Biological Inorganic Chemistry
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Lecture 27
Module 06: Phosphate Metabolism and Cellular Signaling
Phosphatases and Enolases

A very good morning students. So, welcome back where we finished last time and it is in module 6 and lecture number 27. And to consider all these things, because we are focusing our attention mostly on the module what we are talking you see again and again is that of your phosphate metabolism and how much we know about the corresponding signaling process.

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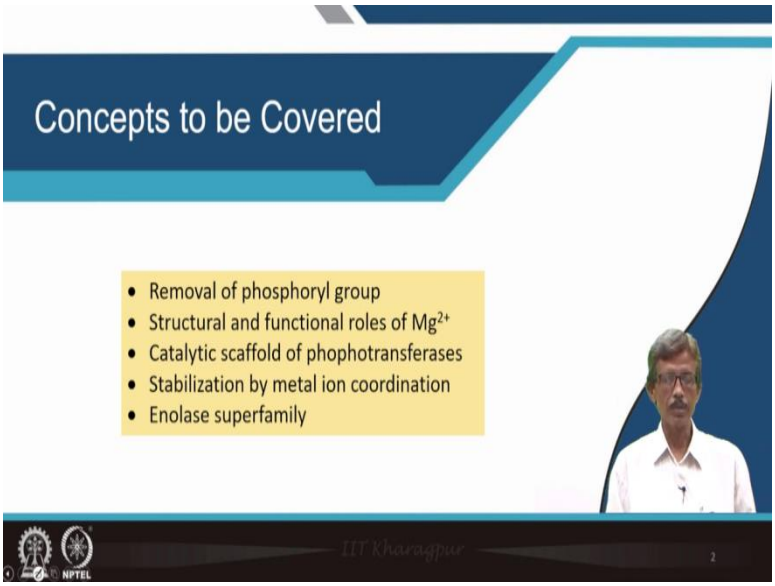

So, to this particular class is devoted for phosphatases and enolases. So, these are the two important classes of biomolecules, we will try to cover and in this particular case now, earlier we have seen that we are thinking about or talking about the addition of phosphoryl group or the phosphate and on to some OH function here will now talk about the removal only and then again the role of the magnesium and because these three classes are basically devoted on the roles of magnesium ions, then the catalytic scaffold of phosphor transferase.

So, if you have a catalytic site and how good it is in terms of its platform, scaffold is nothing but your platform. So, if you have the platform made up of a protein molecule or any other biomolecule and the magnesium is sitting giving a particular structure, then that particular scaffold how is it is important for giving a particular type of catalytic reactions.

So, definitely the fourth concept is basically the stabilization and we want to stabilize in a particular coordination and parliament of that metal and say magnesium two plus and also the enolase superfamily of enzymes. So, in an all so, in all things will be there, enolase superfamily will consider.

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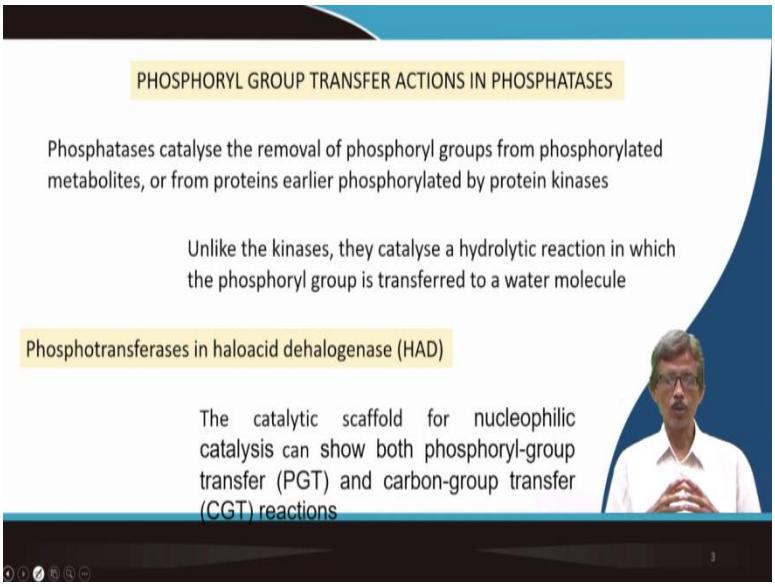

So, in this particular cases, we can define that what our phosphatase is? So, how we can define a very simple thing is that, again we have the PGT action or reactions, so, phosphoryl group transfer reactions we will consider and in this particular cases, when you have the phosphatase is the removal of phosphoryl group from the phosphorylated metabolites, we have seen that you have glucose you can have the fructose you can have the maltose or any other carbohydrate related molecules or the sugar molecules and if you have the OH function on it and everywhere you will find that CH 2 OH function is there.

So, primary alcohol function is there, whether we will be able to phosphorylate it also. So, in truly speaking way that means your enzymes, so, here are the biologically available catalysts. so, how we can utilize those enzymes such that you can get one particular metabolite and that metabolite having CH 2 OH function can be phosphorylated because that phosphorylation and the removal of the phosphate group is very important to give you the energy.

So, not only for the metabolites like the glucose or the fructose phosphates, similarly, also we can go for the corresponding proteins, we basically go for the phosphorylation. So, we have already seen for the kinases now, we will talk about the phosphatases. So, these are the two different types of molecules. So, that is why we have to compare it also that is why we can

say earlier also this unlike the kinases, here, what happens, they catalyze basically hydrolytic reaction that means that so, they can also be considered as phosphatases.

So, we go for the hydrolytic cleavage of a particular type of bond in which the phosphoryl group is transferred to a water molecule. So, water molecule is functioning as a very good nucleophile not that any other hydroxide ion or any deprotonated oil site or the alkoxide site, but it is a simple water molecule which can take out that group and giving you as the corresponding phosphate an ion or the phosphoric acid and iron in that system.

So, one such is a very good example so, we have to take the model examples so we will take one particular model example is that haloacid, what is haloacid? We know or the corresponding acetic acid if you substitute the corresponding hydrogen one of the hydrogen on the methyl function acidic acid is your CH3 COOH.

So, if you substitute one of that H on the methyl function by a chloride function or chloride group, you will get a clone of acetic acid so, which is CH 2 Cl c o h nothing else. So, if you go for any kind of that phospho transfer is the accent on heloacid, dehalogenase. So, you had the haloacid you can go for the dehalogenation reaction that means, you try to remove the corresponding chloride function or the halogen function that was you can have like that of your acetic acid removed to a CH bond is moving to a CCl bond.

So, similarly, if you go back again from CCl bond two against ch bond that is why you go for the dehalogenation reaction. So, a catalytic functions should also be there, so, that the halogenation send can occur within again a catalytic scaffold for nucleophilic catalysis that means, water will come and water will function as a nucleophile and will attack your phosphate puncture.

So, both these two types of reactions we are knowing from our last classes that you can have the PGT and CGT So, phosphoryl group transfer reaction as well as carbon group transfer reactions we can see.

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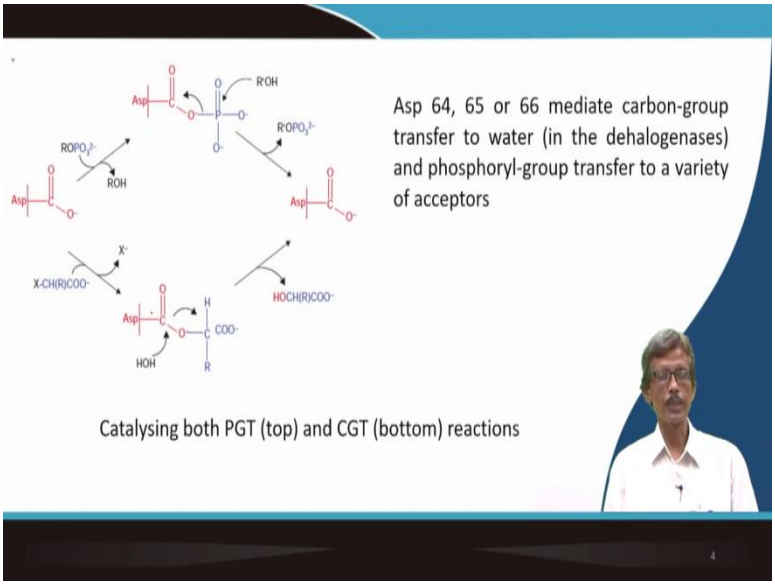

So, what do you find therefore, that if you have this particular type of thing that what do you take that x is your chlorine or chlorine or bromine on a backbone, which is instead of CH 2 now, CH R and CO minus, so, that will be very much similar to that chloro acetic acid. So, if you have that particular chloro acetic acid type of thing and you have the red colored carboxy N of the aspartate anion so, S COH minus or sorry COO minus not CO is the after dependence and it is COO minus.

So, like the acetic acid when we have acetic acid we write CH 3 COO minus the corresponding acetate and ion when we have this corresponding aspartate anion, so, as long as the aspartate and so, it can be your part of the polypeptide chain part of the corresponding protein chain and all these. So, how this corresponding carboxylate end basically and walk in a way that it can attack basically your H CH RCOO minus which is your halo acid anion.

So, while you attack that particular so, halo as it anion is basically responsible for the removal of the x group. So, immediately we have the corresponding C X bond cleavage. So, the CX bond cleavage is basically removing X not as X, it is removing X as X minus So, that if you have the chlorine attached to that particular acid like your chloro acetic acid, the chlorine will be removed as the chloride and ion and in that medium also you can detect it as the way we detect the presence of chloride and an in water medium or any unknown sample or while the detection of sodium chloride in your school days.

So, that also we can detect the nature of the X the way it is removing from the system. So, while doing so, you are establishing the way you are baking the corresponding C X bond you

are forming a new co bond out of the charts on the carboxylate end of the aspartate anion. So, you go for that, so, attacking that then water is now coming and water is not claiming that CO bond.

So, the CO bond is now a strong bond instead of bad what it can go for your thing that is like that of your Ester bond. So, it is C OO R So, bigger thing is that C OO R. So, you see here, your water is attacking here. So, this particular case the water is attacking over here and you will be craving this particular bond that is will be converted to OH.

So, C X bond will be converted to C OH bond. So, instead of your chloro acidic acid, you get the corresponding hydroxy acetic acid or the lactic acid or some kind of that thing will be porn, but when you have the corresponding phosphate anion on the top basically the phosphate anion, it can be your ATP molecule it can be an ADP molecule or any other phosphorylated species earlier we got it through kinase activity.

So, now, if the phosphorylated species is reacting on that aspartate, carboxyl aspartate anion, it is straightaway remove R OH, that means you will again claim that R O bond like your X the bond you will just basically oxygen carbon bond will be claiming and will producing our R OH function over there and the phosphate groups will be attaching on the carboxy end. So this is basically not only your hydroxy end mostly we will see that the hydroxy end is getting phosphorylated but your carboxylate end in also be forced related and we know that the corresponding one will basically get the carboxy phosphates basically.

So, these carboxyl phosphates then finally react with again another R OH type of molecule it can be simple glucose molecule. So then that always will be phosphorylated and we will get back to that aspartate carboxylate anion finally. So, these three basically that aspartate 64, 65 and 66 will mediate therefore, the corresponding carbon group transfer to water in the dehalogenase reactions and also you can have the phosphoryl group transfer to a variety of acceptors only thing that you must have the available acceptors.

So, if you have the acceptors you can have that these two reactions first one is your CGT and which is applicable for your dehalogenase reaction that means, the removal of the halogen function or the loading function from the corresponding substrate or the corresponding halo acid, but it can also saw pgt the phosphoryl group transfer reaction if the acceptor is available over there. So, that way it can catalyze both pgt as well as the CGT reactions.

So, we have discussed one the first two or three discussed is the corresponding CGT and other one is the corresponding PGT reaction.

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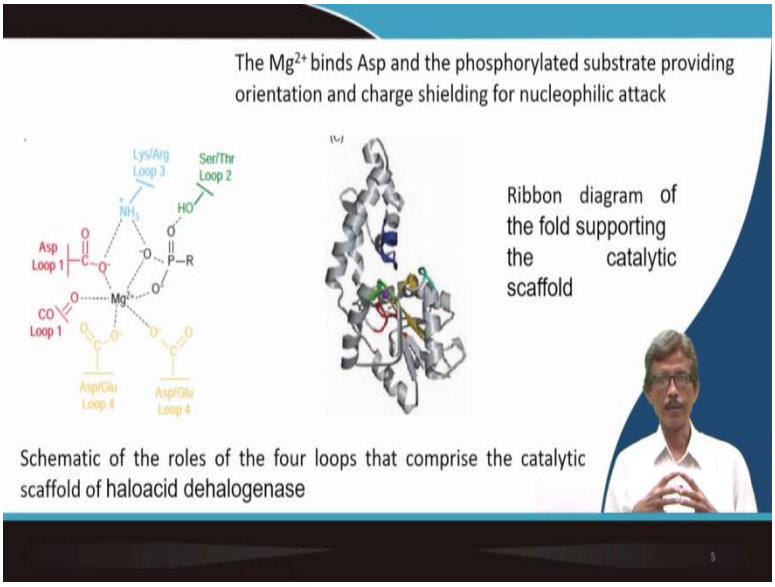

So, definitely you have the presence of the magnesium two plus then magnesium two ion, so is basically a Lewis acid a ketonic function which will be able to grab or which will be able to attract the corresponding and ionic part of the aspartate and if it is available, so, aspartate anion can have the carboxyl function. So, these aspartate ASP O minus and the phosphorylated substrate if you have that substrate as well as the aspartate anion basically we are saying that you have this part it and the substrate is coming for the CGT and substrate is coming for the PGT reaction at the top.

Then we will have to find the orientation of the substrate as well as the magnesium because the magnesium when it is binding, it is only looking for the ligand donor atoms. So, wherever you have the ligand donor atoms, so, you can get the corresponding coordinates and geometry satisfying its coordination number. So, while it is satisfying its coordination number it will put in a particular geometry, it can be a octahedral geometry or a distorted octahedral geometry.

Further also you can have the (())(12:43) that means, you have C 2 OO to the corresponding two plus the positive charge on the magnesium center as well as the negative charge on the aspartate ion. So, it will facilitate finally the corresponding nucleophilic attack of these water molecules.

So, if you look at the orientations basically not only one of the aspartate group aspartate at anon carboxy aspartate that function so, you can have that. So, you can also further other aspartate groups, so, the aspartate or glutamate you can have also, but if you find that the three of these are available, then you can have the serine function or the thiamine in function.

So, basically while you have the folding you can have one particular loop and the loop is providing the COO minus function or the loop is providing another COO minus from the glutamate residue or the serine function or thiamine in function which are providing OH function.

So, immediately when you see that what amino acid coming from the protein backbone what amino acid residue you are talking about whether you are talking about the aspartate residue or whether you are talking about the serine or thiamine residue. So, in the former case it will provide the carboxylated and in the latter case it will provide the hydroxy end so, these are all very much important.

Finally, you see for the loop three loop number three that you can have the lysine residues or the arginine residue. So, these are basically forming the corresponding NH two function or NH 3 plus function, so that is also needed. So, you see the whole network is a very complicated network, but it is centered around the magnesium which we always talk about the corresponding coordinates in chemistry.

So, the metal ion now it is only the corresponding metal and which is the main root metal ion not the transition metal and like iron or nickel or copper. So, these magnesium two plus is basically trying to assemble everything if we consider that okay I am getting more strong bonds with respect to the carboxylate and that means CO minus the charges there and magnesium plus since we only consider that the charge balance, so, two is due to charge balance is important.

So, initially you can grab or you can drag basically two negative charges of these two carboxyl attends. So, you bring those two carboxyl attends to the magnesium center. So, it is forming immediately those two magnesium oxygen bonds from the carboxy ends then you can have also another carboxylate and as well as the phosphate and so, if you gather so much charge, so, that is why the term we have written over here as the charge building.

So, if you have a positive center and if you put more and more negative charge on it, what happens the overall accumulation of the negative charges taking place, that way we get that if

you have a corresponding metal ion in the bio valence state and you bring three bidentate mono negative ligand center.

So, if you are able to assemble three of them, So, overall charge on the complex will be one minus, so one single negative charge will be there on the metal ion complex. So, similarly here, we will have the negative charge build up which is counteracting basically by the corresponding NH three function which is the protonated NH two function for the arginines or the lysine residues from the loop three.

So, basically from the top, the loop three is giving something some kind of positive charge utilizations. So, now, if you consider that though, on the magnesium, the carboxy end that means the corresponding amide and the carboxyl amide and that co function can also so in coordination to your magnesium center.

So, though we are happy by gathering six centers around the magnesium and all of them are oxygen, the heart centers around the magnesium very small magnesium inside, which is 65 pico meter inside. So, we will be able to assemble so many oxygen centers around magnesium but you can have far the hydrogen bonding interactions like that of your NH three plus. So, NH three plus is basically stabilizing this one carboxy and oxygen and other phosphate and oxygen.

So, in between you have done it. So, this particular hydrogen bonding interactions is nothing but your secondary interaction secondary coordination interactions where it is starting from the magnesium two plus forming some coordinate bonds and farther it can be stabilized at the secondary coordination sphere which is stabilizing the secondary coordinate since we are in terms of thermal hydrogen bonding interactions, it can also happen with that of the simple water molecules.

Then if you get this all these things only then by looking at the magnesium center and looking from the viewpoint of coordinates and chemistry you can find that okay this magnesium is there at the center of all these cases, but what about the corresponding rebound structure.

So, the ribbon diagram of the for supporting the catalytic scaffold, so, at the center you have the magnesium but the ribbon folding and all these things that protein environment is also important to know because you will have you see that the from the top basically you can have some vacancy. So, your substrate can enter through that particular pocket. And once the

magnesium is there without magnesium you have one particular structure which can be open structure, then after magnesium it is folding then after substrate it is again folding more.

So, that is why these particular things are very important and schematically if we find that you can have four loops loop one loop two loop three loops for and all these things are there that comprise the catalytic support of halo acid dehalogenase. So, why you require so many loaves, why your magnesium center is important and how it can function finally, for your dehalogenation reaction.

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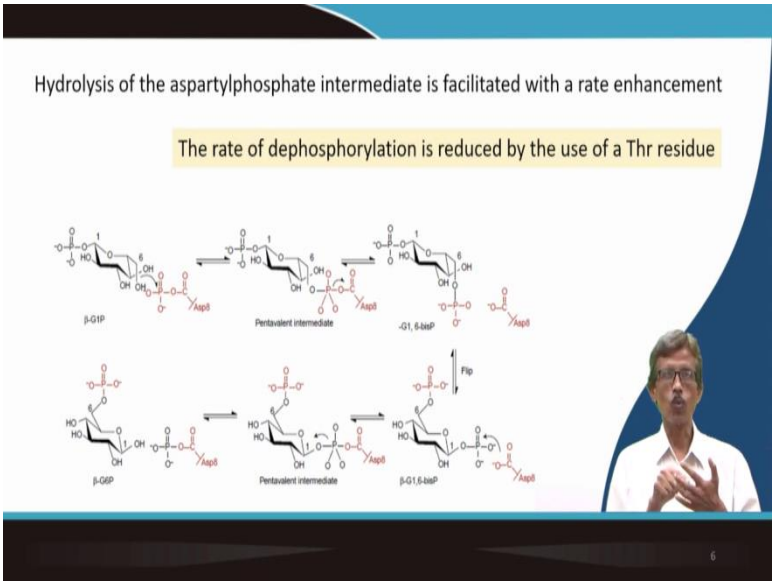

So, basically what we are listening here is that your hydrolysis of the aspartate phosphate intermediate. So, you have the aspartic acid residue, which can be phosphorylated and which basically can facilitate your rate of the reaction rate of the catalytic reaction and is basically the rate of dephosphorylation reactions.

So because now for the first part is activity because that you are breaking the phosphate function which has already been incorporated. So that first part is activity is nothing but a de phosphorylation reaction. So the rate basically can be reduced if you have it thiamin residue. So it is not that we sometimes we require a very fast reaction, because if you have a very fast reaction, so it may not be controlled manner, you go for the corresponding energy release, your substrate should be there, your nucleophile should be there.

So reaching up all these actions ingredients can have some late or can have some time it requires some time. So finally do not perplexed with this big figure is only a very simple thing because but it is the whole diagram whole picture basically what you get. So, you get

basically some sugar molecule from the top, you see there is the sugar molecule on the top and that sugar molecule can be phosphorylated when you have the aspartyl phosphate residue. So, this aspartyl phosphate intermediate is there.

So, that is the aspartic acid as part it as a group and the COO minus group and which has been phosphorylated to the charged oxygen. So that is your aspartyl phosphate. So, just take nicely that what we are meaning it by saying that it is aspartyl phosphate, so it is the intermediate. So, your OH function from the sugar molecule from the glucose molecule or the fructose molecule is basically attacking the tetrahedral phosphorus center.

So, the tetrahedral phosphorus center is highly reactive in this particular environment and magnesium is there but what is happening in terms of that particular reaction is that you can have the corresponding intermediate which is the pentavalent intermediate. So phosphorus when moving to a tetrahedral to a trigonal bipyramidal geometry and all as we have seen that when you have the phosphate group phosphorus is bound to four oxygens center, but when it is moving to a trigonal bipyramidal geometry, all the three oxygen centers will be in a particular plane and you have one above and another below.

So, you have the entering group as well as the living group within that particular axis where you have done the tip of this is the phosphorus. So, in between you have the plane, so, plane is the trigonal planar diagonal plane off three oxygen centers in trigonal, bipyramidal geometry.

So, if you have this trigonal plane, so diagonal plane let it remain like this. So, you have the trigonal plane. So, that trigonal plane will be there then you just basically because the earlier you have the corresponding thing this is a tetrahedral one. So, this is the three and the fourth one is that tetrahedral.

So, in which particular direction we will be going for that attack again it can be or good assignment for you that you draw a tetrahedron nicely within a cube phosphorous at the center of the queue and alternate coordinates you would go for occupiers and buy for oxygen center. So these are the alternate coordinates of the cube.

So, these are two. So, you draw the cube put these and the alternate coordinates of the cube and then you get the tetrahedron. So, now, your OH function from the sugar molecule or the glucose molecule who is detects and it will attack says that your tetrahedral geometry on phosphorus can be changed to a trigonal bipyramidal geometry. So, you get that.

So, then you have the corresponding transport that the phosphate group will be delivered to the sugar molecule and you get basically a heavier function instead of your OH function, you'll have the O phosphate function. So, the formation of that oh phosphate for a thing is important. And you see originally it has one phosphate at the left at the top left you have you see that is the thing.

So you have and again you have a test one phosphate. So basically, you two bulky groups are there around this particular ring, ring molecule that is a hexa six membered ring basically the glucose molecule right like the six membered ring. So the six membered ring you have the balance basically. So, one position you have the phosphate group, which is now inserted and six is originally there.

So that glucose six phosphate was original molecules and then the one six diphosphate you are making so phosphate group transport is digging there and then we get again the reversible one so beta G one six bisphosphate BISP this be the name abbreviated as BISP is the bisphosphate because originally you have one phosphate group function, now we are moving to a double phosphate situation and that double phosphate situation is as that again it can go for this particular one then again the spotted carboxylate function can attack that particular function and all are in equilibrium.

You see all these transformations in some equilibrium. So, some external thing external environment basically drive the reaction in a particular direction, whether a move from left to right or right to left. So some driving forces required such that you get a reaction when you start with that of your one phosphate, glucose one phosphate beta glucose one phosphate so beta GI one P. So beta is an anomaly form a beta. Glucose one phosphate will be converted to blood glucose six phosphate so one phosphate can be converted to your 6 phosphate by doing this simple phosphorylation and dephosphorylating reaction.

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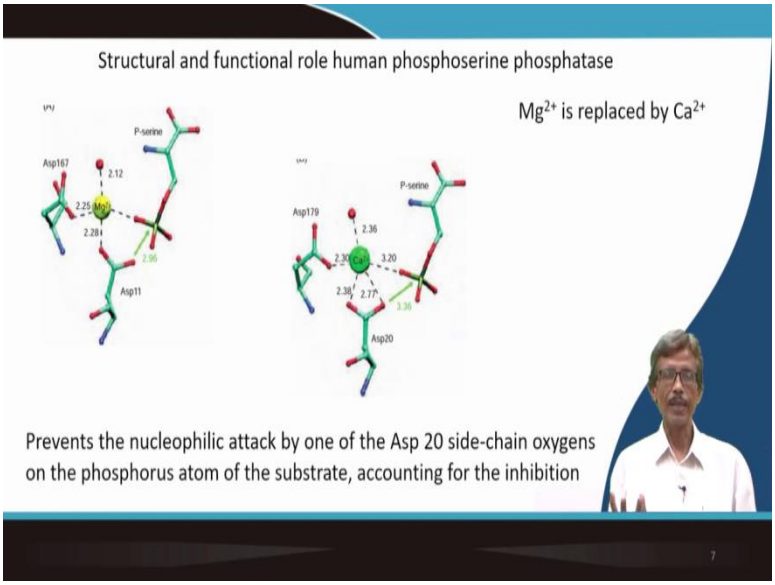

So, it is a very simple one, but you have to understand with that basically nicely then you can have that serine amine acidification as I told you that it is having CH 2 OH function, which is nothing but your alcohol function. So, that can be phosphorylated also. So, once you phosphorylate that function you get a phospho serine function.

So, phospho serine function can be useful again can go for its corresponding phosphatase activity and the magnesium two plus can be replaced by calcium two plus. So, if you have this environment and whether your catalytic activity can be enhanced or decreased or diminished due to the metal and substitution reaction that we basically want to see. So, what is happening basically, whether your coordination environment or the coordination geometric can change?

So, if you go from a smaller metal ion to a bigger metal ion like calcium, you will see that the corresponding coordination of your aspartate group, so, one is case aspartate 11 and another case is not that everything is isomorphous and you gave for the corresponding aspartate 20 and you have the same phosphoserine function.

So, you see now, the two of the carboxy ends are called try to coordinate to your calcium and it is coming away from your phosphate residue which was originally in attachment or very close to at 2.96 angstrom with that particular phosphate unit. So, definitely you have the change. And that particular chain basically is preventing something which prevents basically the nucleophilic attack by one of the aspartate at 20 residue that is a lower number that is aspartate 20 residue sidechain oxygen to the phosphorus atom of the substrate accounting for the inhibition, inhibition of the enzymatic activity due to the metal ion replacement.

So, in our next two classes also further to classes we just consider when we go for the calcium signaling, and calcium subjects and we will again come back to this particular situation we have the example that if you remove this because the calcium ions are the only thing that whether you are able to substitute those magnesium sides by the calcium.

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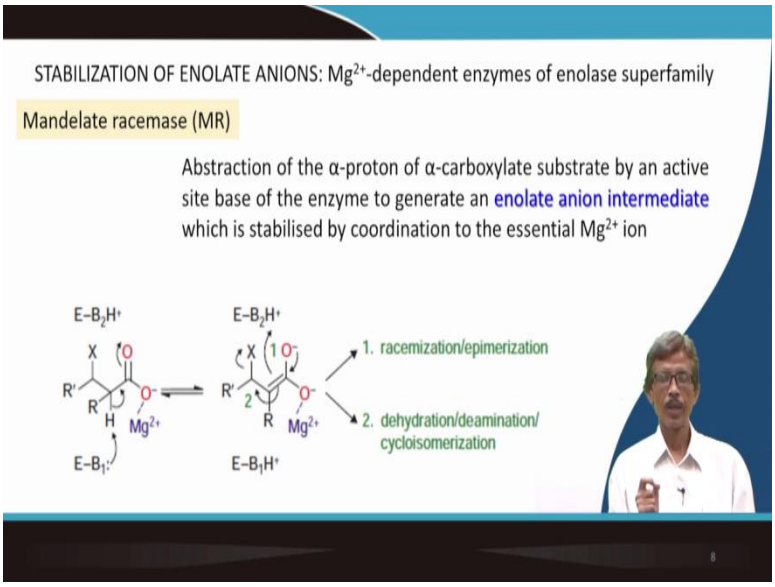

Then finally, we quickly see that the enolase civilization, so, magnesium is also go for that corresponding stabilization of the enolase superfamily and which is the mandelate and resume so mandelic acid if you have.

So, racemization can take place and you have initially the intellect and an intermediate. So, abstraction of alpha proton and the alpha carboxyl itself stayed basically going for stabilization through coordination to your magnesium to center. So, you can have that so you have the carboxy and the carboxylate and for the mandelic acid if you say. So, the magnesium ion is there and which is stabilizing again from the carboxy end. So, that is why we know that the carboxyl functions has a very good affinity for the middle and coordination around magnesium like your magnesium acetate.

If you have a typical salt, what do you get in the laboratory is your magnesium acetate, but due to that, if you have the corresponding CH function is available and that proton can be deprotonated only having that base is available. So base can abstract that through coordination. So, you can go for the racemization or epimerization as well as dehydration de ammunition and cyclo isomerization so, these are all different types of catalytic reactions that what we can see.

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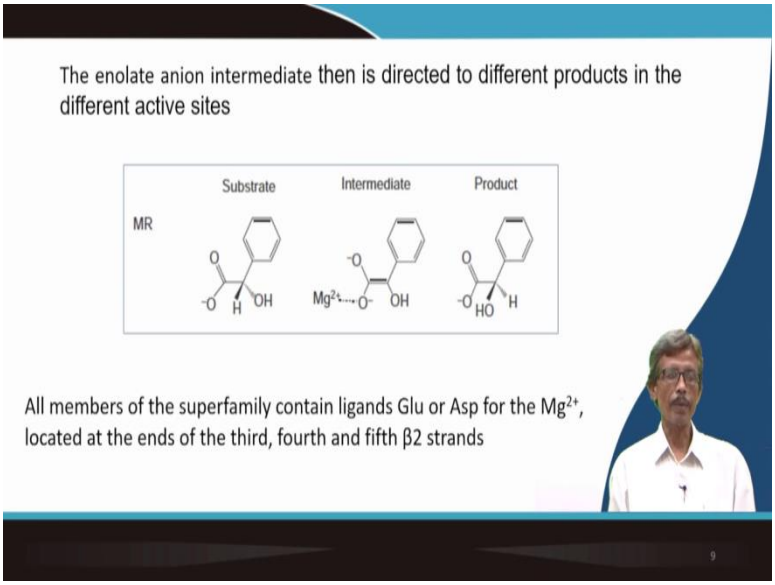

So what enolate anion you can have the intermediate then is directed to different products in the different active sites. So, you have the substrate. So MR is one type of the catalyst and that MR is basically giving you the substrate giving you the product and the corresponding thing as a decimization. So, decimization is taking place at the corresponding carbon center. So, optically active carbon center that is H OH orientation of the H OH is changing through that intermediate which is manganese iron coordinated.

So, all members of this particular super family basically contains like and the glutamate and aspartate anion and they can bind to the magnesium center and you can have the location for the third fourth and fifth beta two strands where they are located.

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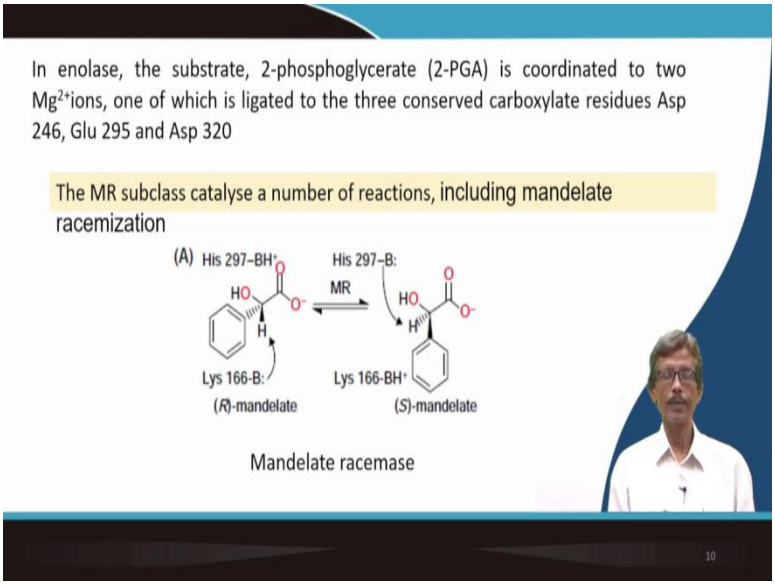

So, in this particular enolase functions in all his super family of these molecules, he always had the two phosphoglycerate. So, if you had if you had the two phosphoglycerate which is coordinating again to the magnesium two plus ion and one of which is like it a to the three conserved carboxyl acid you do so, what we need is the carboxylated residues from the different amine acid side chains and those are binding to the magnesium center.

So, magnesium center is basically dragging in this particular enzyme class and we basically go for the corresponding racemization that R mandalate, so mandelic acid corresponding anion. So, the history in 297 is only functioning as a base. So, that base is the protonated base and that permitted base is basically can go for the deprotonation and that is basically responsible for the abstraction of the proton and magnesium is responsible for the coordination to your carboxyl attained for sewing this particular reaction. So, the class MR what is that is basically your mandalate recimazation.

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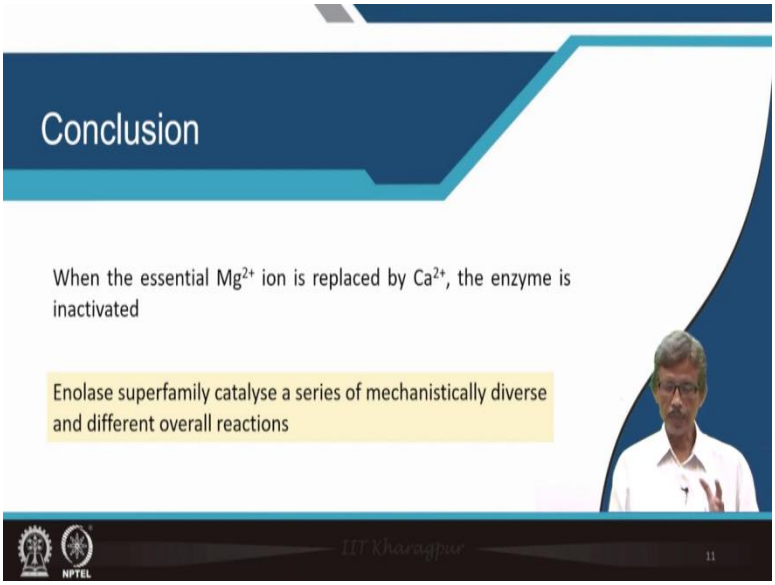

So, in conclusion what we have seen that is a very interesting observation towards the end what we have seen and try to remember it because we will be needing that when you talk about the calcium cases that enzyme can be inactivated if your calcium is replacing the magnesium side then basically the superfamily basically catalyze a city you are mechanically diverse mechanistically diverse sorry the mechanistically diverse then these different mechanisms are they are only one example, I have considered here is only the MR.

So, there are other related reactions where you can have these images and the actions are all these, the actions what I just told you, so, the different overall reactions that means the different products you expect from there.

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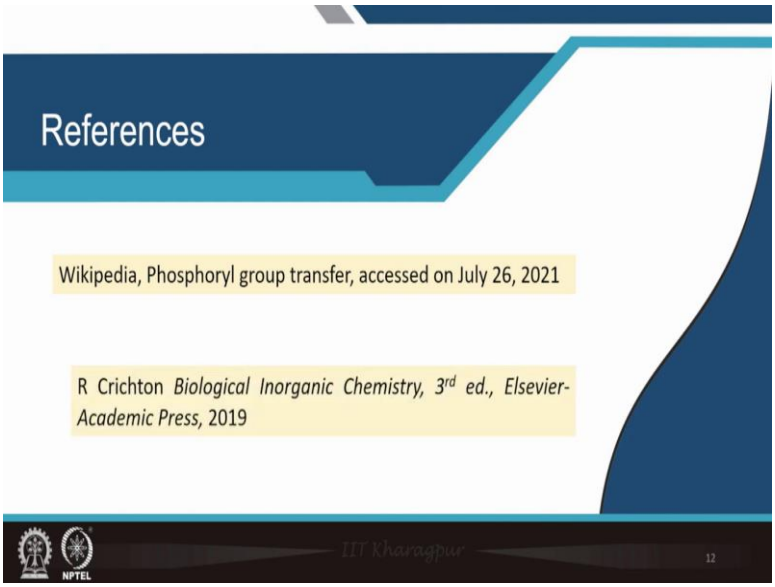

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