Inorganic Chemistry of Life Principles & Properties
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Lecture - 43
Role of Zinc in life - Transferases, ligages and isomerases

We welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the last two classes, we have started looking at there is a zinc containing enzymes, in the first class, I have talked to you general classes and then followed by the example of the alcohol dehydrogenase; explained to you fully just in the previous class, I have taking two other enzymes. Both these enzymes are based on the adding water to carbon dioxide, converting the carbon dioxide into the H 2 CO 3 minus in order to balance the acid base components, the cell or the tissue in the body organs, etcetera.

And the second example, I have talked to you about the peptide hydrolysis particularly, the peptide hydrolysis, I had talked to you in more detail the carboxy peptidase which means that it this particular carboxy peptidase enzyme can only act on the carboxyl terminal of the polypeptide, but not the end terminal.

And secondly, I also try to impressive on these enzyme recognizes the last two residues, and particularly depending upon the side chain of the last residue, the enzyme will work. If it is carboxy peptidase A; it will work on hydrophobic kind of a side chains and if it is a carboxy peptidase B go on and the cationic kind of species, which are there in the side chain and if it is a carbonic carboxy peptidase c, it will be acting on peptide the having a side chain of the negatively charged. So, therefore, it is a very selective. So, therefore, also you have a huge number of peptidases are there. Similarly, I also mentioned we have amine terminal cleavage etcetera and other types of things we are not going into the details.

Now, the next story is just to see we have looked at the oxidoreductase, we have looked at the hydration or the condense bond breakage. Now, let us look at some kind of a transfer. So, you take some group from one and put into the other. So, example here shown here is a betaine homocysteine metyltransferase.

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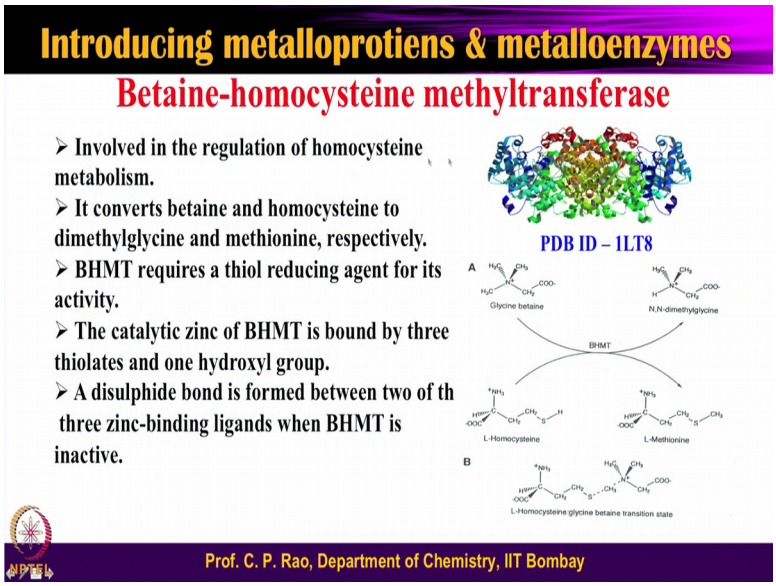

It is basically transfers the methyl moiety as you can see over here this is called the glycine betaine. So, it will go into NN dimethyl glycine. So, this will go into NN dimethyl glycine and this is coupled with the homocysteine going to methionine.

So, what is the difference between the homocysteine and methionine? The one of the methyl group is going onto the sulfur cysteine SH methionine sme that is you keep in mind. So, cysteine is SH and the methionine is SME. So, therefore, you are basically transferring the group one of the methyl group over there. So, these two are coupled together.

So, that is what is mentioned here that is involved in the regulation of the homocysteine metabolism, it converts the betaine and homocysteine to dimethyl glycine and methionine respectively. So, you can see that the glycine betaine will go to N and N dimethyl glycine and another and homocysteine will go to the methionine and that is what we are talking about. So, this is basically a homocysteine metabolism in the process the metabolism takes place when the methyl group is transferred.

In this enzyme, there is a catalytic zinc in this BHMT is a short form for betaine homocysteine methyl transferase; betain homocysteine methyl transferases refer as BHMT. So, in this case, this is the enzyme and this is the structure enzyme, you can see over there and in this enzyme the catalytic zinc of this one is bound by three thiolates and a hydroxyl group. And there is a rapid rearrangement of this enzyme during the reaction

rate goes. The enzyme undergoes oxidative step and enzyme undergoes reductive step, but zinc 2plus does not undergo redox. So, this is again one another case.

So, there is an enzyme undergoing reduction oxidation, but zinc is not undergoing any reduction oxidation. So, here what is going on? It is the cysteine and you know SH and disulfide is SS. So, what is the cysteine and disulfide relation?

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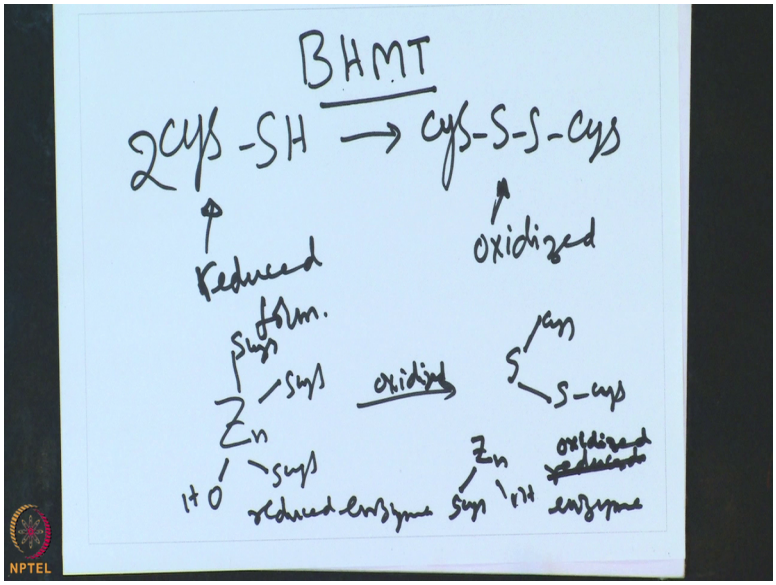

Cysteine to disulfide relation is cysteine is let us say cysteine SH and this will two of such things can give cysteine SS. So, this is oxidized form and this is reduced form.

So, in this manner the enzyme can undergo redox. So, this is the betaine homocysteine methyl transferase methyl homocysteine sorry, betaine homocysteine methyl transferase. So, it is basically that one in this particular reaction there is a redox occurring the redox is not occurring by the zinc, but occurring by the cysteine two cysteine to disulfide.

So, a cysteine disulfide bond is formed between the two of the three zinc sulfur binding ligands as you have seen I mentioned that there are three cystines are there and these three out of the three cysteines and the zinc; two of them will form a disulfide in the oxidized form and in the reduced form, they convert to the coordination. So, zinc having 1, 2, 3, cysteinoil and hydroxide S cysteine, S cysteine, S cysteine, in the reduced enzyme and in the oxidized enzyme, this will think will move away and there is one of the S cysteiniol and other S cysteinoil, this will form a disulfide bond and this moves out.

From here and this is S cysteine and OH. So, this is the reduced enzyme, the other one is the oxidized enzyme, this is not the reduced one this is the oxidized system. So, this is an oxidized enzyme reason is SS bond is there and this is a reduced one the SS is broken to S cysteineoil to cysteiniol thing.

So, this is. So, you have on the left side is a reduced enzyme and the right side, you have the oxidized enzyme so, but when both the cases zinc is in 2 plus there is no difference ok, I hope this is clear in this particular system, what we have here betaine homocysteine methyl transferase.

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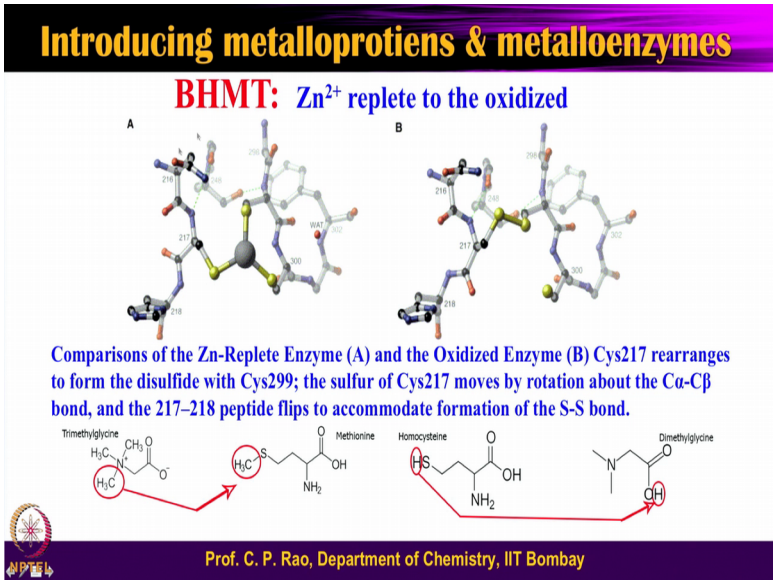

So, let us look at bit more details. So, whatever I mentioned to you that you have a reduced enzyme; where the zinc is attached to one one cysteine another cysteine another cysteine three of them.

These are oxidized enzyme, only the zinc part is not shown here, zinc will goes away and then you have seen that the two of the cystines have become disulphide. So, the cysteine, 217 and 218, this will form a disulfide bond. So, reduced form oxidized form this how it goes in these ones.

And the, and as a result how does this happen? This when this happens, the this to the cysteine 217 should rearrange to form a disulfide bond and sulfur moves in this. So, you have a great change in this particular conformational region of this ok. So, this is the

replete enzyme where the zinc is present, oxidase enzyme zinc is out of the coordination sphere and that is what you call oxidized enzyme and replete enzyme.

So, in this cysteine 217 is the one which is that is somewhere here, this is the one 207 rearranges to form a disulfide with the 299. So, the 299 is with this one. So, it will form a bond with the disulfide bond. So, the sulfur of the 217 moves because the it has to come closer the two sulfur atoms have to come closer and for that there is a rotation about this bond and this kind of sulfur of the moves by rotation about the C alpha C beta of the cysteineoil moiety and then means the flips the 217-218 peptide bond flips in order to accommodate the disulfide bond.

So, from this kind of a coordination sphere to form a disulphide, because two of the sulfurs have to come closer this has to go away. Therefore, there is a movement of this bond and these two coming closer and then forming a disulfide and throwing out this away and that is why this is the things that.

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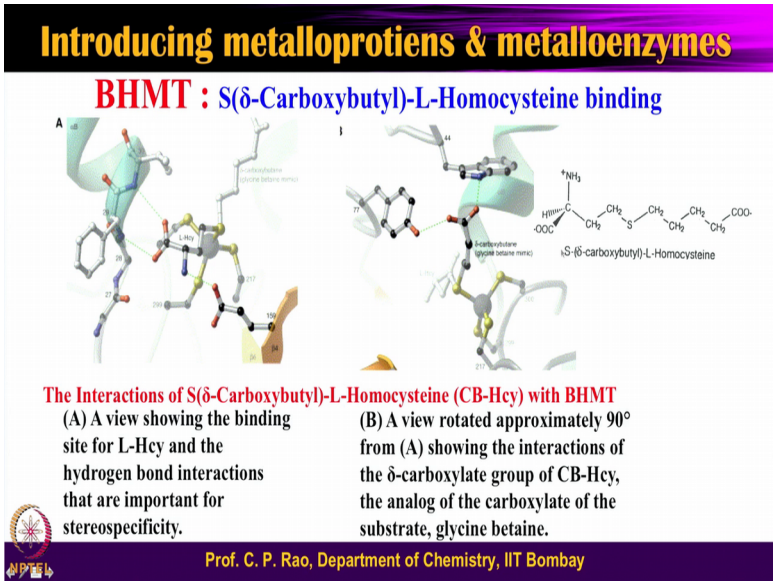

And let us look at another issue which I have not brought to the notice to you earlier, but now I will bring there is a last structure; what is this one. So, this is nothing, but the. So, homocysteine glycine betaine transition state. So, you have to you are forming the transfer of the methyl group before that; there is a kind of a intermediate step, you can see that intermediate step, once the intermediate step is the going to the reaction then the methyl transfer will take place. So and when in that kind of a case, we will get in one

case NN dimethyl glycine and in the other case, you will get the methionine. So, if the methyl group goes over here, this will get the methionine and this will become NN dimethyle. So, this is an intermediate.

So, first the two the basic components will bind to the protein or the enzyme accordingly by the recognition process and then form an intermediate form a transition state and you can see that is the kind of thing. This is still long bond and once this bond is shortened the methyl will be transferred over there and this whole thing is bound in the region of the zinc containing one ok. So, as you can see this is one of the view, this is a perpendicular view to that, and that is how your molecule of the intermediate the two substrates joining together of maintaining this one this bind.

So, this has to have be very close to the zinc as well as the disulfide formation. So, redox is. So, here you can see homocysteine and the hydrogen bond interactions important for steel stereospecificity, you can see that this is the part of the homocysteine which is interacting with this is we called as a recognition. So, in any of the enzyme reaction the substrate recognition is absolutely essential. So, that is what you are trying to see that part of it is. And this is another view of the a view which is about perpendicular to the previous one which is 90 degrees from that showing the this.

So, this will show the carboxylate group of a homocysteine analog of the carboxylate group that is substitute that is the glycine betaine that is interacting. So, you could see both the things. So, you see here this is the carbox, this is one one and you are looking at this ones in the other end 90 degree, you are looking at this one.

So; that means, this particular the two substrate, which are bound over here are basically recognized by a variety of hydrogen bonds around the zinc center in the protein; that means, when this comes in a lot of conformational change is important and that happens. And that leads to this particular transition state, this is the basically a transition state species and then when becomes a shorter, then it will become the regular species.

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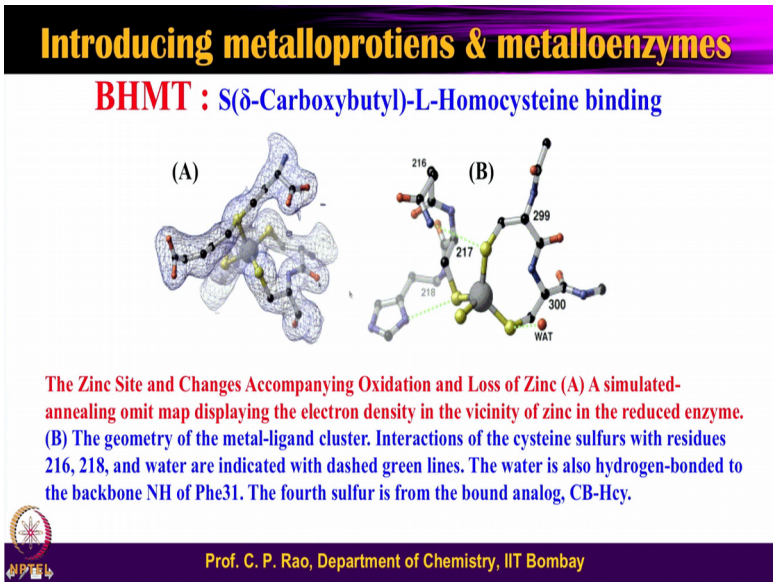

So, the same thing which is shown over there for the butyl homocysteine binding in this both the things and then you will undergo, this is not very important because I have already explained to you. The zinc site and changes accompanied the oxidation and the loss of zinc and this is the A and; that means, zinc is basically out of the coordination sphere that is what it means basically, means is this is basically a simulated annealing omit map that will show you where the zinc supposed to have been there in during the displacement and this is an electron density map in the vicinity of the zinc in the reduced enzyme.

Now, the B what you have is geometry the metal cluster as you can see metal ligand cluster and the interaction the cysteine sulfur with the residues it is 216 and 218 and the water are indicated by the dashed kind of a species over here in lines. So, the water is also hydrogen bonded to the backbone of NH of the phenyl phenyl alanine in 31.

So, the fourth sulfur is from the bound analog of the homocysteine homocysteine; this complex of this. So, that is so, first of all it should bind to the two parts of the thing and this is recognized and then the transfer takes place in the transfer is also triggered by the redox and the enzyme goes from the reduced to the oxidized and that is happening not because the zinc 2 plus undergoing anything, but it is because of the cysteine is undergoing 2 of the cystines and undergoing to oxidation of the of the disulphide.

And this is how the transfer reaction transferees reaction betaine homocysteine methyl transferase and as you can see at the end in this process the glycine betaine will go to NN dimethyl glycine and the homo cysteine will you go to the methionine and this is what is exactly happening in this.

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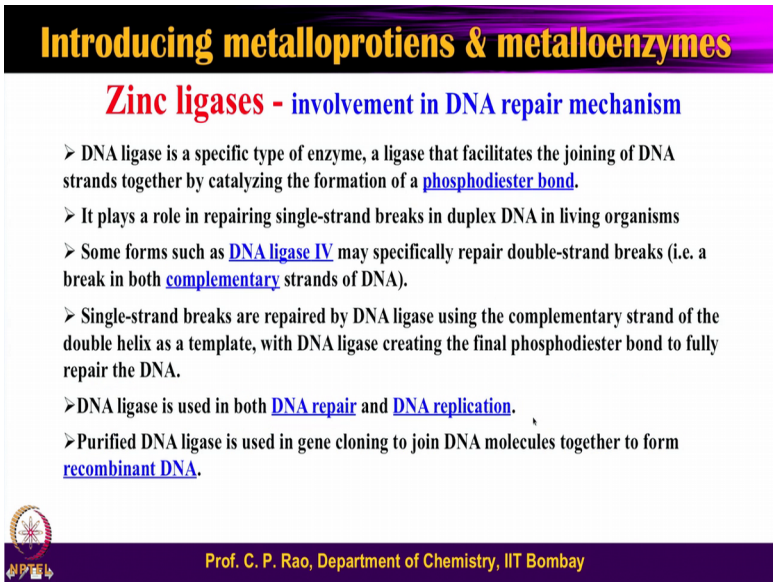

Now, let us look at another kind of a property or a function of these enzymes ligase. Ligase means basically a kind of a repair from the two pieces make a bond. So, bonding binding two pieces to other is the ligase activity. So, the ligase is involved in basically DNA repair mechanism and the DNA is cleaved either in the single strand or it is involved in the double strand there are different kinds of ligases are there.

So, these ligases will re repair this particular broken piece so, but by basically by catalyzing the formation of the phosphodiester bond. So, this is an help in making the phosphodiester bond in this particular thing.

So, therefore, it is basically a repair kind of a thing it can be doing the single strand breaks it can do or it can do the double strand break that there will be different kind of thing; So, repaired by the DNA ligases using the complementary strand of the double helix as it templates. The DNA ligases creating the final phosphodiester bond in order to fully repair the enzyme of the DNA.

So, as you know very well in the body is continuously the DNA cleavage keeps happening; the ligases act on this and repair. So, that is come which is happening because of the chemical assault it can happen, because of the radiation assault it could be sometimes UV radiations can also create this one. So, DNA ligase is used in both repair as well as replication also it is there ok.

One another system which is used in the DNA binding, I will show in the later on, but right now, let us look at this I called the gene fingers of the things that I will show you in a while. So, the purified DNA ligase is used in gene cloning to join the DNA molecules together to form a recombinant DNA also. So, it is absolutely involved in it.

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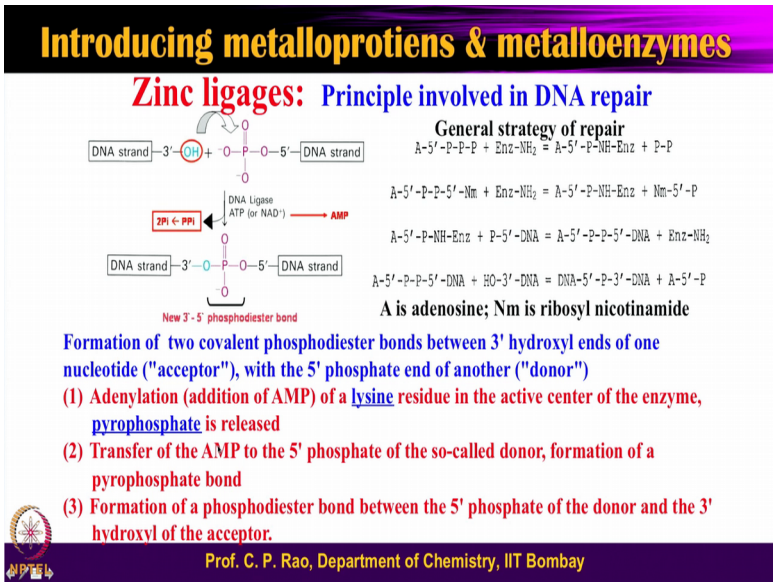

So, as you can see the principle only we I will explain I will not be going into the more detail. So, do not bother about more details of this. Now, you have on the left side 3 prime system, on the right side, 5 prime system and these two joining together. So, when the hydroxyl and the P O thing join together; what will happen a water will be last because its condensation and a die ester bond of the phosphatase formed.

So, in this case, it will be 3 prime, 5 prime because one of the end is coming from 3 prime; the other phosphate end is called hydroxyl end is coming from the 3 prime phosphate end is coming from the 5 prime, therefore, when these two join together, you will get a 3 prime 5 prime phosphate diester bond.

And this is being the when this reaction happens, it will consume the ATP and the ATP will lose out phosphate as well as the amp and that is what you are seeing a one on this direction one on this direction. So, this is a energy process, it requires a lot of energy in this. So, therefore, you can see all of them the 3 prime the 5 prime and the ATP utilization and then finally, bond formation. So, you have a 3 prime strand and the 5 prime strand together.

So, this will lead in the formation the two covalent phosphodiester bonds between the 3 prime hydroxyl ends of one of the nucleotide which you call called as a acceptor and the 5 prime end of the thing which is phosphate which is a called donor in this case, in this terminology, this is the called the acceptor, this is called the donor and then you will find.

So, therefore, adenylation that is addition of amp of a lysine residue in the active center of enzyme will happen because the amp is coming how this will make that one. So, first for pyrophosphate is released out of that pi, ppi, etcetera, pyrophosphate.

So, the transfer of AMP to the 5 prime phosphate of the donor through the formation of a pyrophosphate bond; so, formation of a phosphodiester bond between the 5 prime or the phosphate side and 3 prime of the hydroxyl side, this will form. So, this is the kind of a way; it is a principle based only the principle based. So, you have making the bod. So, these are how you actually repair. So, initially convert into the adenylation, etcetera. A is adenosine NM is the ribosome nicotinamide and these are have to bind to the enzyme bind to the enzyme and then do the reaction.

So, I would say you do not need to go more into detail of this, but you better understand this principle part of it, it is only just the principle part of it.

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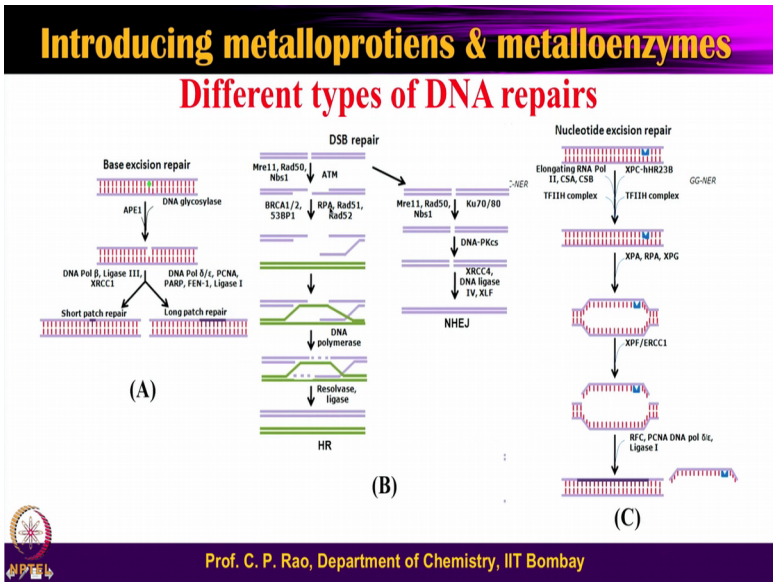

So, this can happen in the way there is explained under A, this can happen under B, this can happen under C. So, there are very many ways of wearing the DNA and please do not bother about all these details of it, the main thing is that the two ends of the broken one are recognized by the enzyme. So, one is that hydroxyl other is this one phosphate and then these two are brought together and these two are branches just stitch together and that is what the principle if you understand this good.

So; that means, zinc enzyme is involved in a ligase property in replication property in DNA binding property all of these kind of things; one another system is also bound to the DNA.

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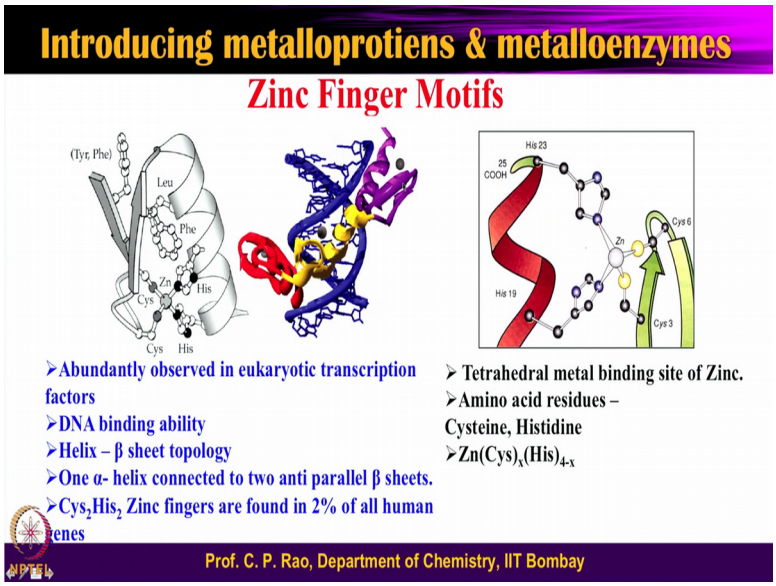

A DNA protein interactions is the zinc finger motives as you can see there are beta sheet alpha helices between it is a kind of a joining between the beta sheet and the alpha helix. So, this is observed in large number of a transcription factors. So, the this can bind very nicely with the DNA. So, helix and beta shape topology; so, you where you have a helix and beta sheet are orienting in this way and the histidines and cystines are coming in the proximity there you will get a zinc finger.

So, one alpha helix connected to the two anti parallel beta sheets. So, cysteine 2 times histidine 2 times are present to the zinc bound binding centers in fingers found in 2 percent of the human genes. So, a lot of human gene binding things. So, this is basically used in recognizing the DNA by protein DNA interactions.

So, what you have you have tetrahedral binding side you have a zinc at the center 2 histidines and 2 cysteines are binding. So, it is a zinc cysteine twice histidine twice. So, you have a slightly different variation cysteine x histidine 4 one; these are all known variety of depending upon different kinds of enzymes. So, is one of the important factor, I am not explaining you any function on this just to explain these kind of things.

One last enzyme, I would like to cover for you is that it is not only hydration not only oxidoreductase not only the ligase property we can also have isomerase isomerizing some groups.

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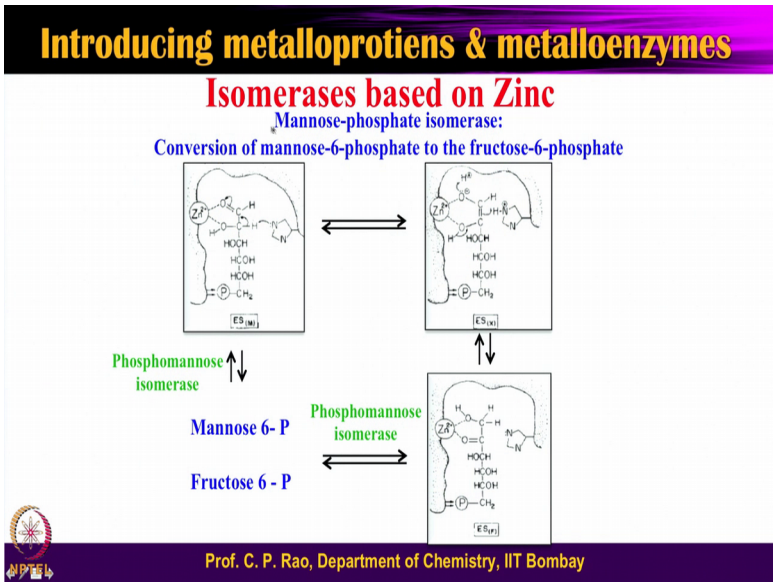

And the example taking is mannos phosphate isomerase; that means, the it isomerases the mannos moiety. So, this converts mannose 6 phosphate into the fructose 6 phosphate it converts mannose 6 phosphate in the fructose 6 phosphate.

So, basically you have your zinc center in the enzyme where the substrate binds and that is mannose 6 phosphate and the substrate in substrate interacts through its C 1, C 2 carbons or C 5 C 6 carbons here through this zinc center and that triggers the conformational change as well as the reaction the neighbor imidazole or histidine will trigger the reaction by the protonation deprotonation steps of this. And this will form an intermediate and then this enzyme will work on this mannose 6 phosphate or fructose 6 phosphate depending upon which direction you are going and they finally, get back to this.

So, this is what this is nothing, but an isomerase. So, you are isomerizing the centers, you can see that you are converting the mannose to the fructose kind of a situation. So, enzymatic reactions are taking place in these ones you can see that. So, you can see that these 2 OH H H in this one. So, this will be opened up when it is opened up you will see the difference between the first mannose part of the and the fructose part of that.

So, say mannose 6 phosphate. So, for this it has to bind, it has to have a deformation kind of a step from over here and therefore, it will it will rearrange because this will take into a double bond system and that will basically take the negative charge over there and that

is what brings in the rearrangement to this. So, you have a enzymes are into this kind of thing ok.

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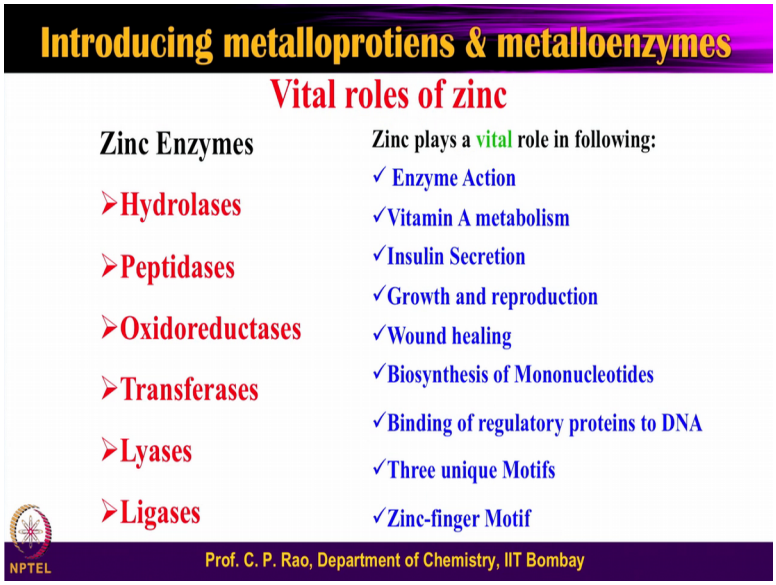

So, what all we have seen in the zinc enzymes we have seen there are 200 different kinds of zinc enzymes are present in the human body. Some of the reactions are water addings and this can also do the condense bond breakage of peptide breakages; you can also do a molecules to undergo redox like between alcohol to aldehyde aldehyde to alcohol oxidoreductase.

Then we have also looked at the lies is the water adding water to the carbon dioxide, then we looked at the transferase; transferase way the methyl group is being transferred; during which again the enzyme undergoes redox. There is a this redox is not at the zincs and not by the zinc ion, but by the zinc bound cystines there are three aldehyde which are bound in the reduced form of the enzyme and when the enzyme oxidizes two of these cystines will come out.

And form a disulfide bond and pushes the zinc ion out. So, in the process that you have or the redox of the enzyme and then the substrates are bound betaine glycine and homocysteine. So, therefore, between these two there is an intermediate is formed and the as a methyl group is being transferred its very ok.

Then one other enzyme that I have explained to you is the ligases in the ligase is that you have the two other bonds the hydroxyl and the phosphate and 3 prime and 5 prime ends are joined together this is a highly use useful in repairing the DNA in repairing the DNA where there is a break has taken place.

So, therefore, enzyme has to recognize the both the terminal of the repair of the broken compartments and then thereby bring them together and make join together to form a bond. It can form single strand case cystenines, it can also rectify the double stranded seasons or breakage all these together and in this ligaes, I have not given any kind of a mechanism, I only just give how to visualize that is good enough.

And isomerase; so, there is one more enzyme here which I am not shown here is the isomerase. So, isomerase is basically I summarizes the carbohydrate position that meets with the mannose towards that of the fructose 2 for this; what is required the mannose has to bind at the 5-6 and there is an attack by the neighbor the histidine imidazole part of that which is involved in the deprotonation of that and this converts the carbon center and then brings a rotation for this and this particular change brings the change in the carbohydrate moiety.

So, thus we have seen hydrolysis liaise peptitade activity in oxido reductase activity we have seen ligase activity we have seen the transferase activity we also have seen the isomerase activity. So, that brings to a conclusion of zinc based story and in the next class, probably and hopefully, I will take up the enzymes corresponding to molybdenum and then we will try to continue on that for some couple of classes or so.

Thank you very much.