Biological Inorganic Chemistry
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Lecture 25
Na+ - K+ ATPase

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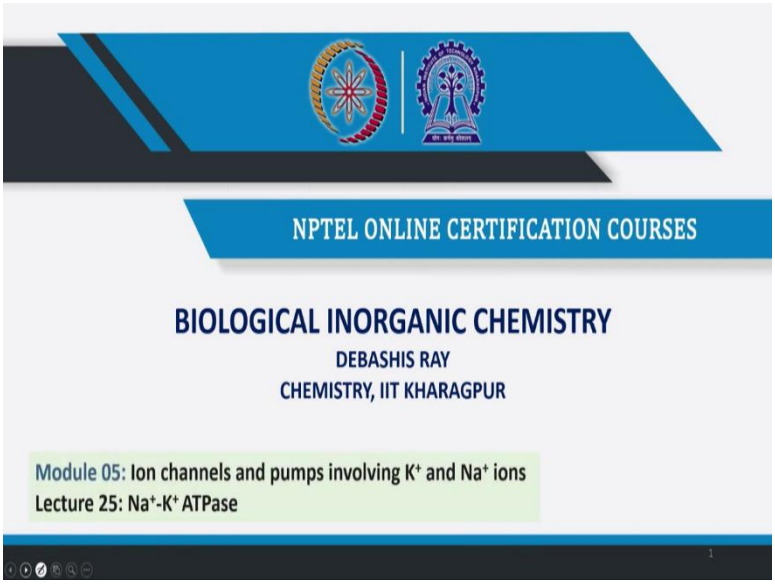

Hello students, good morning everybody. So, where we stand now is basically standing on Module 05, and the last class that is the lecture number 25. So, following the discussions what we have seen so far about the potassium and sodium ions and their involvement in the different ion channels and pumps. Now, we have least at that particular point where we can talk about something which you can consider as ATPase. So, what does it mean basically, why we talk about these ATPases, and how it is depending on the relative concentrations of sodium, as well as the potassium?

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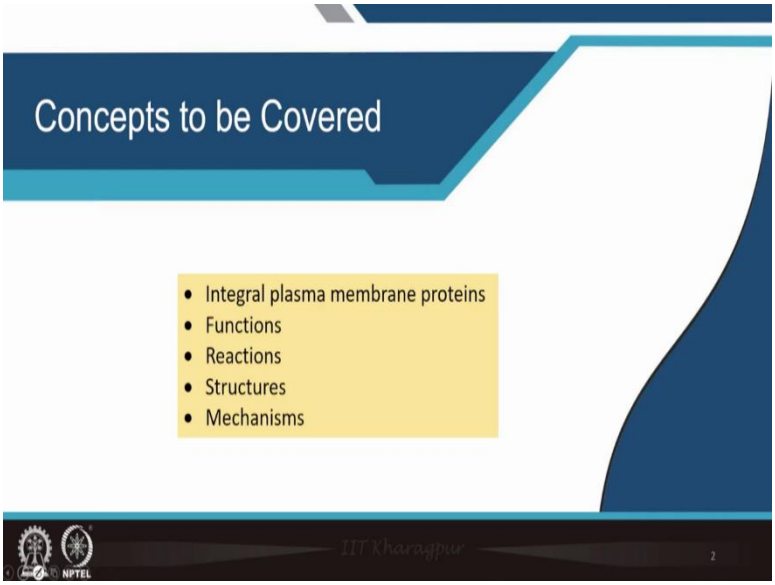

So, we try to cover several parts only, so where we can have the integral plasma membrane proteins on which these particular ATPases will be attached, and these functions basically and their reactions and the structures which is very much important. Now, why selectively the sodium is trapped in one pocket and the potassium is trapped in another pocket, and they are moving in two different directions or the opposite directions, and finally, the mechanisms. Right now, we can have also all these understandings, so far we have gathered.

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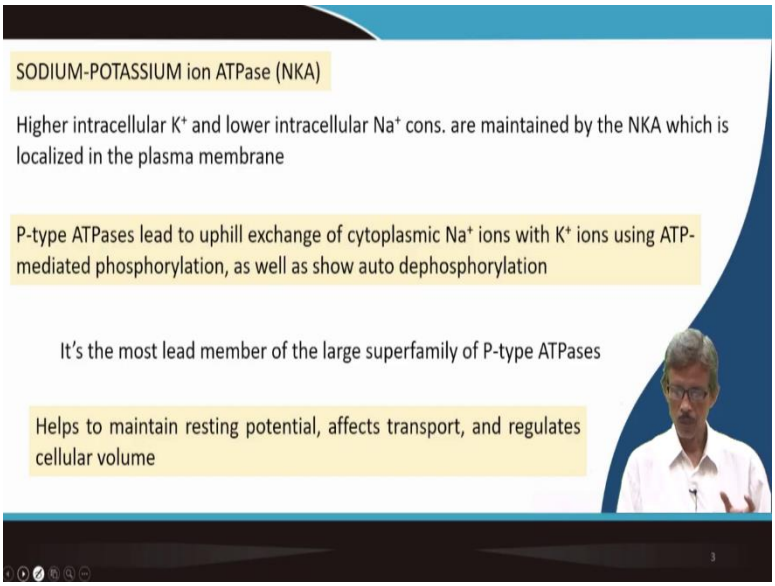

So, the nomenclature of this NKA, is a sodium potassium ion ATPase. Sodium is again from the symbol giving you NA potassium is given from the symbol K, so natrium and kalium. So, that gives rise to the corresponding nomenclature, as a whole, as your NKA. So, this is

important because not that all the time we will talk about or write about this particular sodium ion, potassium ion ATPase is a big statement. Instead of that, we will just write that NKA is the function of these NKAs.

And as we all know, starting from our thing that how we can determine the corresponding concentrations of the sodium ion and the potassium ion in your drinking water or mineral water. Any kind of mineral water, which has been purchased from the market. So, we know that due to that, we have the different concentrations in our system, in our body.

So, we have the higher cellular concentration of the kalium ion, that means, the potassium ion because this is enclosed within the cell, and you can have the lower intracellular, that means, the outside the cell concentration, that means, within this particular case that the intracellular and the extracellular or the intracellular concentrations, we all know now, that we can have the real differences in all these concentrations.

So, how we maintain that? We have seen that we can have the different channels, different pumps and all these things are there, but whether we require something where we can talk about the ase. What is the ase? That is the carboxypeptide ase and all these things. How the using of these corresponding, that cleavage or the degradation or the breaking of the thing.

So, as we all know the involvement of ATP. So, how you can go for the ATPase that means, the cleavage of or the corresponding hydrolysis of one of the phosphate group attached to the triphosphate unit of ATP. So, these NKAs are basically localized in the plasma membrane. So, we think about that plasma that is sitting on the plasma membrane, and then they do all these functions.

So, large number of these ATPases molecules has been structurally characterize, functionally identified and then the corresponding reaction mechanisms have been proposed also. So, there are different categories starting from your plan to bacteria to fungus, to our human body or the other animals or the mammals.

So, one such is that your P-type ATPase. And again, like that of your pumping mechanism, the P-type ATPase also require the uphill exchange of cytoplasmic NA plus with the K plus ion. So, whatever you have, so, you go for first the exchange, and that exchange reaction can also trigger something, that is the importance of all these things. That means, the ATPase molecules that you are going for the movement of the ions.

Then at one point you are seeing that, okay, we can go for the conversion of ATP to ADP, the adenosine diphosphate from triphosphate to diphosphate. So, you can take out one phosphate as your inorganic phosphate that is why we will also write it as capital P and subscript i small i.

So, basically this phosphorylation reaction and auto dephosphorylation reactions also because afterwards also is the reactions all these reactions are reversible. Because, we try to go for the corresponding consumption of glucose molecules, and we try to consume the oxygen. So, if the burning of these glucose by oxygen is producing the corresponding amount of ATP synthesis, but at some point due to the energy released from this particular point, so that oxidative phosphorylation reaction, as well as the dephosphorylation reaction, which can be auto dephosphorylation reaction can balance all these energetic transfers and the energetic balance also.

So, it is the most lead member of the large superfamily of P-type ATPases that is why we are writing as a P-type ATPase. So, they are of different types basically. So, one of them is your P-type. What does it do? So, one single ATPase what it can do? So, it can do basically the control of the corresponding, the base potential, the resting potential, can also control the transport mechanism that means the movement of the sodium in one direction and the movement of the potassium in the other direction.

And while doing so, it can also, we will see afterwards, that it can also manipulate something in terms of its charged balance or the charged species transport it can also go for something related transfer of other ions, including your protons H plus. So, while doing so, it can also transfer the water molecules associated with those ions.

Like, we all know that a sodium ion during the transfer or during the passage of this channel or the gate, it can be dehydrated, dehydrated means the de-coordination that the bound water molecules can be removed, but it is not a bear NA plus only. At least it will have one water molecule coordinated to that particular metal ion center.

So, the volume of water, which is being transferred during this process is also important, and the material transfer from outside of the cell to the inside of the cell is also important. That is why, we can right here also in a fashion that, it regulates the cell volume or the cellular volume. So, the material as well as the corresponding environment, that means, the aquas environment is also important to maintain that particular one in a static condition.

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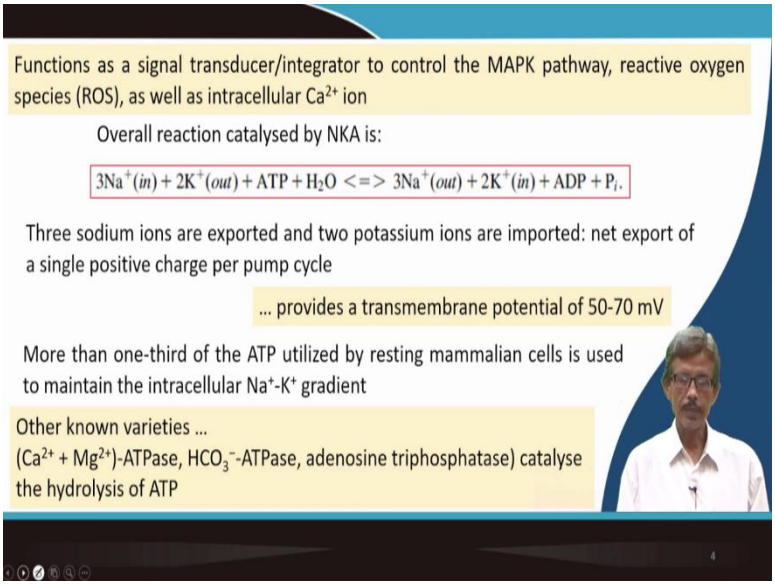

Then, during this particular transfer, and also, we have seen earlier that you have the potential involved the cell potential or the membrane potential. And that the change in the cell potential or the corresponding window of that particular potential can control the transfer of these ions, but it also gives the nerve signal. Like electrical signals it also gives the nerve signals, it can excite our nerves, so that we can do some work, we can think something, our brain signals are also required. So, it can function as a signal transducer.

So, signal transducer, as well as is integrator is basically important for a particular type of pathway. We will not go into detail of all these things MAPK pathway. So, MAPK pathway is a typical pathway basically for the signal transduction and all these things, basically, if we talk in terms of the neurobiology. So, neurological signaling and the biological part also we can consider all these things, but right now, you just only know the name only.

Then, the different types of reactive oxygen species, we all know. Many times we discussed it also that when you go for the corresponding reactive oxygen species, we know that dioxygen itself is also reactive, but other ROS species like your superoxide, peroxides are the oxygen radicals are also there. As well as it can also have some role to play for your balance in the calcium ion transfer and the storage and your assimilation.

So, what is that overall reaction? What can be catalyzed by these NKA? So, up to this point, you should try to remember nicely that what is NKA? What is its basic reaction? How it does? And then finally the cell reaction. It is not the reaction what we are looking for in a test tube. It is the reaction, which is happening in terms of your typical inorganic chemistry, knowledge or understanding that this particular reaction is happening within the cell.

So, you have three sodium ion in, and the two potassium ion, out. That means, the membrane you have, you have inside and the outside of that membrane then you just supply the energy currency ATP, and definitely, the water molecules will be there. Because the involvement of water molecule is there, that is why we have the ase ATPase or the lysis reaction or the hydrolysis reaction. It is nothing, but it is phosphate ester hydrolysis reaction.

The conversion of ATP to ADP is nothing, but your typical phosphate ester hydrolysis reaction. So, definitely, since you are thinking about the hydrolysis reaction, definitely you should have a good nucleophile. And to us, it is not the hydroxide ion or any other nucleophile in hand the water molecule can function as a very good nucleophile for this particular reaction to ultimately give you the product as ADP plus Pi which is your inorganic phosphate anion.

So, the transfer, the number balance, the number balance is nothing, but you have the 3 sodium ion on the left 3 Na plus and 2 potassium ion that is why these, one is exported and another is important. So, one is out and another is in, and the net export is basically a single positive charge per pump cycle. So, if you consider that this is one cycle of pumping mechanism, so you have one extra positive charge, which are getting pumped. So, during this pumping process, your positive charge is getting accumulated, so your membrane potential will also be changing.

That is why it results what? It results a transmembrane potential of 50 to 70 millivolt. So, due to that transfer, due to that hydrolysis we are basically achieving something, which is your transmembrane potential. So, while doing this particular reaction, how much ATP you are consuming, that is important. How our body is consuming? Why we are taking glucose or any other carbohydrate protein and any other food material, because ultimately we are burning the glucose material or the protein material or the peptides, then you are producing your currency your energy currency is your ATP.

Then one-third, so about 33% of that ATP molecules in mammalian cells are used basically to maintain this particular or the difference in gradient, the differential gradient of the sodium and potassium ion along this particular cell membrane. So, one-third of these we consume in that way.

So, not only the sodium potassium dependent ATPases are known, there are many. When we had the calcium and magnesium we will be talking in our future classes also definitely there bivalent alkaline earth elements, calcium 2 plus and magnesium 2 plus and there are also the

ATPases are there. But remember all the time that we are talking the basic ATPase. But if you do not have the sodium and potassium dependence for this particular transfer, we do not get something, which is dependent on potassium ion or the sodium ion.

When magnesium is coming, and if that magnesium is responsible for the coordination of the diphosphate unit, 2 of these oxygen units on PO and PO, which is coordinating to your magnesium and it brings the strain on the end phosphate unit, such that, you can clip then due to the attack of the nucleophile water.

Then the bicarbonate-based ATPase is also, that means, the bicarbonate dependent ATPases are there, then you can have the typical adenosine triphosphate for all these things, so adenosine triphosphate itself can be hydrolyzed and basically these are going forward the hydrolysis reaction.

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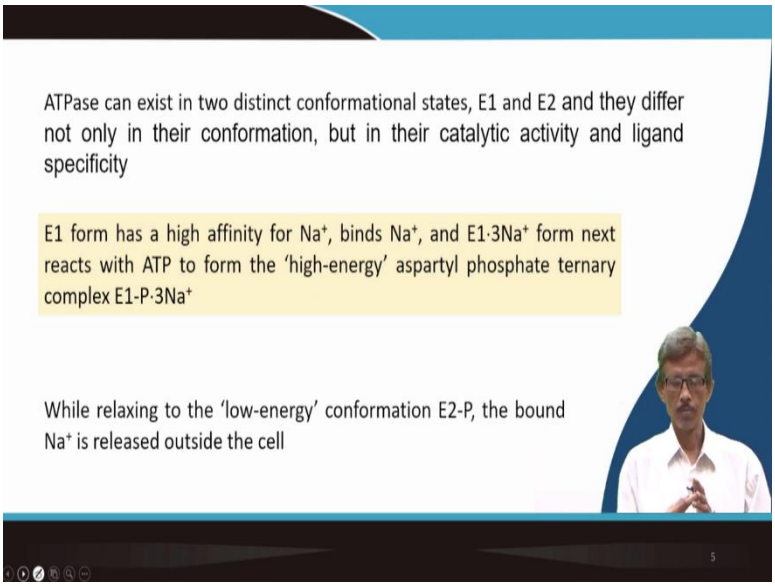

So, how this can present in some other forms. So, if we consider that is not the two energy levels E1 and E2, but you can have the conformational states. People first defined it as some nomenclature in terms of E1 and E2 is not the conformation C1 and conformance C2, but not also in terms of your energy states E1 and E2, but it is the level. The people who are working in this area, they basically leveled it for that particular purpose.

And they differ not only their conformation, that means, the protein structure, the tertiary structure and its conformation, but also their catalytic activity and the ligand specificity. That is why, all these things are very complicated and very difficult also. It is not that one day you

find one structure you will be happy. Because you know only one part of this thing, you do not know the other part or the other structures and other activities for the other species.

So, when it is E1, how this E1 will be related to your NA plus. So, this one particular form the conformation or the structure. The tertiary structure of the protein is such that, it will have high affinity for NA plus, that means, the corresponding binding constant, if you allow to find the corresponding binding constant like your ligand anion binding to your sodium plus like your EDTA 4 minus, we know the corresponding binding cost and for that.

If it is higher, we can consider that E1 form has a higher affinity for NA plus and in a natural way it can bind NA plus giving you E1 center dot 3 NA plus, is not full stop or decimal plus it is centered dot. So, E1 centered dot 3Na plus like the formula when we write 5 water or crystallizations or 3 water of crystallization along with one formula of say sodium acetate or any other species, which will react now.

So, this particular form with the attached sodium ions can react with ATP forming a high energy, as aspartyl phosphate. So, phosphate you are getting, but that phosphate is now attached to the aspartic acid or asparted at residue in the ternary complex. Why we call is a ternary? Binary is these two forms E1 and the NA, the metal ion and E1. E1 form is the E1 conformation of that protein part, huge protein part.

When it goes for the ternary form, and the ternary form is definitely high energy form, which you have the E1, you have the phosphate aspartyl phosphate and then still you have the 3Na plus. And when it goes for the product towards the product side, that means, you move to a low energy conformation state which is E2-P, and the bound Na plus is released outside the cell.

So, movement from E1 form to the E2 form and still you have the bound phosphate now, you can go by that way that E2-P is forming. So, E1P.3Na plus is converting E2-P, and then, while doing so, your attached sodium will be released now. So, that the reaction wise or the mechanism wise you can think in that particular way.

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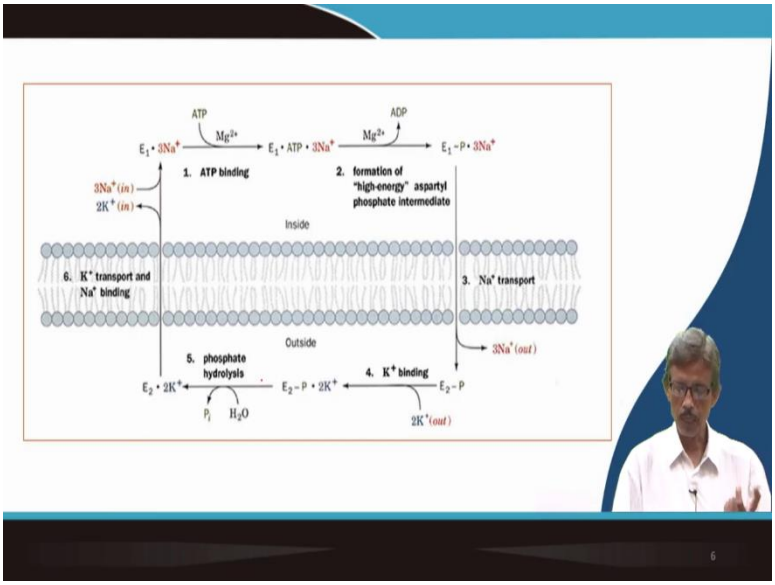

So, this is the whole schematic for that particular case, where you will get all these things. You give some time. So, we are not spending much time on this particular diagram, but you will give all these things. You will have the magnesium, the involvement of the magnesium for ATP binding, that we will again discuss when we will talk about the magnesium. The corresponding metal ion we will be choosing because our next week classes will be devoted to the magnesium, and the calcium.

Then that particular case that the membrane is there. So, you have the potassium transport and the sodium binding, so these are the steps basically. The number one, step number 1, step number 2, step number 3, step number 4, 5 to up to 6. So, this is the whole catalytic cycle starting from number 1 to number 6, where the ATP binding is taking place then the corresponding metal ion binding. And finally, you have the transport of K and the sodium binding. So, that way is basically the whole full cycle is completed and you get the transfer of sodium and potassium, and the expense of the corresponding hydrolysis of ATP molecules.

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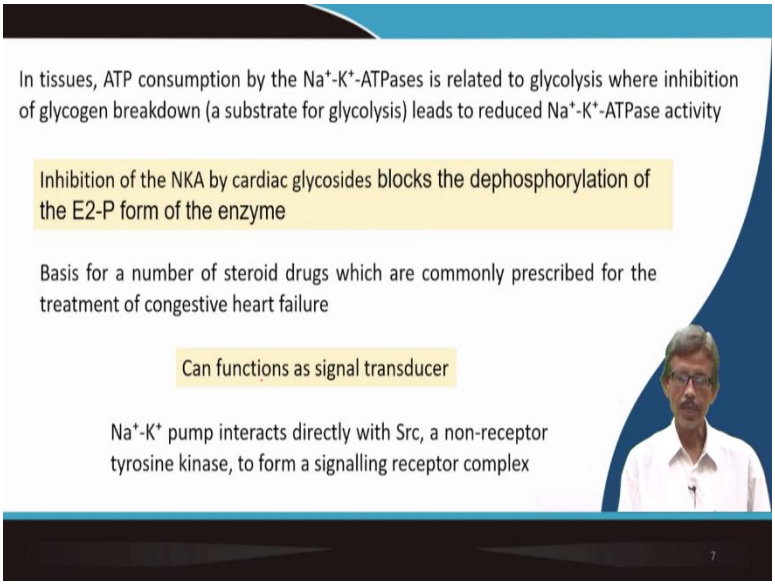

So, in tissues what do you find that the ATP is consumed by these ATPases. Sometimes it can be found that in the case of glycolysis. So, we know what is glycolysis, the glucose bonding process, the glycogen formation and all these. And sometime it is the typical, the glycogen, the polymeric form is going to be hydrolyzed or the breakdown leads to a reduced sodium potassium ATPase activity. So, these particular glycogen process or the glycogen degradation process or the glycolysis can also be correlated to this particular ATPases.

So, inhibition of these NKA by cardiac glycosides basically, which blocks the dephosphorylation of the E2-P form of the enzyme. So, E2-P means, that you have the phosphate group still attached to that particular biomolecule, but you require something where you can go for the dephosphorylation. So NK by cardiac glycosides, so cardiac glycosides can play some role for your D phosphorylation reactions.

In a similar fashion, the cardiac thing since we are bringing it to our attention, so they can also be related to the heart diseases or heart failure or congestive heart failure. Sometime, people prescribe the steroid drugs, and which are basically given for the treatment of this heart failure. So, not only that particular, how it works basically. How the steroid drugs are working? So, they are basically working on this sodium potassium ATPases.

So, sodium potassium pump is there, the proton pump is there, all these things are interrelated. So, all these things will also be required to know by the doctors also for giving a right drug or right medicine for getting all these things. And while doing so, we have seen that since you have the potential and that particular potential membrane potential control

transfer of these two basic ions sodium plus and the potassium plus can also be required, as a signal transducer.

So, it can produce signal, it can excite your nerves, and it can give many different signals for your work. So, these pumps basically sometimes we call is as the pumps also interacts directly with SRC. What is the SRC? SRC is nothing but a kinase or kinase we call. So it is a tyrosine kinase, and kinase is nothing, but is going for the phosphorylation part of the tyrosine residue. Tyrosine residue is nothing but your phenol in it.

So, phenol is having OH function. So, if your age is replaced, and I will be able to attach some phosphate groups at it, then you can have some these tyrosine kinase formation and signaling receptor complex, and finally, it can go for your signal transduction.

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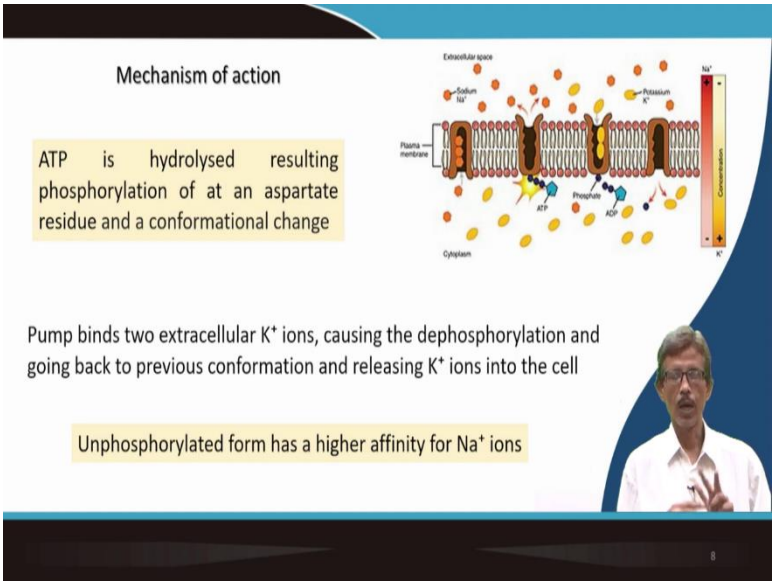

So, this particular figure we know from our very beginning that is there, and the channels are there and the different abbreviated form for this extracellular space and the cytoplasm and the corresponding membrane. And the bigger way we have showing that what are your sodium ions, what are your potassium ions and the ATP molecules and ADP molecules and all these are present, and you have this corresponding on the right hand side the corresponding scale.

The higher sodium concentration and the lower sodium, so it is lighter. And the reverse is also for the lower potassium concentration and the higher potassium concentration inside the cell, within the cytoplasm. So, how we see about? If the figure is given to you, you should be able to say or you should be able to tell now, because we have reached a point where we have known all these things, the mechanism of action of what? Mechanism of action of your

ATPase molecules which are dependent on the relative concentrations of sodium ion as well as potassium ion.

So, the mechanism what we can state immediately with relation to three, four points or basically the three points that ATP will be hydrolyzed. And once it is hydrolyzed, you are taking out one phosphate unit, and that phosphate is utilized for your asparted phosphorylation and there will be a conformational change that we have seen, E1 is moving to E2. Definitely that is a conformational change.

So, that conformational change from E1 to E2 is basically triggered by your ATP hydrolysis and that has again been triggered by the corresponding balance or imbalance of sodium as well as the potassium ions.

So, this particular pumping mechanism what we can consider, so it binds two extracellular potassium ions, then causing the dephosphorylation. So, you have now sodium is binding initially then you have the potassium. And finally, we talk about the phosphorylation of this potted residue then the phosphorylation, and going back to previous confirmation that means, it is changing to again E1, and releasing now the potassium ion, then it will release the potassium ion. So, grabbing the sodium ion, as well as releasing it, as well as the grabbing of potassium ion as release is the typical mechanism what is being performed by this particular machinery.

Then, you have the unphosphorylated form has a higher affinity for the sodium ion. So, the phosphorylation basically will control the amount of phosphorylated form is there between these two ions. And we will also see, we will also correlate all these things related to that of your presence of magnesium ion, as well as calcium ions in future.

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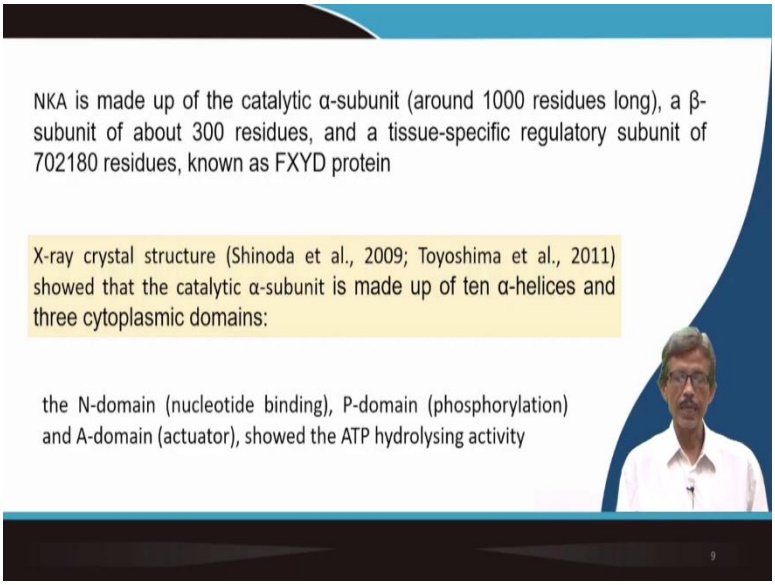

So, it is basically made up of the catalytic alpha subunit around 1000 such units are there, amino acid residues are there, and then beta subunit, 300 amine acid residues are there. And then one particular regulatory subunit, and which is tissue-specific. So, one part of our tissue and another part of our tissue will have a different such machine. Machineries are there, but it is FXYD protein.

And it is very huge one. So, it is 702180 residues, amino acid numbers will tell you how big it is. So, the tissue-specific regulator is there, that means, it is a tissue protein. How people identified all these things, all these regions and all these areas basically or all these domains which are important? So, critically if we want to know that so, it is not a very old story, it is only 10 years old story when Shinoda in one paper they reported it, and again the final one can be reported as reported by Toyoshima in 2011.

So, crystal structure has told us that, okay, we know about these alpha subunit having 1000 residues of amino acids. Then these 1000 residues are basically divided into 10 alpha helices. That means, one helix is composed of 100 amino acid residues, and three cytoplasmic domains. So, you will have these three cytoplasmic domains and those domains basically, why we are going for this typical x-ray crystal structure.

Not only about the different helices, amino acids, all the different amino acids, but specifically we try to locate all these different domains. And depending upon their function and their action, we can find out that we can have all these domains nicely in our hand, such that, you can have N-domain, and we can also consider that it is the N-terminus end, but it can have some other biological origin of naming.

We should not go there, we should not bother on these things, but you should have the abbreviated one such that you can differentiate like we define XYZ or ABC or PQR. So, A-domain, P-domain… N-domain P-domain and A-domain one is required for nucleotide binding, another is for the phosphorylation what we are discussing right now, and another is the actuator. So, all these together can basically giving for your ATP hydrolyzing activity.

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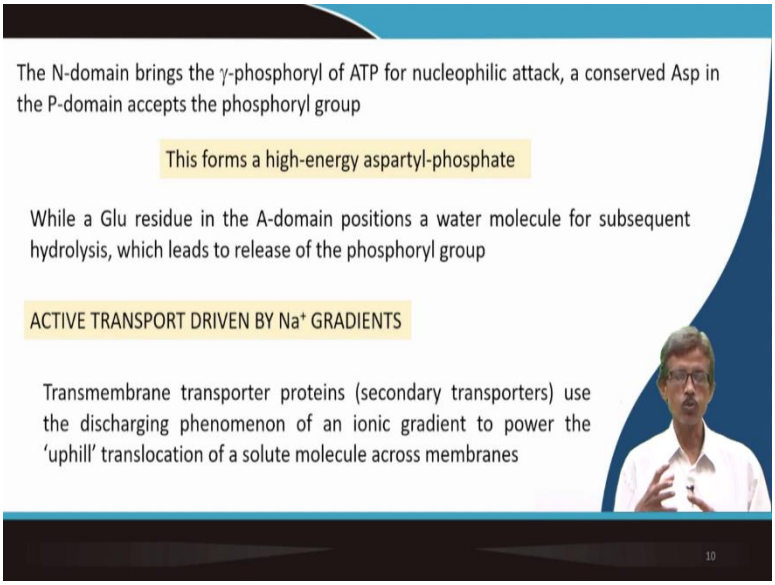

And this end domain basically brings the gamma phosphoryl group, that means, alpha, beta, gamma the third one of ATP for the nucleophilic attack a conserved asparted in the P-domain accept that phosphoryl group. So, that is why you have the phosphorylation reaction, and is high energy there.

So, I already told you that the high energy form you have, and another one is the low energy form. So, this high energy form is your aspartyl phosphate group bearing thing, then the glutamate residues. So, until and unless you determine the entire structure and specifically locate some point where the amine acid residues in that particular domain is basically giving that particular function.

The way we know that the amine acid residues or the peptide carboxy function the CO function is required for your corresponding interaction with the metal ions. So, the glutamate residue is for the A-domain positions a water molecule is also required for instance, a water molecule for subsequent hydrolysis because you have to abstract or you have to take up the nucleophile which is your water, and then, you have the corresponding release of the phosphoryl group.

So, what we find that the active transport, which can be driven by sodium gradients all as we know from our very beginning of all these classes of this week, that we are talking all in terms of your sodium gradients, that means, the differential sodium concentration.

You have, therefore, the transmembrane transporter proteins we call them the secondary transporters. They can also be used for maintaining that ionic gradient, and also power the pump for uphill translocation of the not only ions, but also the different other solute molecules.

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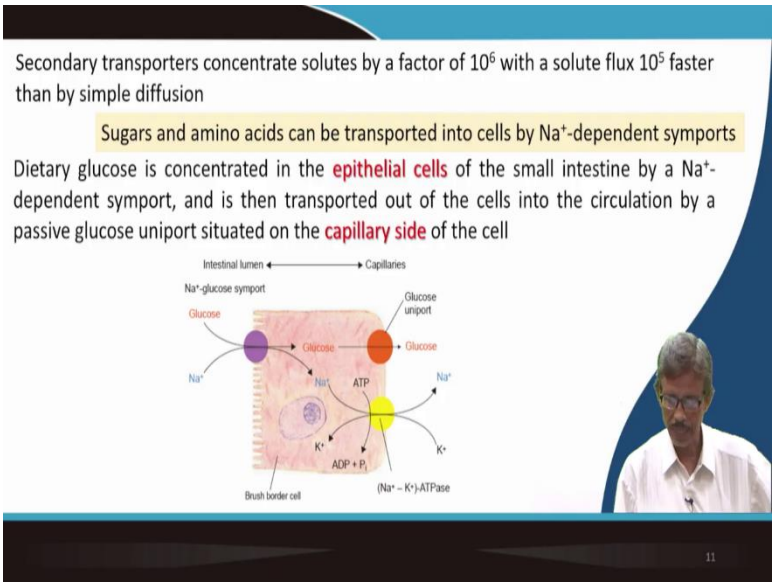

So, these secondary transporters basically are very important, because they go for a solute concentration change by a factor of 10 to the power 6. You see they are so efficient. It is very difficult sometimes to move 10 times higher or lower. And a solute flux, which is 10 to the power 5 times faster than the simple diffusion.

So, only the diffusion, if we consider that, no, nothing is there, no pumping mechanism is there only it is permeable like osmosis through the membrane for simple diffusion, but it is not so. You require a very high rate. So, the rate of the reaction should be very high other we cannot have the signals, we cannot work and we cannot talk even for taking these sort of classes also.

So, the other two ingredients like sugars and amino acids can be transported through cell through these molecules as sodium dependent transport. So, when we have the dietary drugs and its concentrations in the epithelial cell, how these are absorbed. So, sodium dependence symports are there and then the capillary side, the capillary, where the capillaries are there

which are responsible for the taking up the sugar, taking up the amino acid, taking up the glucose.

So, this is a big one which can have the interstitial rumen and all these things. So, the glucose import is there and the corresponding involvement of your sodium potassium ATPases are there.

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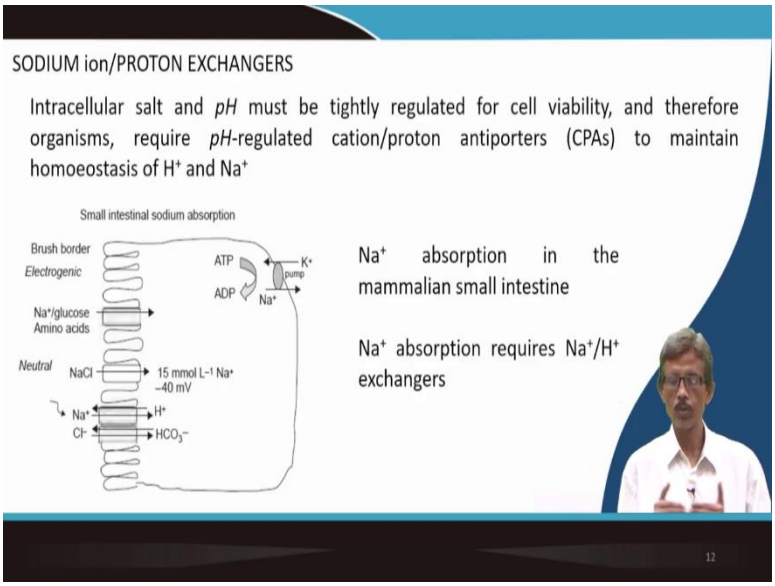

Then finally, we have seen, we have to see that the proton exchangers are also there, and the pH can be regulated. Do not worry for all these, we will come back again and again in other classes also that how the proton can also control, the proton exchangers can also be involved in your corresponding calcium exchange or the magnesium exchange.

So, right now, you should be accustomed with this particular case that you have the small intestinal sodium absorption where we go for the corresponding absorption of sodium, where you are consuming a sodium chloride, and how much sodium chloride is being absorbed by your intestine.

So, the sodium absorption in the mammalian small intestine. We all know, we are taking sodium chloride, but now we know how sodium chloride is being absorbed by your small intestine. And the sodium absorption basically requires also the exchange of the sodium ion and the hydrogen ion or the proton that is why the acidities and all these things in our gastric juice is also dependent.

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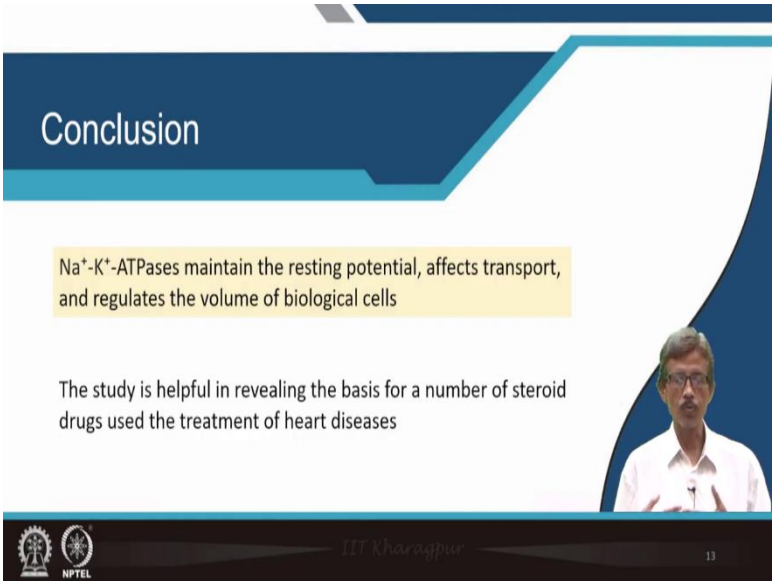

So, now, we should conclude here, as that, we are studying these ATPases for monitoring the resting potential, for transport mechanism and regulation of the cell volume also. And this can also be helpful for knowing something, which is having some medicinal aspects or medicinal chemistry aspects, that some drugs and how they are important for some diseases and how they are related to the corresponding balance of your sodium as well as potassium.

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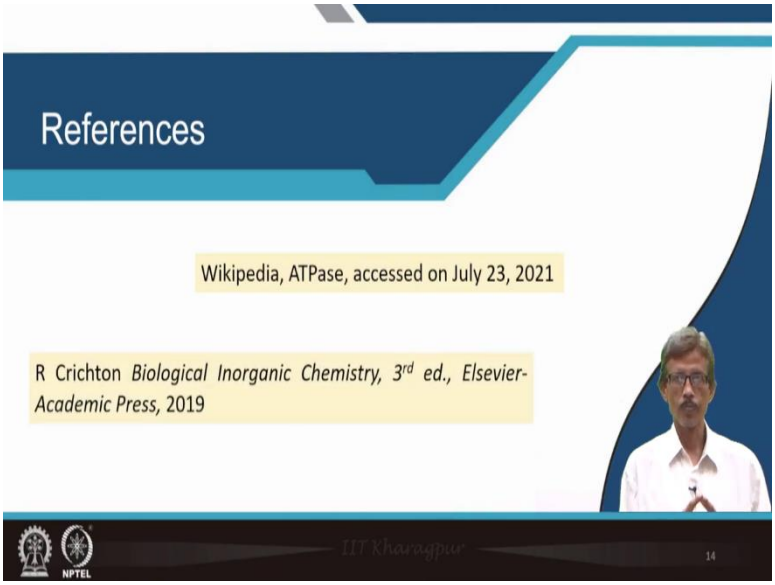

Finally, the references. Again, we just only focus our attention only two parts. One is the Wikipedia part, and another is your book part, which is your Crichton's book. So, thank you very much for your attention.