Biological Inorganic Chemistry
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Lecture 26
Module 06: Phosphate Metabolism and Cellular Signaling
Mg 2+ Dependent Enzymes and Kinases

Hello, good morning everybody. So, where we finished last time from there we basically tried to continue and is a different module is module 6 now, and we have reached to lecture number 26 and after sodium and potassium will now bring you or give you some sense on the role of the two other metal ions.

So, firstly three lectures will be devoted to magnesium two plus and the remaining two will be on calcium two plus. So here in this particular class, what we want to see is that a very important thing when our body or any other living system undergoes is the phosphate metabolism.

And sometimes we find that this particular metabolism is also directly related to the signaling process of the different cell systems. So, definitely we will talk about the magnesium two dependent enzymes and kinases. So, this typical term how we define and how we can understand about the different kinases we will see.

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So, in our human body, how the different amount of magnesium is getting distributed, as we have learned so far that how we can consider about the corresponding concentrations of sodium as well as potassium within the cell and outside the cell that means, this concentration

is also very much important in understanding their relative amount what is available within the cell and outside the cell and how these important metal ions are particularly for magnesium two plus, because we do not know much about the magnesium chemistry magnesium (())(2:13) chemistry, apart from main group metal ion chemistry and organic chemistry starting with that of your important (())(2:21) reagent.

So, magnesium dependent enzymes in glycolysis processes and how we can introduce, so, we are just separating out this introduction of the phosphate anion or the removal of the phosphate anion here we will only consider for kinases and others the introduction of the phosphate groups.

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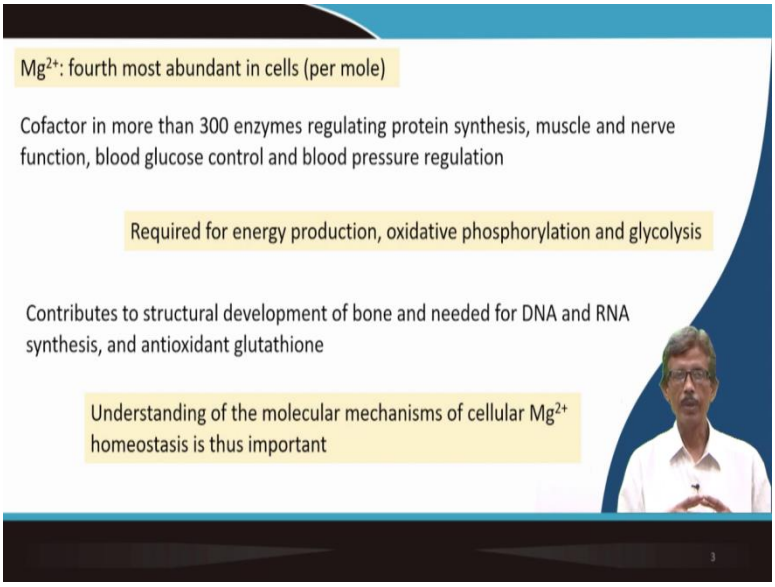

So, if we consider that apart from our the relative abundances, what we have seen last time, that the carbon, hydrogen, oxygen and all these and the different metal ions, so, in terms of that magnesium is the fourth most abundant metal ion, which is available within the cells in terms of its per mole concentration.

So, more than 30 enzymes so far has been discovered. So, it is very interesting that earlier we are talking about that we know about 300 or more or 500 enzymes based on zinc, we know that zinc is the most important and the most essential metal and for our system. And nowadays, we take gene supplements, zinc food supplements and genes in terms of their different medicines.

Now, if we just allow, because day by day, we are just enriching our knowledge in terms of these enzymes how they are fully characterized, whether you have identified the presence of

the magnesium ion or not and there are different reactions and functions. So more than of these 300 enzymes, the cofactors of all these cases are required basically starting from the protein synthesis, very essential part of our survival for our livelihood, then the muscle and nerve functions.

That means during that binding or during the movement of these manganese magnesium based different biological molecules, we will find that the nerve signals can be transferred, then two more important thing, what we all the time we are bothered about. So magnesium is important in controlling the blood glucose amount as well as blood pressure.

So finally, also we will see that it is required because these are the importance's where we can think about where we can think about their control, but what is the actual reactions? The direct involvement of the, that magnesium ion magnesium two plus for the different reactions, so we will basically talk about three things, one is your energy production.

Obviously, we will be knowing when I talk about the involvement of magnesium for energy production, definitely we are talking about the ATP related thing. Because we know that the ATP is your corresponding energy currency. So, we can produce for its energy can be obtained from the system if we go for the hydrolysis or can be consumed from other food material or the glucose material or the carbohydrate material for its production.

Then it whether you have the oxidative phosphorylation reaction, so, oxidative phosphorylation reaction that basically also drives many many reactions in our system and the glycolysis which is the most fundamental reaction for the glycogen synthesis and the glycogen degradation also.

Apart from its major contribution for bone formation, and the structure development, it is also needed for two other very important molecules like DNA and RNA. So, magnesium dependent DNA RNA synthesis, we should be knowing and also the synthesis of antioxidant glutathione in our body.

So, this typical understanding for the molecular mechanism because how they are working, so, we will not be able to go in detail of all these things, but we will focus our attention on the magnesium binding, magnesium interaction and magnesium related coordinate chemistry because we want to know about the initial distribution of the metal ions in the environment.

That means, the magnesium two plus homeostasis is therefore, important because your if your cell is not having the enough amount of magnesium for its all these activities, you will have some problem.

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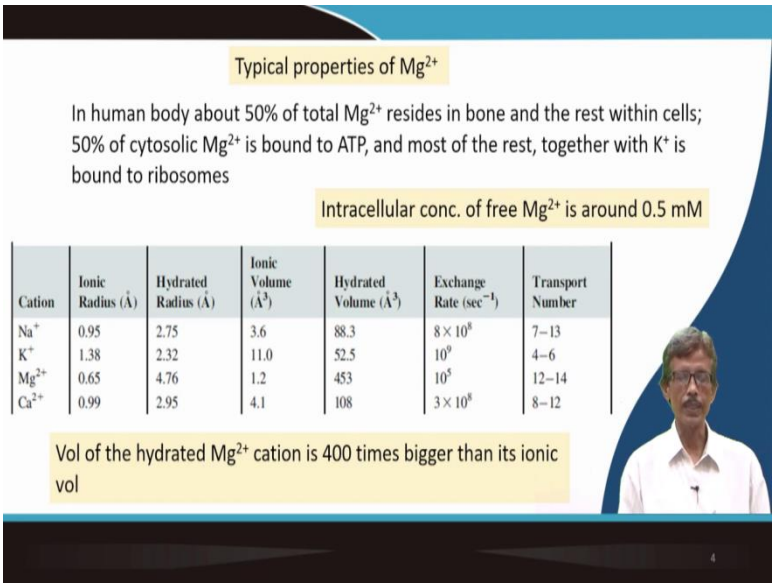

Cation	Ionic Radius (Å)	Hydrated Radius (Å)	Ionic Volume (Å^3)	Hydrated Volume (Å^3)	Exchange Rate (sec^{-1})	Transport Number
Na^+	0.95	2.75	3.6	88.3	8×10^8	7–13
K^+	1.38	2.32	11.0	52.5	10^9	4–6
Mg^{2+}	0.65	4.76	1.2	453	10^5	12–14
Ca^{2+}	0.99	2.95	4.1	108	3×10^8	8–12

So, what we see now is your the different properties, so, not that the metal property, but the metal ion properties, which are very important. So, when you have the magnesium because everything will be getting from our food source or any other sources, we will be getting magnesium as the magnesium ion.

So, 50 percent of the total amount is basically residing on bones and remaining within the cells. So, 50 percent of the of that again so, 50 percent in the cell again another 50 percent of that of the cytosolic magnesium two plus is in the bound form. So, you see how good is in terms of its corresponding coordination or the correlation to the diphosphate unit of your ATP molecule.

So, that is the most important part of knowing the magnesium related coordinate chemistry or bio coordinates in chemistry or the biological role of magnesium we can find out that the phosphorus oxygen bond two thirds phosphorus oxygen bonds from say ATP or ADP molecule, so, that phosphorus oxygen that oxygen basically which is bound to already to phosphorus can function very good amount of coordination.

So, we know that that phosphorus the phosphate in itself where no oxygen is there, the tri phenyl phosphate and all are very good ligands to the metal arms. So, similarly, the ATPs

would also be a very good ligand for these magnesium ion and the remaining 50 percent along with k plus is bound to the ribosomes.

So, the distribution basically we should have some very good idea about what are the is distribution because people will be talking about and people are doing research also on identification or the monitoring of the magnesium two plus ion how in your cellular conditions or your cellular cells basically, how you can detect the presence of magnesium two plus.

If somebody says then I know that the concentration is point five millimolar concentration within the cell. So, how can you find out so, there should be some technique spectrophotometric or spectral fluorometric techniques which can be there, which can basically give you the corresponding informations about the total concentration of magnesium.

But before that, already we have seen the two other metal ions the mono valent metal ion like sodium ion as well as the potassium ion and in our the last two classes of this module basically are devoted to calcium two plus. So, if we consider all of them all four together and we will all know like your periodic table, the electronegativity values the ionization potentials values all these things you can have also the table for ionic radius.

So, these ionic radius what do we get here is that sodium potassium, magnesium and calcium. So, if we consider that how they are very you see if we consider simply the ionic radius of sodium plus which is 0.95 compared to magnesium it is only 0.65 angstrom. So, whatever you are getting used to the charge basically, so, due to the charge your size is less and which is less than that of your sodium.

So, so far whatever we have learned about the typical coordination chemistry or bi coordination chemistry with respect to sodium as well as the potassium whether that can be applied to magnesium or not we typically see this particular case also, whether it will be possible to substitute some of the positions of magnesium because we know that typical presence of magnesium is important for its actual function, if we substitute that magnesium by some other metal ions, the calcium or sodium will not be able to extract out that information or that particularly activity.

So, you see, the smallest one having by positive charge to plus positive charge is very important. That is why it is the hydrated radius is also very high is 4.76 on stone. So, is

basically gather more number of water molecules. So, the smaller size is important is not that it will just can go for the binding it can function as a very good ionophore to that of your corresponding crown ethers or cryptids.

But, these two quantities are smaller, typical ionic radius and the hydrated radius bigger hydrated radius. So, you can have also the ionic volume and the hydrated volume also hydrated volume is also important hydrate radius as well as the volume which is 453 angstroms is a very big amount.

So whenever, they will be moving for cellular signals or going to the ATP side or going to some other engine side, we will find that this particular volume movement is important. So, you have to allow for that much space to move the hydrated magnesium two plus ion to carry that particular ion in terms of that much big volume of five 453 angstrom then the exchange rate is also not very high, but it is also 10 to the 5 order and the transport number.

So, these all these fundamental quantities which we can tag around magnesium tubeless are important in understanding these functions, because what do we see we see the functions, we see the mechanisms, but we try to understand in terms of its corresponding the activities or the properties in terms of the magnesium two plus ion.

So, we have seen here that the hydrated volume is very big and is four times bigger than its ionic volume. So, always that is why the properties and all these things are important when you talk about the synthesis in the laboratory the synthesis of say (())(12:26) reagent, the methyl magnesium bromide or methyl magnesium iodide.

So, R Mg x we can talk about the reactivity of that magnesium in terms of his metallic reactivity in the metallic condition with that of your Rx, but here since you have already the ion on so, never forget these are a MIs. So, we are thinking we are talking all these in terms of the MIs, the metal ions.

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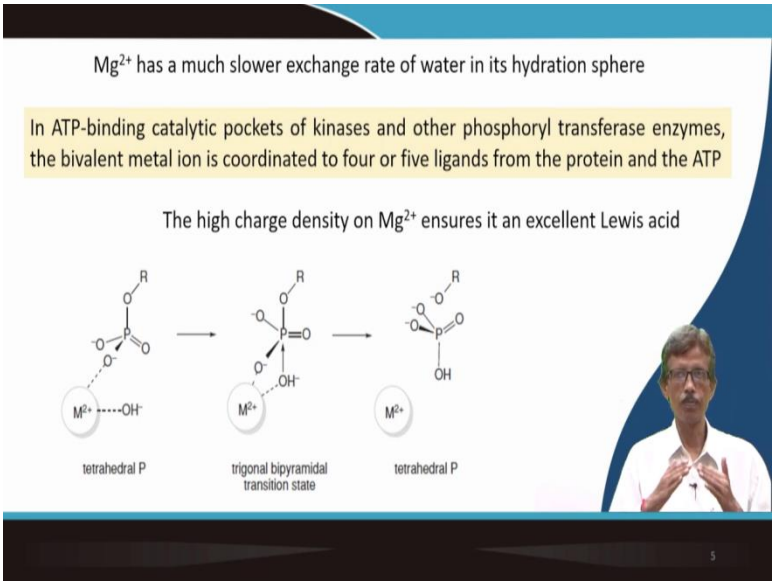

So, what do you see now, that the magnesium two plus has a much slower exchange rate of water in its hydration spear. So, since the charge is very high, so, it will be tightly bound. So, the exchange rate will also not be very high and it is you will give you a slower exchange rate.

Now, you see that already we have discussed already we have seen and we are able to understand that the diphosphate part. So, the triphosphate part of ATP molecule having two oxygen donors on two phosphorus ends, so, P O and P O or P O minus and O minus charges are there. So, either you can have a charged bidentate like an having to minus negative charge or a neutral P O P O coordination to the metal ion because the charge will ultimately balance you can have the corresponding delocalization of all these charges.

But, we should know that the hard oxygen donors from the phosphate units are coming and binding to the hard magnesium center which is smaller in size also. So, you have the typical catalytic pocket and if the ATP binding pocket is available over there in that catalytic pocket and magnesium can come and bind to that particular pocket.

Then other phosphoryl transfer is enzymes so, phosphate binding, activating hydrolysis and transfer. So, those are your phosphoryl transfer is enzyme. So, the phosphate group basically will be able to move from one point to the other by the use of your magnesium two plus ion. So, when the magnesium two plus ion is there now, we can think of we can go back to your typical inorganic chemistry or coordination chemistry books or classes that what is your coordination number.

If I ask a very simple question related to that example, what I am giving because they are formula school days you are studying the grignard reagent. So, grignard reagent we know we have studied many reactions and all these things but if I ask you a very simple reaction not that in a solid state if you are able to make it or synthesize it in the solid state, but in solution, when you will be doing some reaction with some substrate or some reagent, you are RMG x. So, you have taken in some solvent. So, we are ethyl or THF tetra hydro furon we usually take. So, in that particular solution, what is the coordination number of magnesium two plus when we dissolve that Grignard reagent in that particular solvent.

So, coordination number is always important whenever you think of a metal ion whether the environment is a biological environment or a protein environment or your metal ion is present in your test tube in presence of water in presence of other solvent like little bit complicated solvent like dmso, because, we have seen that the dmso can give you some environment some kind of corresponding typical environment which is very much similar to that of your protein environment.

So, either four or five so, it can be either tetra coordinated or penta coordinated. So, you must have the corresponding number of proteins donors or the ATP donor groups like oxygen sub the phosphate units can be available and can come around to your magnesium and it will bind to that particular magnesium center.

So, coordination number is always important. So, in this particular class basically when you want a master in coordination chemistry, you will always think in terms of the magnesium two plus as if you are saying that I am only seeing first the magnesium coordinates and chemistry. So, this charges there and high charge density definitely as I told you compared to your sodium and potassium, it is giving a corresponding very useful Lewis acid corrector.

So, whatever phosphate you just simply give any phosphate group so, it is basically alkyl phosphate or phosphonates you can have So, R O groups are they R O can be your typical water molecule like that of your phosphoric acid, R O can be your art is your alkyl function for alcohol, it can be your glucose function or it can be your ATP also.

So, you have the magnesium two plus and it will be interacting with the O H or O minus only. So, if some charge from the OH ion, your hydroxide and so, the interaction is basically the typical hydroxide type of thing. So, one you can have the one coordination side can be occupied in this tetrahedral phosphorus.

So, the phosphate is present is that it redl one and is try to interact with the magnesium center, but that magnesium center already we have seen that it can have very many number of bound water molecules. So, those water molecules that they are and at some point that water molecule having some different PK Hello compared to a free water molecule. So, it can go for deprotonation.

So, if it goes for the deprotonation if some bases also available in the protein pocket quickly it can go for your hydroxide bound form and that hydroxide basically when it is activated by the metal ion and it is under control of the phosphorus center also can now form a strong bond in the second case, when you have the trigonal bipyramidal transition state.

So, when you have the trigonal bipyramidal transition state the original O minus can still be bound to your magnesium two plus center, but it can lose then up the size that finally it can transfer the already bound hydroxide to grow from the middle and center to the phosphorus center and you have the movement of the umbrella on the reverse side.

So, earlier you have this particular say and then it is moving in that direction. So inverse and is taking place around this phosphorus center again we are getting back the corresponding tetrahedral phosphorus.

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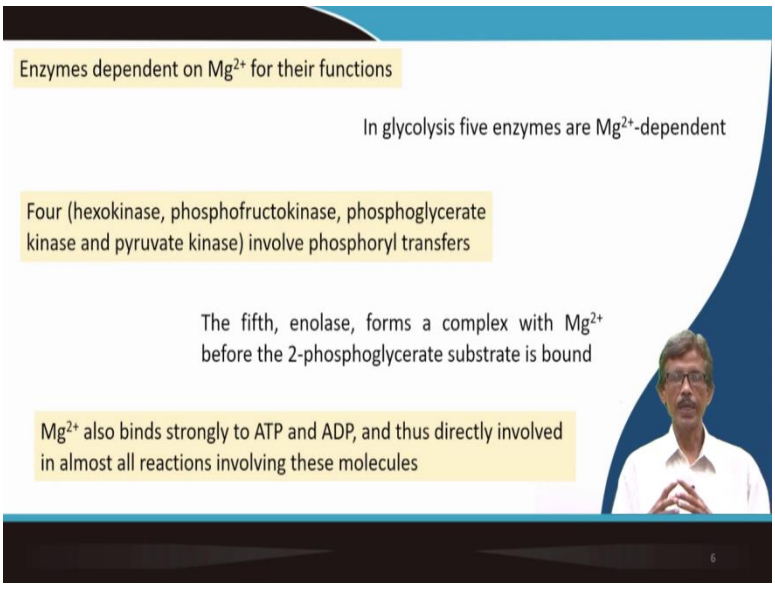

So, let us see quickly what are the enzymes those are basically dependent on magnesium two plus for their functions. In glycolysis, we have five enzymes, which are dependent. So, if you can have 10 or more number of these at least five you can have for this particular reactions, then four out there and these four we have we tagged them for the different reactivity

patterns, but the basic reaction is your typical phosphate group reaction for hexokinase possible fructose kinase, then phosphoglycerate kinase and the pyruvate kinase.

So, all these cases they are all kinases. So, these kinases are basically responsible for the phosphate group transfer from one to the other. Then we will see also the acknowledge in all so, in that means the double bond is there and OL is there. That means the hydroxy group maybe is there already. Like keto in all tautomer ism we know from our school days, the keto enol.

So, if you have a keto profile as well as you have the double keto groups also that can go for the IN OL formation that tautomerization to IN OL. Similarly that OL O is deprotonated you get the in all its function. So, that can be stabilized basically is not that form is forbear that form we are getting, but you have to stabilize through middle and coordination.

So, as we have seen that it is binding to your ATP and ATP molecules, and that is why you can consider that they are basically involved in all the reactions, where we see that ATP and ATP molecules are there you cannot see that , we had ATP and ATP molecules only without magnesium because in almost all cases, you will find that the activation of the ATP molecules or the stabilization of that ATP molecules required the presence of magnesium two plus.

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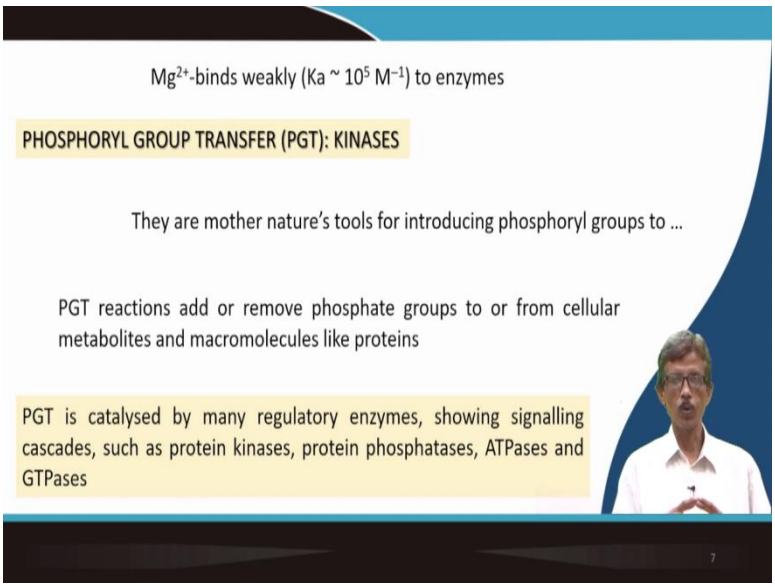

But in all these cases, there is no such strong binding unlike your transition metal ions like iron or copper the binding of magnesium two plus two the enzymes and their corresponding polypeptide donor groups or the donor at home is relatively weak and only the corresponding

association constant or the corresponding formation constant of the corresponding complexes is also less is only 10 to the 5 mole inverse.

Then, we just simply confined to say that we can have the PGT in kinases what is PGT? PGT is nothing but your phosphoryl group transfer reactions. But the mother nature's tool basically the mother nature's weapon or rather you can say it is tool or weapon for introducing the phosphoryl groups to is basically introduction not the removal in case of phosphotases not in kinases, and phosphatases.

We will talk about the removal of those phosphate groups. So, we're from the PGT reactions are basically important to add and remove the phosphate groups to or from the cellular metabolites when we are metabolizing glucose, some intermediate we get basically out some corresponding glycogen assimilation, we can have something where you have to have go for the corresponding attachment of the phosphate groups or sometimes the macromolecules like proteins can also be phosphorylated.

So, this sort of reaction is catalyzed by many regulatory enzymes, which can also give us the corresponding signals such as protein kinases, protein phosphatases ATPases and GTPases. So, you see not only these are very important for these kinases reactions, but is also important for many enzymes to functions because the proteins are having OH functions, either that OH from is coming from the alcohol end or is coming from the phenol end and you can have also the core different the fire dinucleotides.

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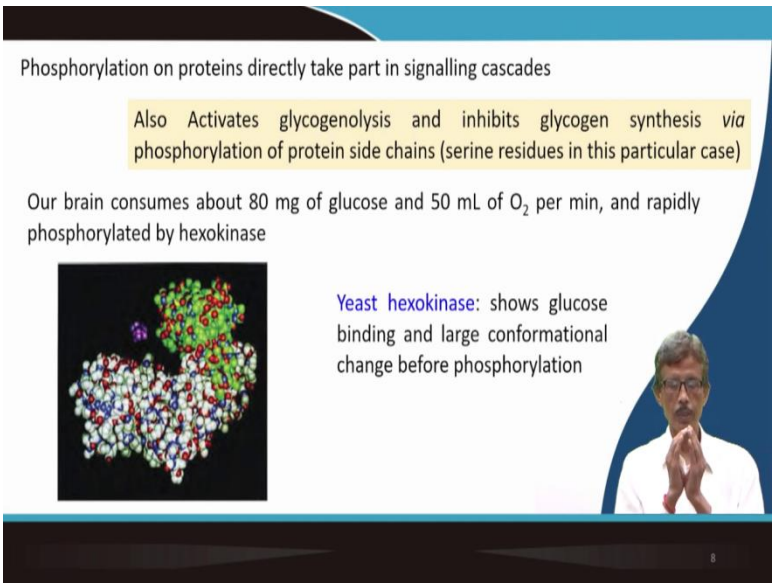

So, when you go for the phosphorylation reaction on proteins, it directly takes part in the signaling process. And it also activates the glycogenolysis that means, the hydrolysis and innovates sometimes also the glycogen synthesis by a phosphorylation of the protein side chain, what is that side chain, it is the alcohol end there is just no I told you that alcohol and of the serine minor acid residue.

So, serine has the alcohol function OH function is there and that OH each function can be phosphorylated. So, that is the basic idea. So, whenever we are talking that you have the serine function, whether it can be phosphorylated or not that serine has a very useful functions in the enzymatically wild also in absence of metal ions some time.

So, when our system is basically consuming these two together glucose as well as the oxygen molecule, the amount is 180 milligram and amount is 50 milliliters per minute. So, you see that our system is always we are supplying the fuel and we are supplying the oxidant your glucose is your fuel and O 2 is your oxygen and that is the quiet for the phosphorylation of the hexo kinase.

So, hexo kinase you have you see, this particular group is coming in the pocket basically is entering their strike to move there. So, what is moving there so, that we will see in yeast hexo kinase why how people have studied all these things is very important. And people have studied all these nicely on some model system model life living system, which is your yeast.

So, is yeast hexo kinase basically solves the glucose binding so, glucose molecule is coming. So, it should have the bigger hexo kinase unit and when it is coming and then finally binding to that particular pocket, so, it is in the cell it can be open up and it can close also a little bit when the glucose is entering and there must be some conformational change before we think of the corresponding the accent which is your phosphorylation the reaction.

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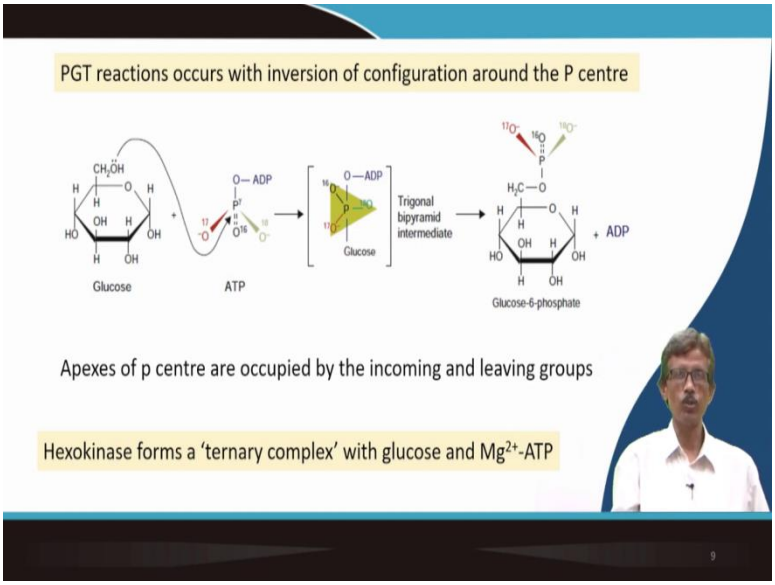

So, these phosphorylation acid reaction can occur from the inversion configuration around the phosphorus center we have also seen is now is another typical example I am showing you, you can have alcohol you can have water also because that the very basic coordination environment around the phosphorus which is important is your trigonal bipyramidal environment.

So, your glucose monitor is coming with your CH 2 OH like your serine can attack your phosphate unit and the phosphate unit and all oxygens are substituted by your the different types of oxygen centers. So, that basically gives us a different character of these and we can find out the inverse and pauses also.

So, what we are doing we are going for the glucose six phosphate how quickly and how easily that can be phosphorylated by your ATP molecule. So, in ADP bound to one phosphate It is nothing but your ATP molecule. So, it is basically the transferring, so, this basically the glucose is taking up there. So, already we have the ATP energy rich molecule in our hand. So, the two epochs is not small P is capital P center is the phosphorus center is occupied by the incoming and living groups.

So, one is your incoming group and another is your living group. So, glucose is coming and these ATP is moving basically as from the transferring one phosphate unit to you.

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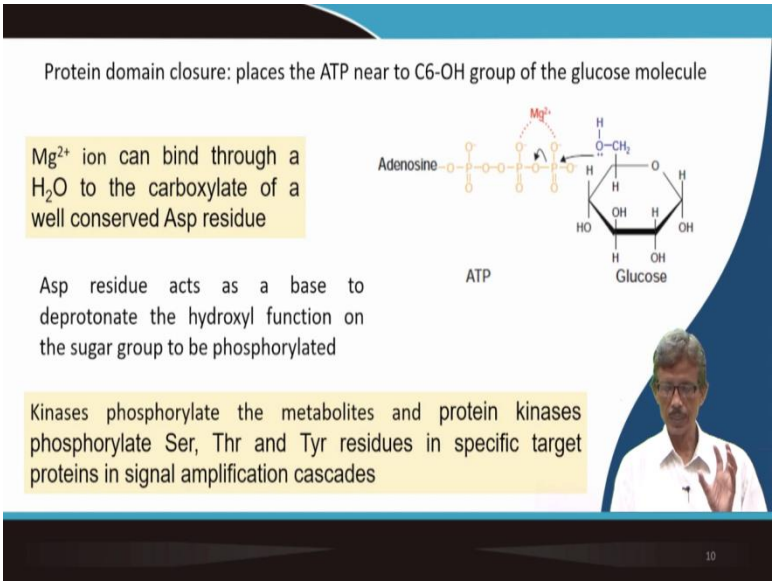

So, you are getting a ternary complex out of that when you have the interaction of the magnesium two plus with your ATP molecule. So, what do you see now that the protein domain close that so, glucose is entering, so, you will have the protein domain closer places the ATP near the C 6 OH function of the glucose. So, you have that so, how you attacking that So, I that is why they are in these two phosphate units are bound to the magnesium is basically binding through a water molecule to the carboxylate of a well comes up asp residue.

So, asp residue is there and that aspartate residue is important which acts as a base to deprotonate the hydroxyl function on the sugar group. So, sugar, the glucose unit you have. so, if you have that aspartate residue, if we can deprotonate that you need so, instead of OH it is O minus, which will be required for your phosphorylation reaction.

So, the kinases phosphorylate, the metabolites and the protein kinases phosphorylate the siren unit, the only new unit or titles in DC do so, all are having OH function either alcholic OH or phenolic OH So, those are basically phosphorylated there is no problem. So, only you have to find out which amino acid in the protein chain can supply you the function and that it functions can be phosphorylated.

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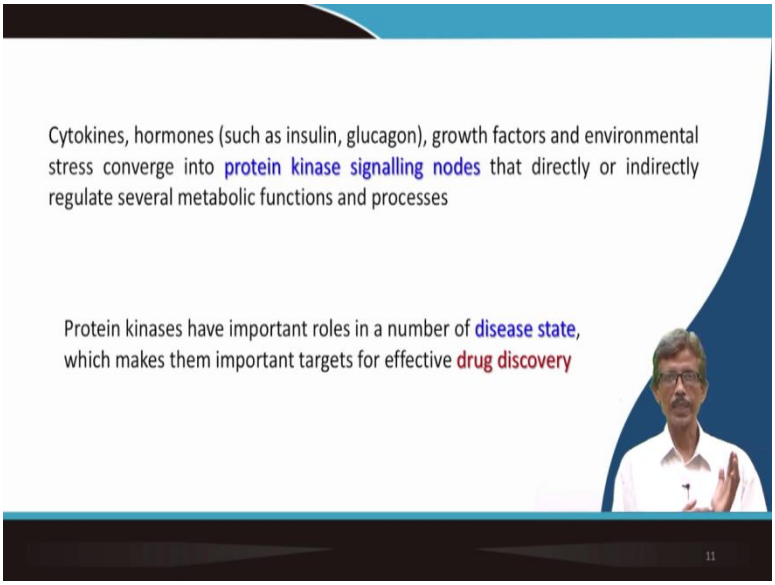

So, note that many other important molecules in this cytokines, we know all about cytokines, cytokines are also can be related to this particular one hormones that they are because all these are important several growth factors are for protein kinase signaling nodes, because we have to go for the signaling processes that these hormones this cytokines and all can be activated and give some important property or the function.

So, these protein kinases have important roles also forced in several cases the disease state which makes them important targets basically for effective drug discovery. So, if you consider that we can stop the function or we can enhance the activity of these protein kinases by something by some molecule which can go and inhibit these functions, we can have something which can be developed as a drug molecule.

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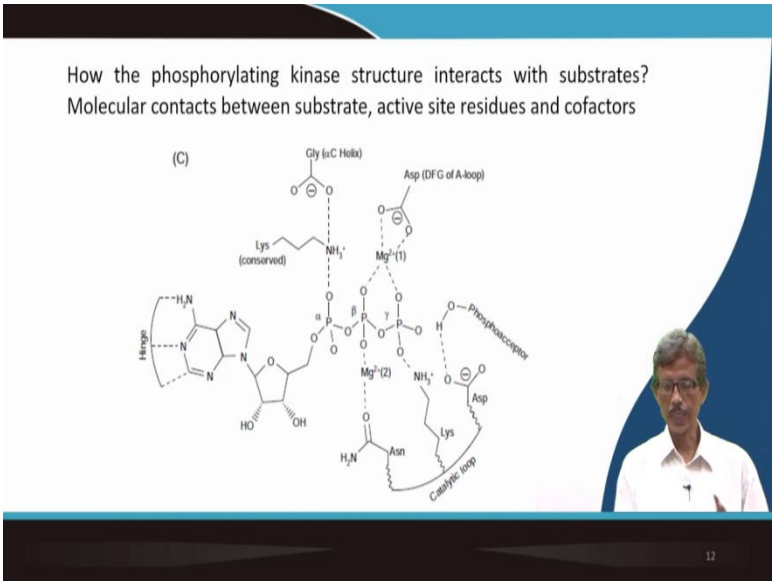

So, this phosphorylating the kinase structure interacts with the substrates how? We have to know about the molecular contacts between the substrates and the active site residues and the different cofactors that is a very important thing. So, three dimensionally from this particular structure so, it is basically the visualizations you can close your eyes and try to visualize is the thing that you have the magnesium something is coming and binding to your magnesium but above all you have the substrate molecule.

So, being substrate molecule will also come and where it will come it will come in the active side center and the different cofactors are also there. So, from a big diagram what we try to understand you see now, commonly that you can have the left hand side you have the hinge and hinge is basically taking care through some hydrogen bonding interactions on the nucleotide base.

So, at any part of the base and on the low or do you have the catalytic loop catalytic loop is supplying some amino acid as you do so, side by side s and L is an ASP So, my request to all of you that whenever you see these figures try to understand is what type of amino acids we are talking about.

So, you should be knowing the corresponding abbreviated form of ASN LYSN and ESP. So, SP we all know the esparted groups. So, that is why we have esparted acid group and esparted anion, which can be phosphorylated. So, the first part at when it is bound to that OH function, which is nothing but your phosphoacceptor.

So, separated of that group apart from the other two amino acids within the catalytic group is therefore, important to channelize this thing because one magnesium at the top which is your magnesium one, which is important and apart from that, you can have another magnesium two, which can have some role, but is not clearly identified till date.

So, the interactions not only with the phosphate the phosphate and ions can also stabilize this particular lysine residue which is a conserved residue through only hydrogen bonding interaction. So, not only coordination the dotted lines we are showing as the coordination bonds also, sometimes if you have a very strong bond and at the bonding distance the coordinate bonding distances around say 1.9 or 2.1, we cannot consider these as the typical bonds.

So, these are the interactions like your hydrogen bonding interaction. So, it can be a little bit stronger than hydrogen bond interactions, but it can be also weaker. So, because you just in a transient moment you go for these interactions and then the magnesium will be leaving that particular place once the reaction is achieved.

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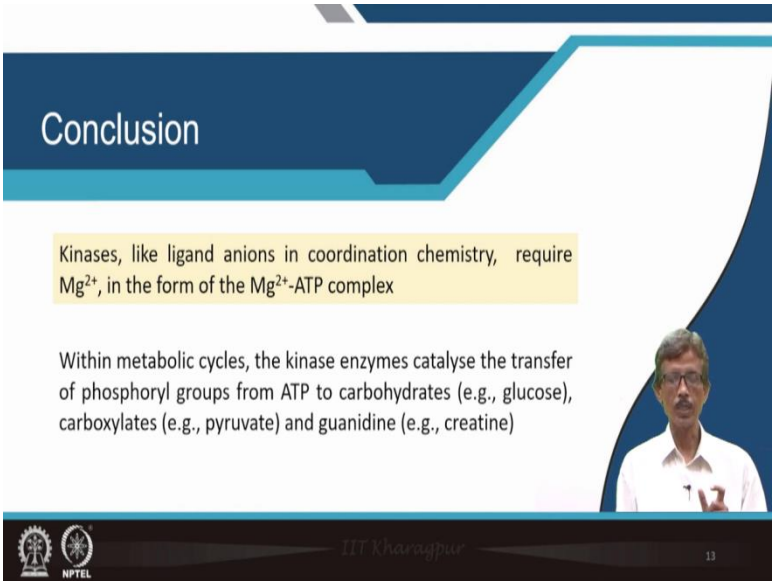

So, the conclusion part what we have seen what we have noticed in this particular class is that we have studied about the kinases you should be able to define what are kinases and how these kinases are important, since the charges are there on the phosphorus oxygen bonds, and the O R can be neutral with P O double bond or sometimes we do not write as P O double bond with it the coordinate bonds because these are all basically when you determine the structure, they are having the phosphorus oxygen single bond distances.

So, either the charge oxygen or the neutral oxygens all in between can give you the corresponding neutralization to your magnesium two plus center and it can form or it can bind to that of your ATP complex. Then the different metabolic cycles, the kinases are important the kinase enzymes are important which are basically catalyzing the same reaction, we are talking all the time about the phosphate group transfer reactions.

So, groups from ATP can be transferred to the glucose or any other carbohydrate molecule can be transferred to the carboxylates like aspartate. Now, you can have the pyruvate So, the Pirate Bay guys is cycled, we all know and then the guanidine is a very important molecule another type of molecule you can read it, I do not have that much time to take about a talk about all these things, but what is guanidine and what we can get it so, if you have keratin, so keratin factor that is there in our system so that keratin can also be phosphorylated.

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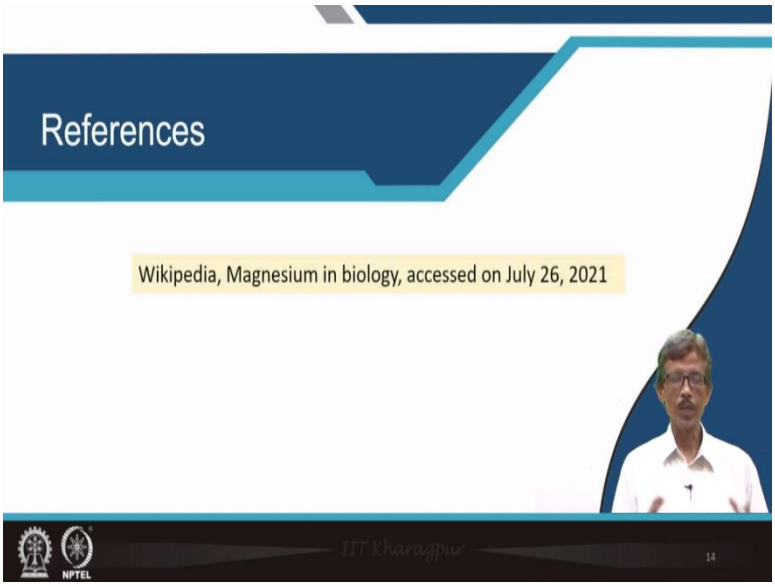

So, books are simply from the Wikipedia page, also the magnesium in biology and the Crichton book also. So thank you very much for your kind attention.