Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology, Kharagpur
Lecture 45
Redox Catalysis by Manganese Ions

Hello, good morning everybody. So, we have reached to the end of your module number 9. And in this lecture we will be talking about phosphatases. So, what is that alkaline phosphatase and a group of those phosphatases, we call is the phosphatases, and one simple example we will find out afterwards, the adenosine deaminase, what is that.

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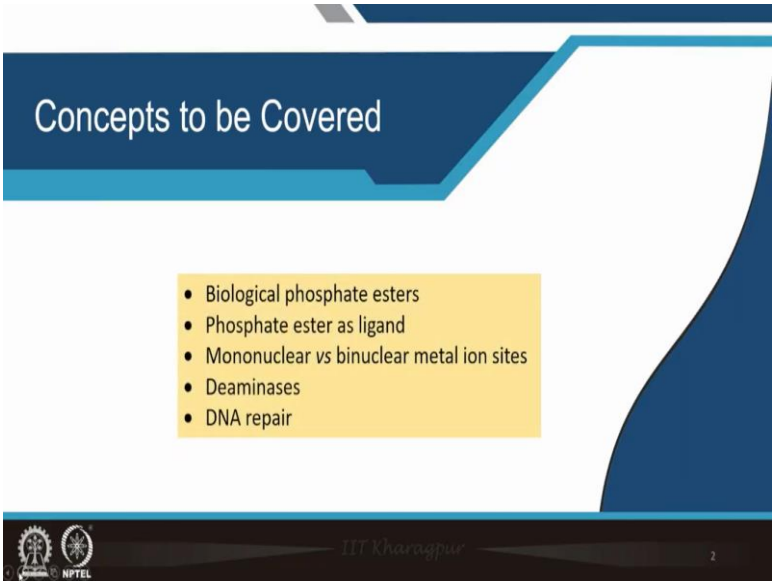

So, definitely we will tackle about the different phosphate esters and mainly we will talk about the corresponding cleavage. That is why we are talking as ase, the phosphatases reactivity, and how the phosphate ester can function as a ligand. That is important. And we will talk about the zinc ions, whether you have one zinc center is fine, or you require two such zinc centers for this catalysis. Then what we mean about the diaminase reaction, and finally the DNA repair in this manner.

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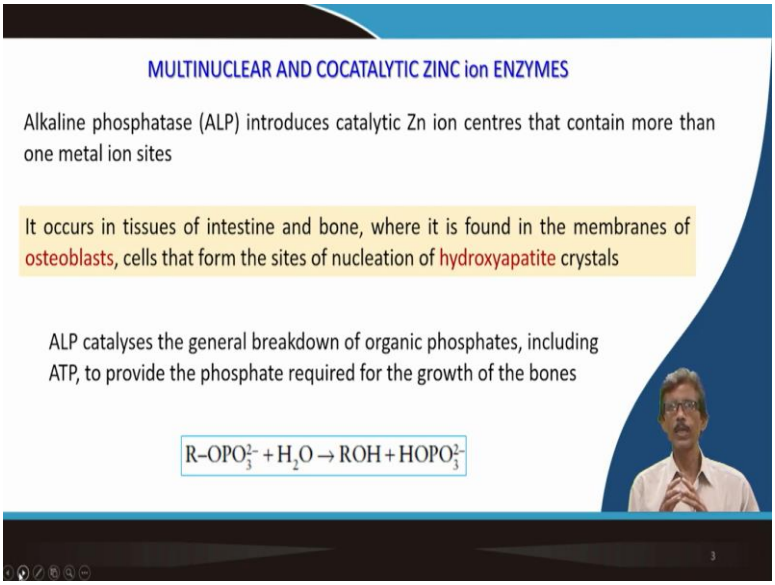

So, if we go that okay we just go little bit beyond the mononuclear entity what we are talking about your carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase, all these we have seen so far that one zinc center with the tripodal thing. You put one zinc that is fine and that is your catalyst, very simple catalyst.

Now, if we need the help of another supporting metal ion center which is adjacent to the first one, you get a binuclear entity. And sometimes we find that these binuclear entities can also be sustained like we have also seen that you have superoxide dismutase. Why you require another zinc center when the copper center is your catalytic one?

So, it plays basically the structural role. So, it is like your coenzyme so you can have one particular center which is cocatalytic in nature. So, we abbreviate nicely as ALP, which is AL for alkaline and phosphatase P, which basically gives us the idea that we must have some zinc ion center. And sometimes we can have more than one such center where we get these things. Why these are so important to study, important to know their activity, particularly their enzymatic functions?

So, in membranes, in our body membrane also, and the membrane of osteoblasts, it is there. And sometimes the cells which are required for our growth of our bone, our teeth, or any hard tissues, we require the calcium-based mineral. We know the name of that calcium-based mineral is nothing but your hydroxy apatite. So, how we can grow from the solution?

If you have to grow the crystals of hydroxy apatite, so these basically, these zinc enzymes will help in supplying many, many number of these phosphate anions, the inorganic

phosphate anions. So, that is why in terms of your nonmetallic bio inorganic chemistry part, knowing phosphate, their assimilation, all these things are important, but we are taking the help of the metal ion like zinc plus.

But what we are talking about the growth of something that hydroxy apatite, taking the help of the corresponding combination of the phosphate groups to another metallion, say, calcium because we know the calcium is there for your bone, for your teeth, and all these things. So, let us see how we can degrade the organic phosphate molecules. We should know a little bit about that organic phosphate molecules because we know what is phosphoric acid which is H three PO four. How you can make that phosphate as your organic phosphate, like your organic thiol.

Inorganic sulfur we know as your H two S, inorganic sulfur, as you know as sodium sulfide, or sodium bisulfide. But when you put that SH function or a thy ether type of S function in organic backbone, we get the two different amino acid who are having sulfide bearing groups, because these are required for this sort of hydrolysis. So, any alcohol function, or any phenol function or any glucose moiety if you are able to convert that ROH function to ROPO three or two minus, we will get the organic phosphate.

And when we are talking about the corresponding phosphatase activity which is nothing but the hydrolysis activity, and that hydrolysis reaction is basically triggered by the addition of the water molecule from our primitive understanding that if it is getting hydrolyzed, you have to take up the two parts of the water molecule, the H part and the OH part of that water molecule and we will have the two products and one will be added to one half and another will be added to the other half.

So, that way you can generate the alcohol, you can generate the phenol back or you can generate the glucose or sugar molecule by taking the H phosphate (ion) anion. It is not the typical PO four three minus, but it can be your HOPO three two minus or H two PO three one minus.

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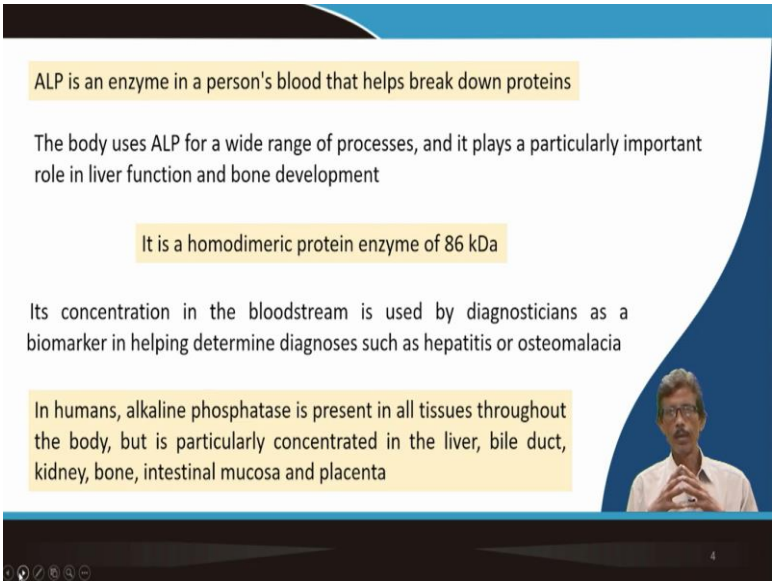

So, different protonated phosphates varieties we can have, where we are that enzymes. So, in our blood, and if any person's blood we try to measure, the concentration of those enzymes because you have to use that and it is helping the breakdown of the protein molecule, and protein molecule having the phosphate backbone. Not the amide backbone. That we have already seen for the carboxy peptidase activity.

Then the another counterpart is that of your amine carboxy amine peptidase. There you basically trying to break the amide function, the CO NH, such that you can have COH and NH two function apart. So, how we use this particular LP molecules?

They are used in different or varieties of reactions, and they basically are very important for our liver function and bone development. So, if the liver function is dependent on this particular enzyme, so if there is any abnormality in your liver function, doctors will prescribe you go for the estimation of this particular enzyme, or if you have some bone density problem, not due to of your aging but some other purpose, then people can think of or because these are the biological marker. You can measure the concentration, and you can find out the role of these ALP molecules or ALP enzymes for our well being.

So, when we characterize it, we find out that is a homodimeric protein that means two parts are same, its not hetero one. So, homodimeric protein enzyme of not very much, or not very less also, it is around 86 kilo dalton in molecular weight. And in blood, it is used by diagnosticians that means the diagnostic test what people can do, and can be used as a biomarker in helping to determine our diagnosis such as two disease conditions. One is

known as hepatitis, we know the malfunctioning of our liver or osteomalacia which is related to our bone deformation.

So, is therefore present in all tissues, because the tissues are basically directly getting those phosphates for their bone formation, for the cartilage formation, for the ligament formation. But most of the time, it will be concentrated in the liver, in your bile duct, kidney, bone intestinal mucosa, and placenta.

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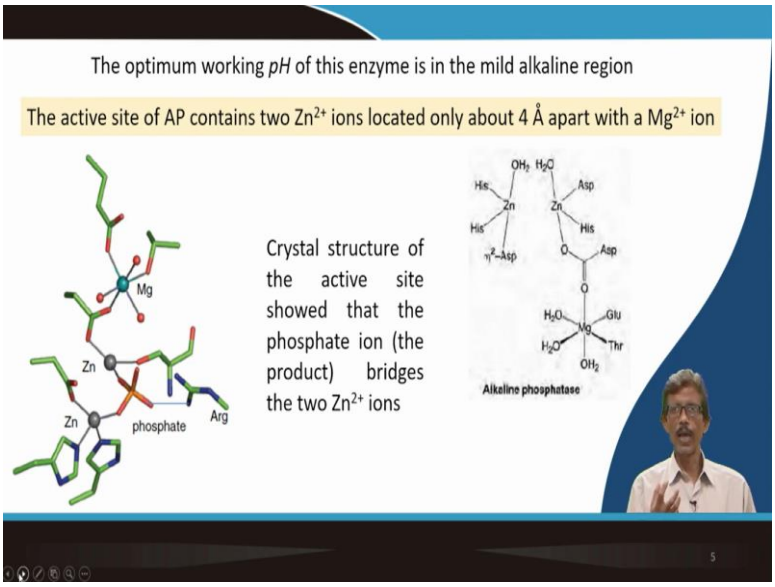

So, these are the areas, and we will find that in all these different areas we can have different such phosphatases. Their names will be different one, two, three, etc will specialized their presence. And we can find out their working activity. So, as the name tells you is working or it is functioning in the alkaline medium. That means the basic pH above seven. So, if we find that if you have a working pH of this enzyme is mild alkaline, not very strong acidic one, it is just above seven, if it is 7.5 pH and the base is not so strong, but the concentration of the hydroxide ions from the water medium or the aqueous medium is not high, only thing that some of these groups of water can be converted to your hydroxide ions.

So, we all know that not only the basic medium, but acidic medium can also work nicely for your hydrolysis reactions that we will see also for another group of molecules where the acidic medium is fine for your activity. So, you have two zinc centers, and they are quite long distance, mostly for hemocyanin we know, for any other dinuclear sites we know what are the metallion metallion distances, but here it is around four angstrom apart.

Along with these two zincs, we can have the magnesium. So, definitely, we are slowly making our life complicated by bringing the second zinc center, then not only zinc, another one which is not zinc, which is magnesium. And we all know, magnesium also can take some useful role for hydrolyzing your phosphate backbone, or the ATP to ADP conversion. So, how the active site looks like, the chemical drawing, as well as the structure drawing?

We can see that you have a phosphate ion. After hydrolysis, what we see, we have seen now that you have HPO three two minus you have got. So, that phosphate anion, protonated phosphate anion you can have, and that phosphate anion is your product of that hydrolysis reaction of these alkaline phosphatase molecules.

So, once they are formed, the bridging groups which were originally there, maybe some amino acid residue is required for bridging the two-middle ion center, or simple water molecule or the hydroxide O function is required to hold these two zinc ion centers. So, that particular point can be replaced by your product of this hydrolysis reaction which is your phosphate anion.

So, you can have a good separation. Why your zinc zinc separation is that big? Why it is not close to 3.4 or 3.5, what we have for your hemocyanin copper copper distance? Because already we know that the zinc ions are little bit bigger than your copper two plus, but is due to the three dimensional or tetrahedral nature of the phosphate anion.

That have to be accommodated over there and when the bridging is taking place, you will have the phosphorus center O and O. So, it is basically the bridging O of the phosphate part and O for the other part. So, its basically a three-atom bridging, but sometimes, you can have to accommodate the three-dimensional part, the other two PO (groups) bonds, then that is why the distance will change, which is unlike your other three atom bridging groups like your acetate. Simple acetate is also O-C-O bridging.

So, if you look at the molecule, then, so we will find that amine acid residues, we level it, so chemical drawing, I will not ask you maybe this particular drawing, but you should know about this drawing. If the drawing is also given to you for alkaline phosphate, you should be able to identify these positions. Even the positions are all vacant, you should be able to fulfill those positions.

These are the very simple thing what we can understand. You can play around with these where you have the histadine, where you have the acetate, where you have the other water

molecule and the coordination to the magnesium ion, and why the coordination around zinc is different from that of your magnesium, not only in terms of the groups which are available to coordinate to your zinc center, but also the geometry, the coordinates in geometry, as well as the coordinates in environment and the corresponding arrangement because, in your case, manganese, you know that is (a) not a transition metal ion.

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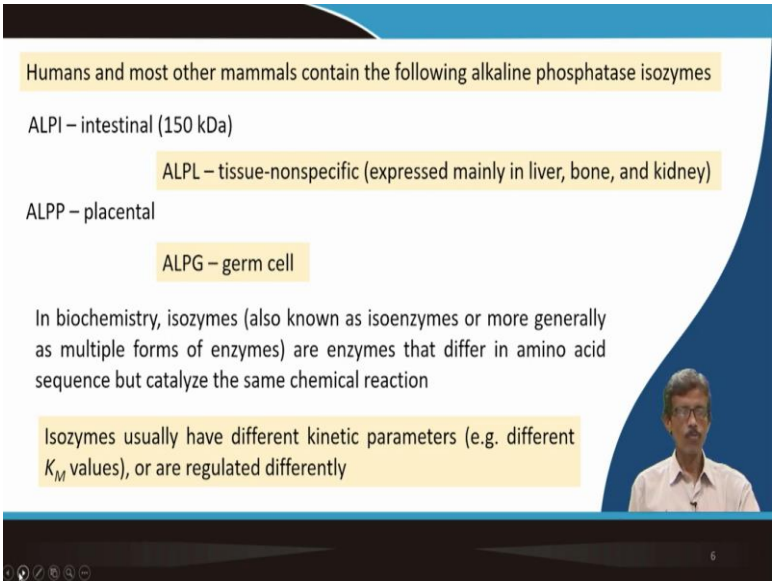

It is a main group element, but still it is favoring a octahedral coordination. So, many mammalian sources, we all have the alkaline phosphatase, but those are the different types of enzymes. So, these are isoenzymes and isoenzymes we call as your isozymes. So, when it is available in your intestine, and is of a bigger size, or the longer phosphatase molecule of 150 kilo dalton is known as LPI intestine. I for intestine. Then LPL. So, L is for liver, related to liver. So, tissues, non-specific tissues, expresses mainly in liver, bone, and kidney. Then placental origin is L, LPNP PP. Then the germ cell, you put G.

So, these are the basically the suffix around ALP molecules. And the definition why we call them isozymes that differ in amino acid sequences, but catalyze the same chemical reaction. So, their origin is different, their positioning is different, but they will all go for the phosphatase activity. And since they are going for the phosphatase activity but the amino acid sequence is different that means its origin is different. Your ligand backbone is different though that ligand backbone is holding the metallion, but if you go for the corresponding enzyme kinetics, you follow the enzyme kinetics, what will you find?

When you try to see the enzyme kinetics, we will see that the parameter which can be derived from there, the Michaelis Menten constant, or many other constants we can derive by

studying the enzyme kinetics. Once such is your km value. So, they are different, the magnitude of km, the rate of the reaction will be different. That means, the reaction for your phosphate group transfer, like your ADP to ATP, as we call it the phosphate groups, but it is the removal of the phosphate group is the hydrolysis reaction. So, the rate of the reaction, and the mechanism of that particular hydrolysis reaction will be different.

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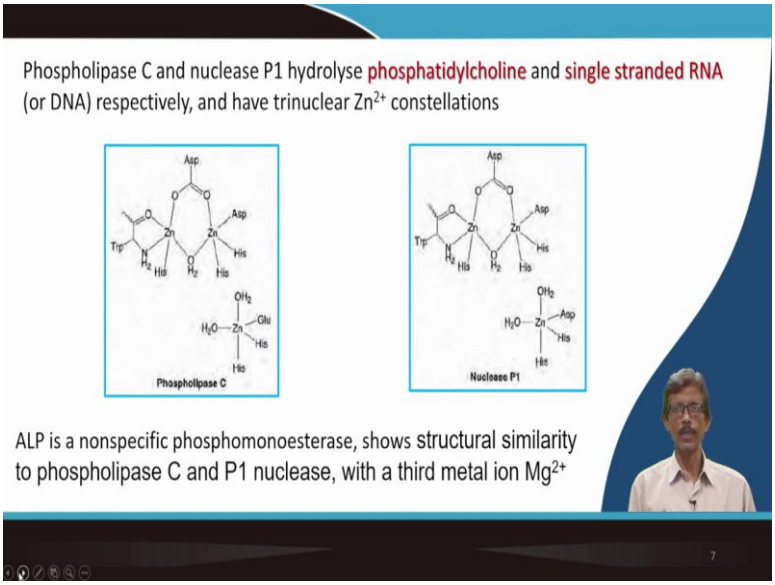

So, now we will see, we will talk about two other molecules of more complicated one, but related to that particular ALP molecules. One is phospholipase C, and another is nuclease P one but they all follow the hydrolysis reaction, but your substrate molecules are different. One is phosphatidylcholine. So, colin, you know.

So, colin is a zwitterionic molecule, very important in our system. So, try to learn that also what is colin molecule. And when the phosphate group is attached in place of your OH function is a phosphatidylcoline, you go for that hydrolysis. Then more importantly if you have the DNA and RNA molecules, and if they are single stranded, they again work on all these things.

That is why they are known as nuclease P1. That means showing the phosphatase activity, but on the nuclear basis. So, once the name is given to you, you should remember, immediately recall that okay its nuclease P1, B1 is for the phosphate removal, one of the inorganic phosphate group can be removed from that part, but it will be working on the nuclear basis, or the nucleotides, or the nucleosites.

So, this is the phospholipase C. So, if you try to remember the previous one, then you can try to correlate it also, but in this particular case earlier one, two zinc as well as one magnesium, all three are connected, interconnected by the bridging amino acid residues from the protein chain. But here you see the zinc, the third zinc in phospholipase C is sitting quite far, a little bit away. But there is no such common amino acid residue which is bridging the third zinc to that of your second or the first zinc.

So, it is isolated one, and all the zinc centers are having coordination number of five, and they are basically remaining like this, which is critically observable for the third zinc, the ZN three, which is trigonal bipyramid in nature, in terms of its geometry. If you go for the nuclease P 1, is mostly related, and you can have the differences in the corresponding, the mononuclear one, the mononuclear zinc environment, the amino acid residues, the change in the amino acid residues. But interesting you will find that in case of binuclear zinc, you have a bridging water molecule. Many times, I have told you that when you do not have the hydroxide O bridging between these two metal ions, you can have the water bridging between these two metallion centers.

But for this mononuclear part in nuclease P 1, you see in the trigonal bipyramidal geometry, you have two different types of water molecules, one is the basal water, another is the apical water. So, their reactivity pattern for these two water molecules will be typically different, if at all these zinc center is responsible for your hydrolysis reaction.

So, is a nonspecific one. Sometimes, it is not related to a particular type of substrate, but it is phosphomonoesterase, and it is structurally similar to these particular two types of phospholipase and nuclease with a third metallion where you can have in case of our esterase is the magnesium but now you have the zinc center.

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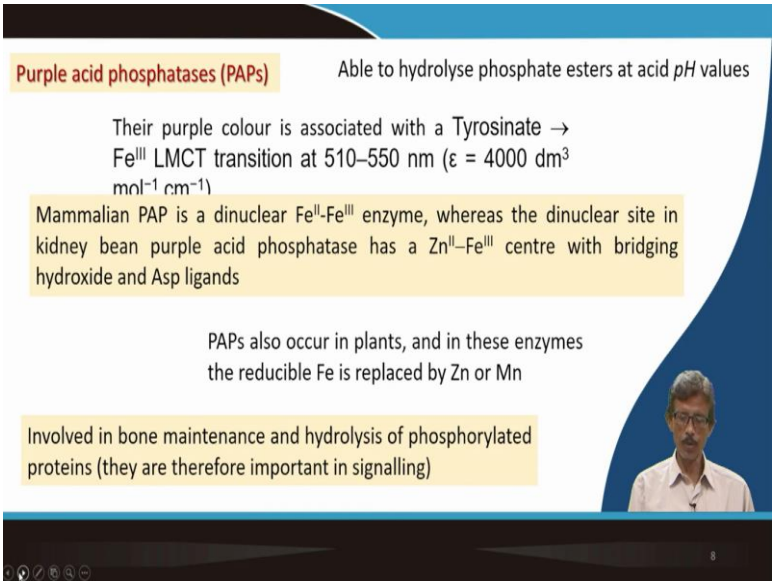

Then we will just change the medium of your reaction. Earlier we were talking about the base condition or the basic condition. Now, you bring it to the acid condition and these two conditions we already know very well from our early days, from our childhood days even, from your school knowledge that both these two mediums, either it is acid catalysed hydrolysis, or is the base catalyzed hydrolysis. That means the medium is either you are having HO minus, or HO plus. If it is simple esterhydrolysis, we call the acid catalyzed esterhydrolysis, or base catalyzed esterhydrolysis. Now, it is, instead of simple organic ester, now it is a little bit, we can call it as inorganic phosphate-based ester. Why the name is purple acid phosphatases?

So, first from the right-hand side you try to learn can call it as a PAP, is a very good abbreviation for many other big molecules also, the ligand molecules we call a very simple organic molecule which is also abbreviated as PAP, which is phenylazopyridine. So, in which context we are using that and why we are going for this abbreviation?

Because we do not waste our time in writing so many letters in placing the thing. That is why, when you tell this thing, you write PAP. It is your purple acid phosphate. So, why the acid medium, and why it is purple? So, in acid medium, your pH value we can, but the color origin, is color origin is simply that of your understanding what we learnt earlier in our, again our college days, that how you detect the presence or the unknown sample of phenol. We take the help of ferric chloride. We put one drop of dilute ferric chloride in a phenol solution, we know that is there will be red violet coloration, or the purple coloration that is due to the charge transfer transition.

Similarly, if you have the in the tyrosine residuals, so there will be some tyrosine residuals will be available and that not only signifies the presence of your tyrosine anion, but also the presence of your iron in the trivalent state. So, that is why you get the corresponding transition. So, ligand to metal charge transfer transitions we see, sorry this is covering that part.

So, this concentration as at what particular point, say, around 500. So, we have now for these what is the difference, it is the PAP molecules are different, a little bit, and they are different in the form that what they have that both of their iron centers are the iron. So, that is a little bit a different one. And if we can have mammalian PAP, it is iron iron center like your hemerythrin molecules.

But if we go for any plant origin, like your kidney bean, kidney bean purple acid phosphatase where you have the zinc. That is why we are bringing these, we are giving these example under the category of zinc ions, enzymes, or zinc metalloenzymes, but we take the help of another metallion so that is why it gives the typical example of hetero dinuclear cystine.

But what are there? You have the bridging hydroxido group and bridging aspartite group. So, you can have the occurrence in plants and you can have the reducible iron center replaced by zinc or by manganese in this purple acid phosphatase. So, if you have the phosphor related protein, which are very important for signaling proccess also and in bone maintenance also that means you need to have the corresponding removal of phosphate from some point and incorporation of the phosphate in the hydroxyapatite molecule. What is that active site?

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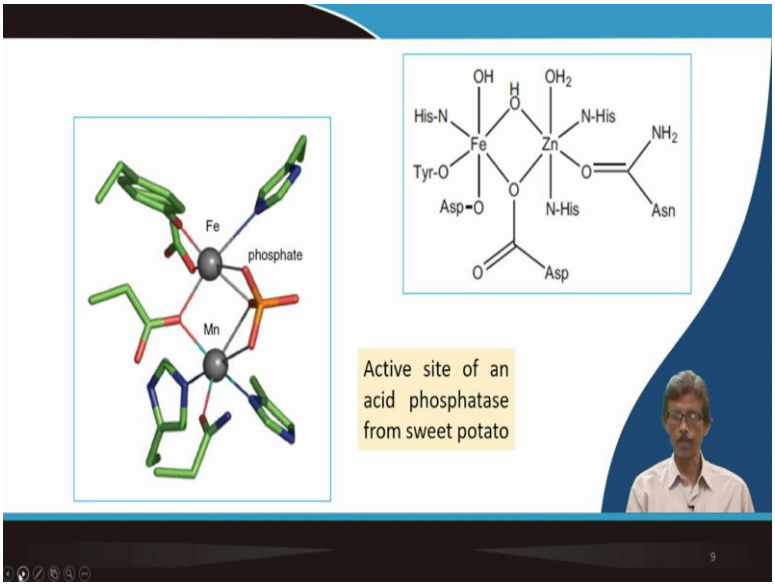

So, active site of that acid phosphatase from the sweet potato origin is only different. So, now it is basically iron manganese that can be reconstituted if you have iron and zinc. The zinc center can be substituted by manganese also, such that spectroscopically or even for excess structure determination, you can monitor nicely that particular active site. So, isotopic metallion substitution is possible.

That is the original one, you have the iron zinc center can be substituted by manganese also, and is hold nicely by the hydroxide O bridging from the top. Here, it is from the right, and by the corresponding amino acid residue and amino acid residue you see, that n is written is not histadine, it is the wrong one. It is basically the carboxylate end. So, the carboxylate end will be there for your bridging, and n is your terminal one. So, do not confuse between these two adjacent positioning of these two groups. So, the carboxylates CO function, that aspartite origin is going for your bridging purpose.

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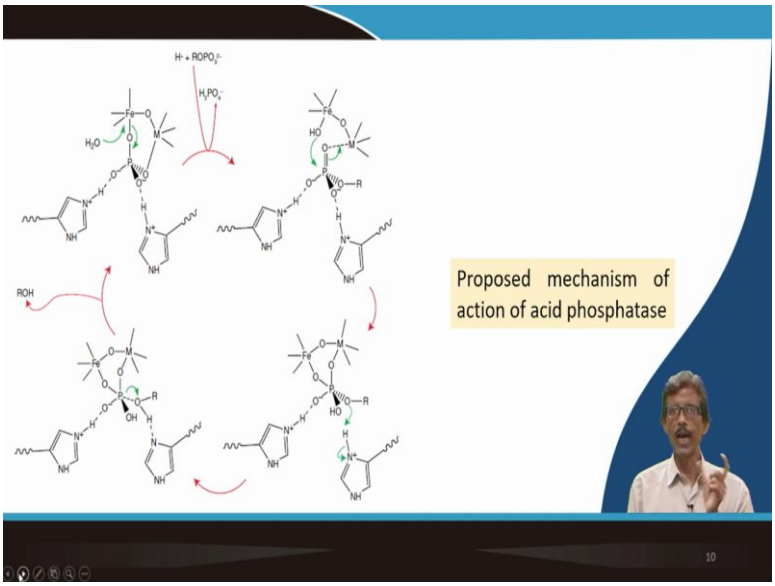

So, the original one, original active one, and the substituted metallion one zinc are there. Now, how the reaction goes in stepwise manner? From the very simple reaction, it is the hydrolysis reaction but when it is metallion catalysed, your thing will be very much complicated when you have to propose the catalytic cycle.

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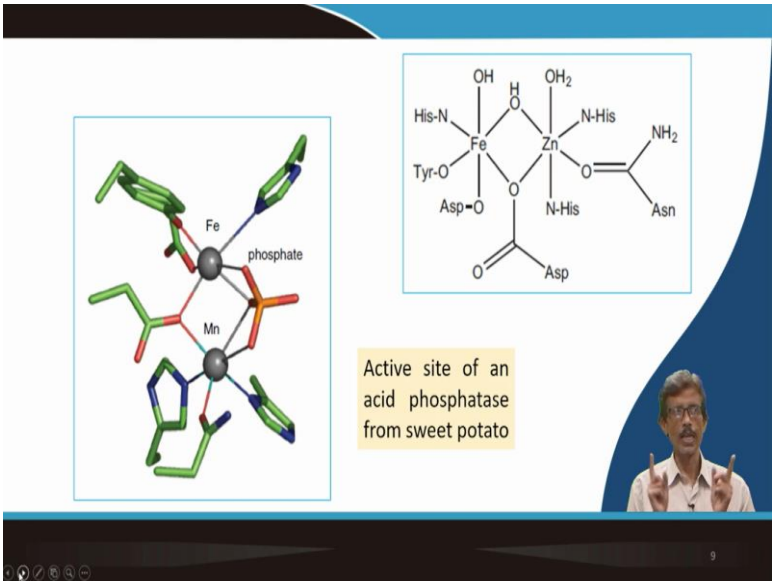

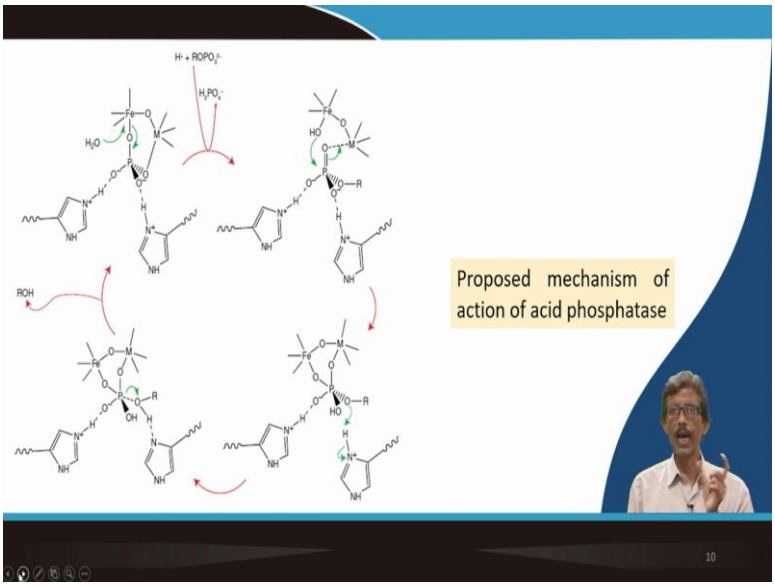

So, you can start from some particular place where you have the iron and other metallion. It can be either zinc or it can be your manganese or sometime, if your is human thing, human PAP, is basically iron iron center. But you require a binuclear motive and that binuclear motive when it is there, and one of the amino acid residues what is a bridging you, there we have seen that is the aspartite residue.

The aspartite residue is bridging these two metallion centers, and that aspartite residue is, if it is not there, that will be occupied by the phosphate group or (the) form one other part of the phosphate groups. So, the phosphate group is coming over there. And if you can see that okay, I have the originally from the top left basically, you see that the typical inorganic

phosphate is there, and which will be replaced by your organic phosphate group that means ROPO three two minus.

So, organic phosphate, we are getting. We are releasing inorganic phosphate. So, that is why the curly arrow, red arrow, is giving what you are taking. We are taking an acid medium, the organic phosphate, and we're releasing the protonated inorganic phosphate. So, that organic phosphate, you have to bring close to those two metallion centers, the iron, as well as this M. So, Fe and M, you have to bring from here. So, these Fe you have, and then you have the M, and already you have one bridging group that means the hydroxido group is there. So, that hydroxido group you cannot remove from there. So, that will be remained there.

Now, thing is that you have to break the corresponding RO function. So, that RO function that can be there, then R is there. So, another residues will be coming from the lower part also. The positioning of the organic phosphate group, then the attack of this particular one, so the two metallion centers are basically responsible for holding whether you have inorganic phosphate, the product molecule, or the organic phosphate, but when you are cleaving from the other arm of the POH or POR bond, you require some other acidic unit, that means the protonated individual unit from the right side, the lower part of the right side.

So, that is basically required. So, it is getting interaction with that particular group, and then is basically when it is protonated nicely that ROH. That means it is happy with that ROH product, then that bond will be clipped. So, that bond will be longer and you can cut it, still under the control of your metallion center.

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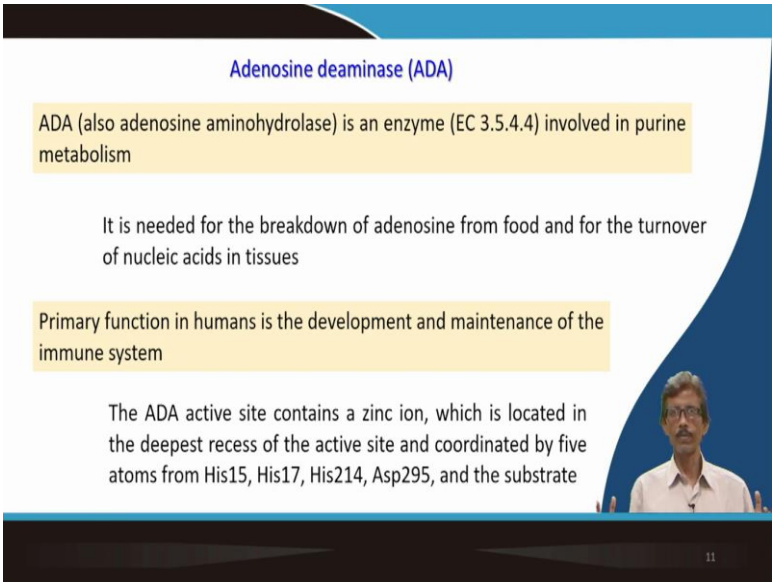

So, lastly, we will just quickly see what is called about the deaminase. I told you that how it is responsible for your DNA, or the RNA business also. So, you have to take out the amine function from the adenosine. And it has corresponding typical EC number, which category it is coming. So, three dot five dot four dot four in purine metabolism.

So, purine we know that the nuclear basis, and those purine type, one type, adenine and another purine you should know. So, two purine and pyrimidine. Two pyrimidine, two purine we all know. So, for the purine metabolism, we should take the help of this enzyme. And for the breakdown of the adenosine from the food for the turnover of the nucleic acid in the tissues, if the tissue is in demand of your nucleic acid and adenosine if we are getting, or biosynthesized in our body, or it is available from the food material also, you have to go for its corresponding deammunition reaction, such that it can take part in your purine metabolism process.

So, it is basically the very fundamental working principle for this in the development and maintenance of our immune system, is very important, particularly during this Corona period also, (we are) every time we are talking about the immune system. It's not only the zinc which can increase your immune system but your immune system is always important. There are very important molecules we call immuno regulatory molecules.

So, ADA, ADA. So, these ADA active site, you are zinc and the coordination sites fulfilled by the other amino acid residues. You have three histidine residues and one aspartite residues. So, altogether four as well as the water molecule if you have. So, you will have the five coordination sites occupied around this zinc.

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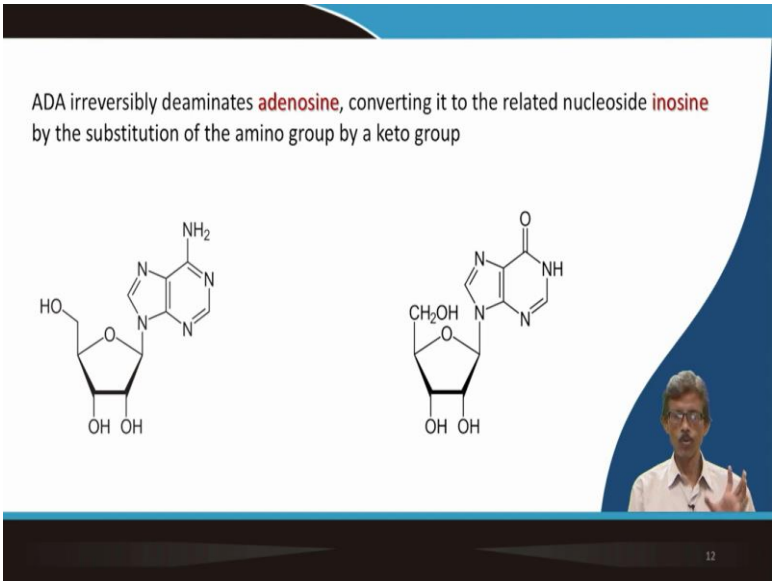

So, irreversibly is basically the deaminase the adenosine like the sugar units, which sometimes the sugar can be converted from OH to H. Here, (is) we are converting NH 2 to O C double bond O. So, CNH two is their molecule and you have to convert the top NH 2 functions. Only one NH 2 function is there.

So, it is easy to remember also nicely that I have to remove that NH 2 function if the remining part you write as R, RNH 2 is typical example of your organic molecule. So, that RNH two, and if you take the adjacent carbon of that R, then that will be converted to a ketone in this particular molecule.

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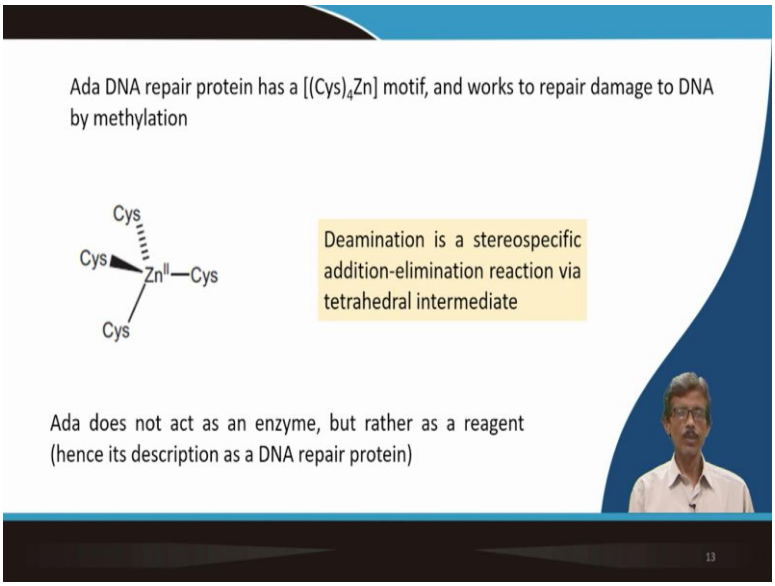

So, adenosine will be converted to inosine. When the amine function is not there, it will be simply inosine with that of your sugar molecule attached to it. So, you have the ribose sugar still present with it. So, they are basically for the repair protein, and has a all coordinates in site fulfilled cysteine residues also sometime. You can have the histadine residues also some time depending upon your origin or the working for the different places.

So, if you have this diaminase protein, and then another one is your repair protein therefore. There, you have the histadine residues, and now you have all sulfur residues for coordination to your zinc center. And, when, this particular one is a stereospecific addition elimination reaction by a tetrahedral intermediate, we see that this enzyme is therefore required for your DNA repair work, and it can also be used for your description, where the DNA is modified.

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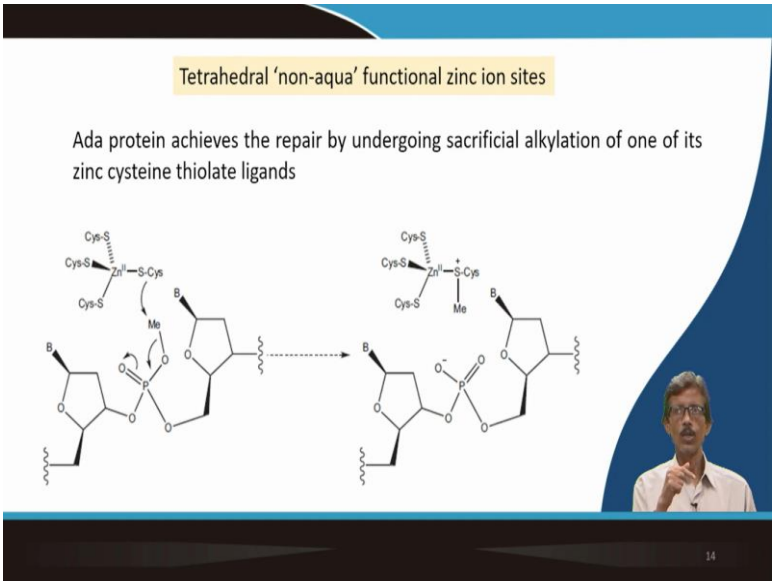

So, you have this non-aqua function zinc iron site, ADA protein is achieved for repairing work and how it is repairing. So, you take all sulphur, zinc environment and the cystine sulphur is already coordinated to zinc site but that can take up the methyl group from your already present methyl group in the phosphate backbone.

So, that movement of the methyl group from your phosphate backbone to that of your sulfur, so sulfur group of the thiolate group attached to your zinc center can take up and it will be converted from cystinate sulfur to a methionine sulphur where you have the corresponding ether function S, minus will be converted to S me.

So, that is why when it is bound, when it is bound to your zinc center, we show the basically the chart but it is not required. The thiolate can be converted to thioether.

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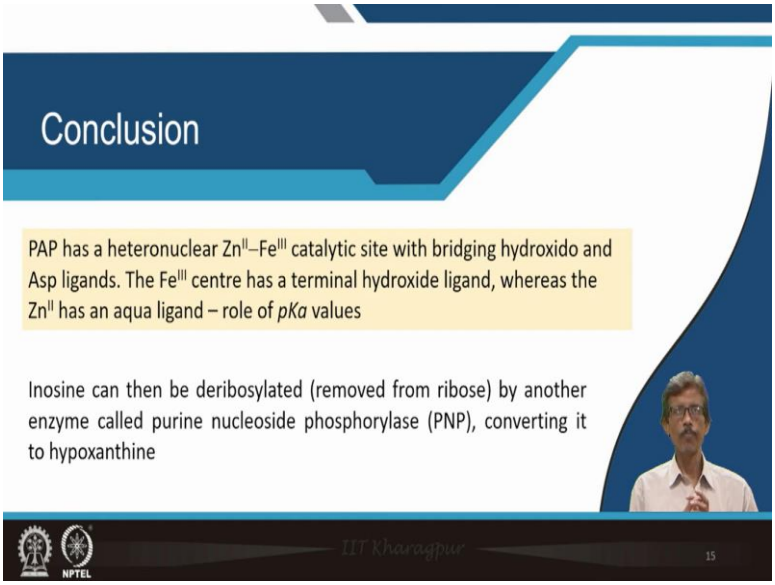

So, what do we have seen in case of your purple acid phosphatases? You require a heteronuclear cysteine, or a iron iron cysteine. And you can have the terminal hydroxide O ligand. And in case of zinc, we have the ACO ligand, that is why you can also understand in that way that the iron cannot sustain the water coordination. It immediately go for deprotonation giving you the hydroxido group, but zinc can sustain that water coordination.

And in case of your deamination reaction, once the inosine is formed, attached to that of your sugar unit, but in the next step, it is be deribosylated or ribosylated. The ribose sugar can be taken away by another enzyme which is purine nucleoside phosphorylase, PNP, to convert into another interesting molecule, very important molecule which is your hypoxanthine.

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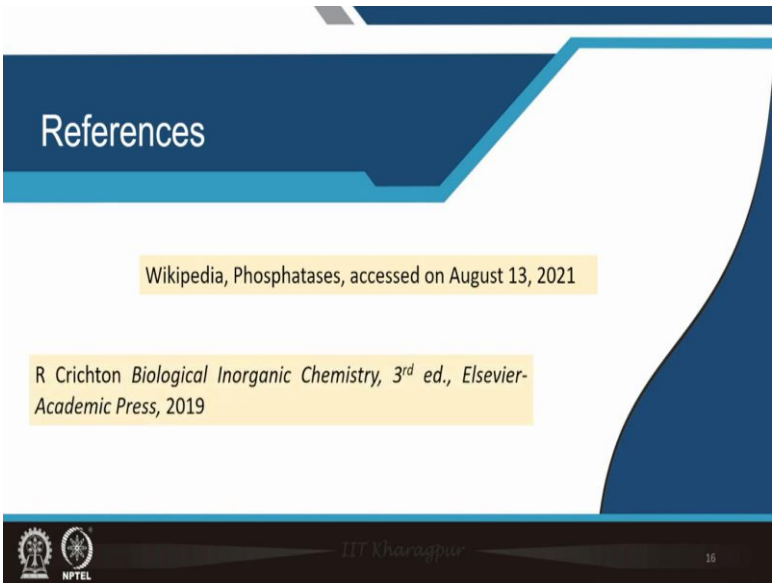

So, we will start you start studying. After immediately can be starting from this class also, the phosphatases is from the Wikipedia page. And the book with you. So, much for your kind attention.