions. So, you are taking from inside and then you are making into the outside this is what is basically demonstrated.

So, you can see the region, the region is connected by the sodium ions in the peptide protein region and to the next site. And here at this stage the water interaction will lead to the replacement of the. So, similarly the next conformational change will lead to the release of all these one by one. So, the third one will come first; the second site one will come next; the first site ion will come next; so, this is how the sodium ion flow.

And the same thing can be seen from here the flow as it goes here when the conformational state is changed then there is a opening up of this and then it will drop down. So, at one conformation, the three are intact. When the phosphorylation takes place, the conformation changes; as the conformational state changes the ion in the first will go to the second, second will go to the third, third will come out into the in it is released into the cell so where the water is present here. So, in other words, it will be into the cytosol.

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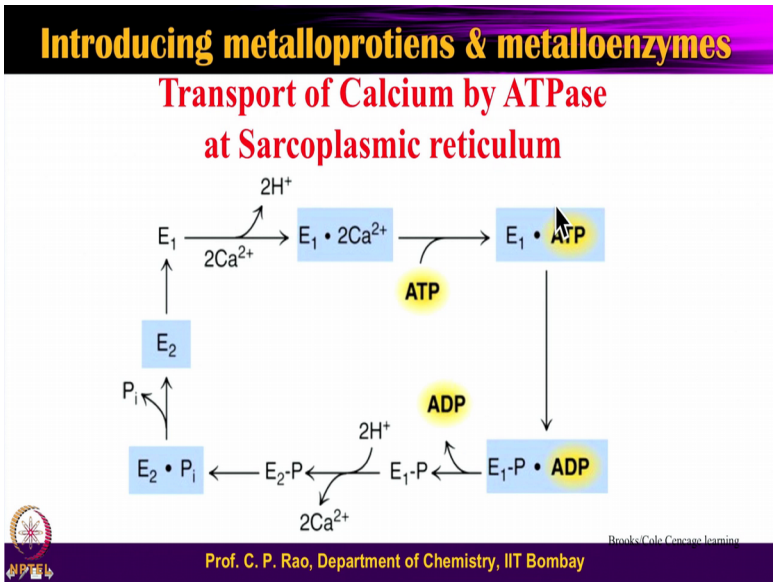

So, I hope with this you understand the sodium potassium ATPase pump. Similarly, you can also look at the calcium ATPase pump and the calcium ATPase pump is coupled with the protons. So, calcium is 2 plus. So, your proton is 1 plus. So, you can understand each calcium ion is coupled with 2 protons. And you should also understand when this kind of a coupling takes place then you need to understand that there is a ph variation and that is what I explained in the earlier slides too.

Just like the sodium potassium pump for the calcium ATPase pump and let us say original confirmation is E 1 in presence of the ATP the calcium binding the calcium binding will release the two are the protons and at this stage the ATP activates. So, you have a ATP complex of the even and this ATP complex of the E 1 is activated to convert to the ADP which means this is ATP is hydrolyzed and their ADP goes out and the phosphate part is added to the protein, so that is what is called the protein phosphorylation.

So, E 1 P will convert to AD 2 kind of a structure and during this that it has an ability to release the calcium ions because in presence of the H plus ions. So, in presence of the H plus ions then this will release that one. So, now the protein has a different configure the conformation. And at this conformation again now it loses the phosphate, so dephosphorylation and goes back to the original state of the conformation. So, it is very analogous to what happens in case of the sodium potassium ATPase. It is from the

calcium proton pump. So, in other words calcium ATPase will goes in the similar kind of a type of transport.

Now, you understand the major things that happens in this is either the sodium or potassium. Let us take the sodium sorry sodium potassium ATPase that the initial conformation of the ATPase has a affinity to bind to the sodiums, the sodiums are taken in all the three sodiums are taken in. Then the ATP activates this and then ATP is hydrolyzed to ADP and that will result in a phosphorylation of the protein, and the phosphorylation of the protein will change the conformation, and the conformational change will release the will release the sodiums.

And at that stage, the conformational state is suitable for the potassium to bind to that. And this is in a different conformational state. And at this stage, once the potassium is bound, then the dephosphorylation takes place. When the dephosphorylation takes place potassium come out too. So, it is the same kind of thing between the calcium and the protons as well.

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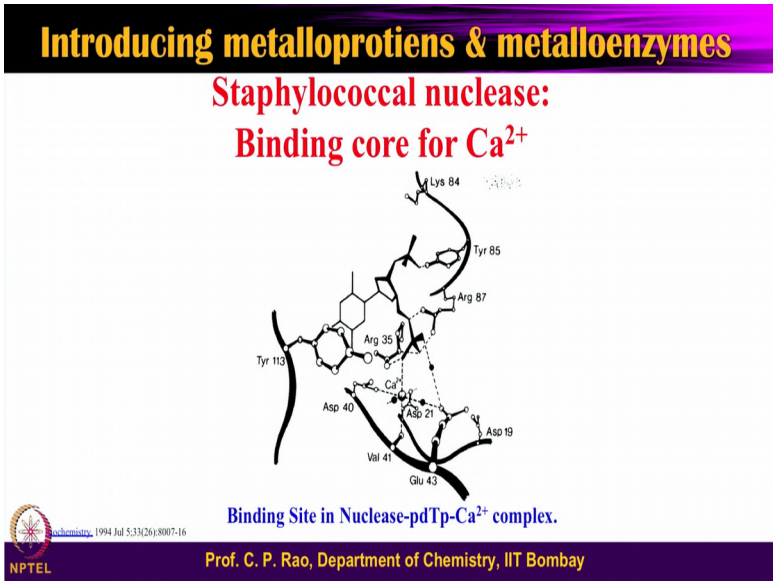

Now, look at a little bit more on the one more protein or one more enzyme of a calcium containing enzyme. So, this enzyme is a staphylococcal nuclease. And this is a calcium. Calcium is at the active site or catalytic site. Now, you can look at that the calcium is bound to the protein this, these are all coming from the protein. And then what does it do it does hydrolyze the sugar bond connected with the ribose. So, it will separate the ribose

versus this one so that is the nuclease activity of this. So, this is the main binding core for the calcium as you can see where the substrate is also of course, bound in this. So, binding site of the nuclease of the calcium complex.

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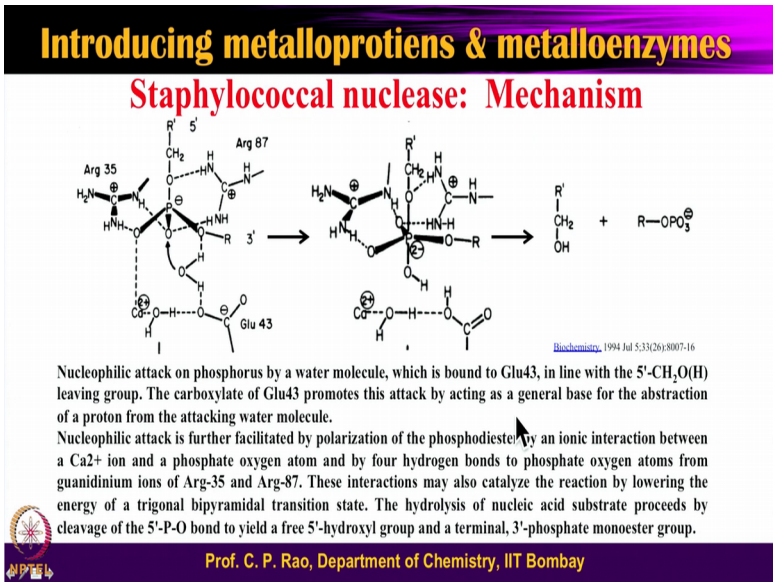

Let us look at the kind of things that happen in this. What does basically it does. So, it is a phosphate and sugar bonding I am sure you are aware in the nucleotides. So, it is that particular bonding which gets hydrolyzed. So, separate the phosphate, and separate the sugar part of it, so that is what is happening

So, you have seen in the previous slide that you can see that and this is the nucleotide part which is attached over here. And this is very clearly can be seen the region of interest. So, the region of interest, this is your calcium, and this is the phosphate, and this is the phosphate with the sugar connectivity. And it is this one which gets hydrolyzed.

So, to get hydrolyzed, so you have kind of an attack on this. And this nucleophilic attack comes from a water molecule which in turn is activated by a glutamate residue. So, glutamate residue in the vicinity of the reaction center will activate a water molecule and then make this water molecule into OH minus and that is brings in a nucleophilic attack. And as a result of this, as you can see this, this transforms into this in this thing as you can see 1, 2, 3 trigonal, the one on the top one on the bottom. So, you get trigonal bipyramidal intermediate stage. And here the phosphorous is in the tetrahedral state and

here trigonal bipyramidal state. And this is an essential kind of a transition state to which this particular reaction goes through.

Once it goes the attack of the water as a hydroxyl which is activated by this glutamate. And it is not only this just this role of the glutamate is important, role of this arginine is important, arginine-35 and the role of the arginine-87. So, role of arginine eighty 35, arginine-87 are equally important in the whole process of reaction converting into transition state and converting into product all three stages of this , but however glutamate 43 has a specific role in activating the water.

So, the initiation of the nucleophilic attack is basically carried out it basically carried out by this particular water which is assisted by glutamate for 43 and whereas, the phosph nucleotide is stabilized by the arginine-35 on this side, and arginine-87 on this side. And additionally as you can see that there is a N H 2 group because the arginine which is essential when you break the p o bond you need to stabilize this bond by this O minus by a protonation and that is what is you can see the O O is a so this is O O that because of OH CH 2 R prime. So, this is the ribosal part or ribose part and this is the phosphate part. And the phosphate center gets a OH from this side which is as a nucleophilic attack on this.

So, the entire the phosphate binding is not only stabilized by the calcium center, but also stabilized by arginine-35, arginine-87. So, therefore, at the reaction center these act like a secondary coordination sphere and there is an importance. So, many times we think that the metalloenzyme means the substrate will directly bind to the metal center nothing else is required which is not true in some cases of course, the metal center is required here. And what is required is the secondary interactions or secondary sphere interactions.

The secondary sphere interactions means interactions coming from the protein; in this case arginine-35, arginine-87 which will ensure that this nucleotide is perfectly placed over this, so that the water attack can take place at the phosphorus; otherwise it will not happen. And that leads to the intermediate step which is transition state; and the transition state is pentacoordinate the phosphorus and this will further protonate and break to the phosphate in this one.

So, thus we have seen the clear cut mechanism of an enzyme nuclease which is the activated by calcium 2 plus. You can see that direct reaction is not at the calcium 2 plus,

but calcium 2 plus is essential for this to hold this whole situation. And the secondary interactions are necessary for the holding the substrate. So, it is a total system which is playing an important role not just the calcium 2 plus alone, so that is why we talked that the in these enzymes there the metal activated enzymes basically.

So, one of the example I have shown very clearly here is the how a phosphate the nucleotide hydrolysis takes place 2 phosphate and sugar, 2 phosphate and sugar going from a nucleophilic attack of the water via this particular glutamate glutamate is base that will pull out the proton it will make OH minus. This OH minus will interact or make a nucleophilic attack and this and then make the transition state. And this at this transition state the protonation will lead to the r prime CH 2 OH and this so that means, you need an alignment of the phosphate moiety with respect to this water. And this whole thing is ensured by this group here that group over there arginine and the arginine over there.

So, these are the kinds of activities that particularly takes care in this particular enzyme. So, in a fact let me conclude that we have been looking at in the last three to four classes, the role of alkali alkaline earth ions and there when you come to the role of the alkali alkaline earth ions, it is the sodium plus potassium plus and it is the calcium 2 plus and magnesium 2 plus. We have looked at their relative concentrations inside the cell, outside the cell in intracellular, extracellular, we have try to understand that most of the enzymes inside the cell or activated by the potassium plus and very few or from the sodium plus and we have seen that the there are large number of variety of enzymes kinases, phosphatases, mutases, synthetases all these kinds of things are activated by potassium plus.

And we also analyzed several crystal structures known in the literature. And we have looked at the potassium plus or sodium plus binding centers. And they the binding could be the 4-coordination, 5-coordination, 6-coordination, 7-coordination, rare cases 8-coordinations to all these we have tried to look at. And then and then try to compare these relative affinity of these ones the calcium 2 pluses have greatest affinity has compared the magnesium 2 plus greater than that of the potassium plus greater than that of the sodium plus this we have tried to understand the affinity of these ones.

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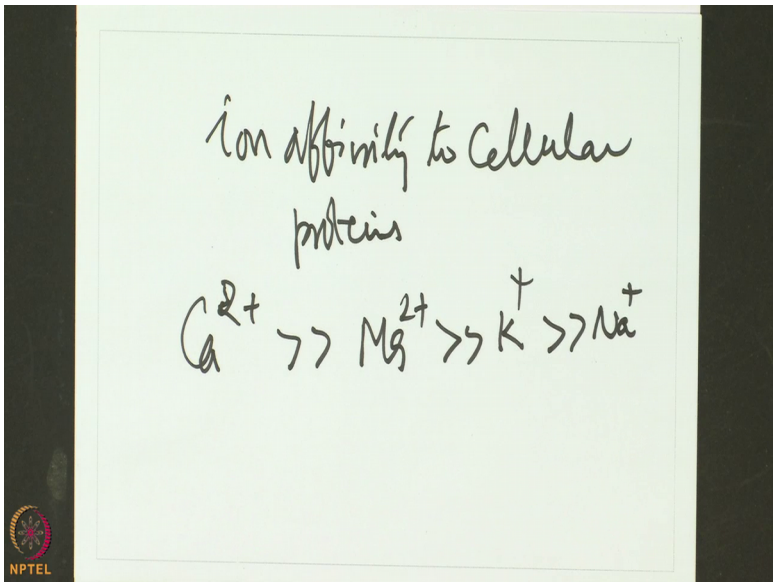

And in the cellular proteins the ion affinity to cellular proteins is calcium 2 plus is much much greater than magnesium 2 plus much much greater than potassium plus is much greater than sodium plus. So, and I have also explained to you that in case of the magnesium and calcium concentrations if you compare, magnesium concentration is much greater than the calcium, but however magnesium ions do not activate the calcium enzymes because of the selectivity of the calcium towards the carboxyl kind of groups. And this is where the nature has modified certain glutamic residues by adding one more carboxylic group, gamma carboxylic group to make into the carboxylate containing proteins. And therefore, they become very specific towards the towards the calcium binding.

So, calcium versus the magnesium kind of a competition has been taken care by the nature by adding more carboxylic groups. So, more carboxylic groups are more favoring the calcium 2 plus as compared to the magnesium 2 plus. Then we have looked at the magnesium 2 plus is involved in a large number of enzyme reactions kinases, phosphatases, mutases all these kinds of enzyme, synthetases all these. And particularly a lot is involved in phosphorylation and dephosphorylation. Magnetism two plus is involved a lot in the phosphorylation and dephosphorylation. In the next part, we have tried to understand the ion binding and the ion selectivities.

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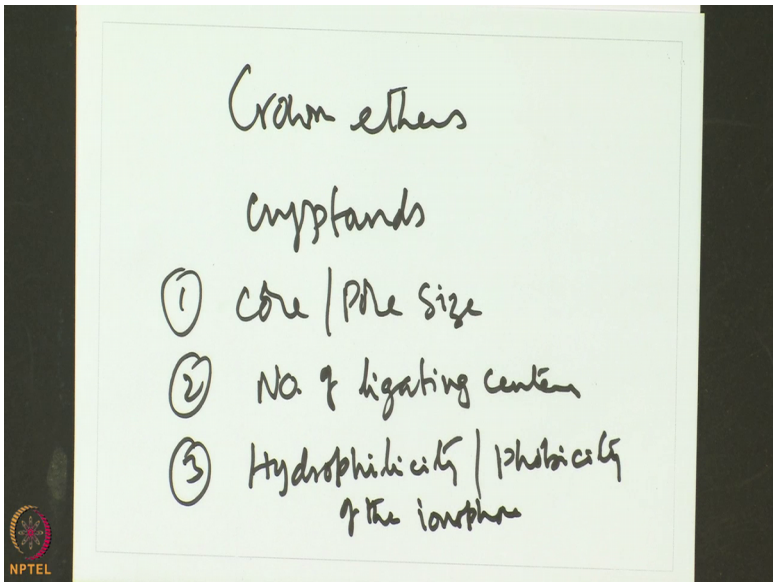

So, we have looked at the crown ethers and we have looked at the cryptands explained through crown ethers and cryptands. So, what we have tried to look at is one is core size, core or pore size. And 2, number of ligating centers. And we have looked at hydrophilicity or phobicity of the ionophore which is exposed to the environment and that will influence the binding strengths of these ions basically because there is a competition between the water and the ligand a water and the ion. So, these are the kinds of things that.

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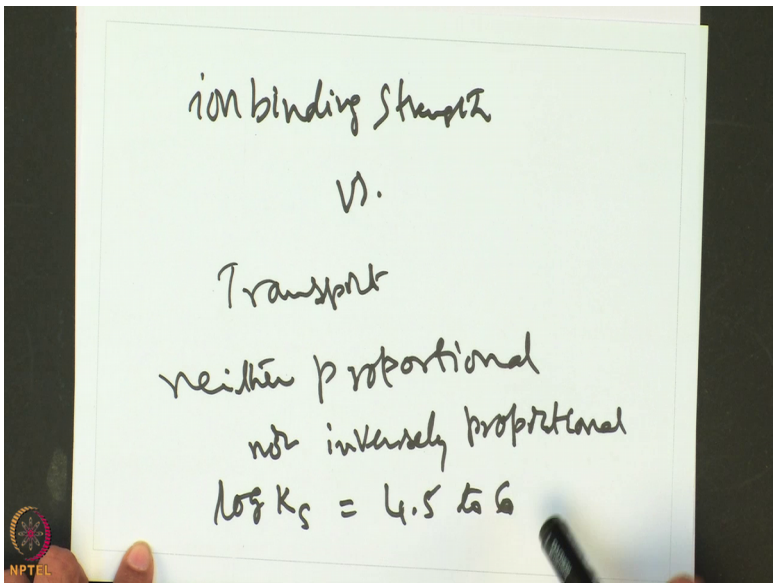

And then we have looked at in the in the next stage we looked at ion cell binding strengths; so, ion binding strength versus versus the transport. So, it is a neither proportional nor not inversely proportional. So, these what we have explained ah. And therefore, we said log ks which is in the range of 4.5 to 6 is the most preferred one rather than the lower the k s log k s, it is a problem it will break down before transports higher they higher the log k s is it will key hold it will never release. So, in either case we have a trouble and therefore, these 4.5 to 6 seem to be very and this matches very well with the with the with the natural ionophores.

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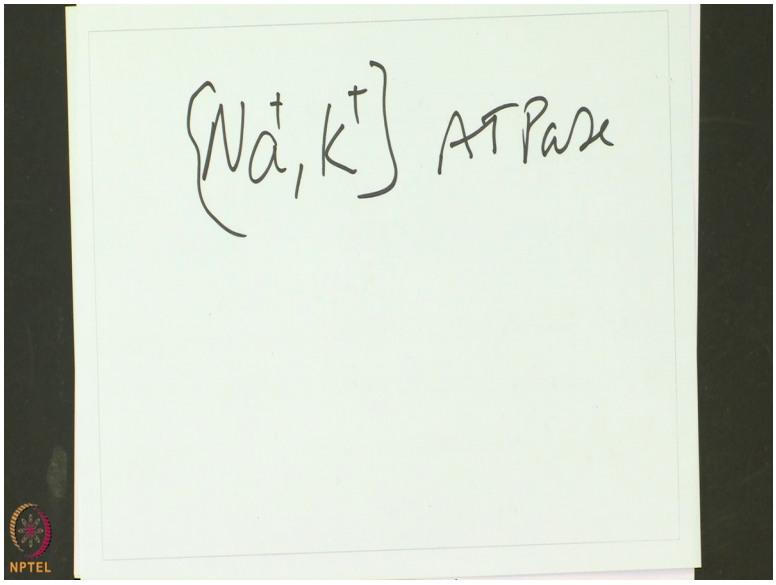

And in the next aspect we have talked about the talked about the ATPases. And sodium potassium ATPase so you have the initially it binds to 3 sodium ions initial conformation. and then the at that stage the phosphorylation is triggered. And when this phosphorylated it loses its affinity and loses the three sodium ions; and at this stage it is the potassium ion centers can be activated or bound. And then and then and then in presence of the magnesium, again the dephosphorylation takes place and the dephosphorylated one releases the potassium ions and this whole thing will be cyclic kind of thing. So, therefore, sodium potassium ATPase.

So, this has the maximum amount of ATP consumed for this kind of thing. The last example I have showed was there for the nuclease where the nuclease you break the bond between the phosphorus and the sugar bond is being broken. So, therefore, sugar is

separated from the phosphate, where the nucleophilic attack. Nucleophilic attack is not directly from calcium center for by water which is activated by glutamate and the whole system is configured to be oriented by other arginines present in the near vicinity and going via five coordinated transition state and leading to the breakage of these two the sugar and the phosphate part of it. And in the next class, we will start with the transition metal base catalysis.

Thank you very much.