Metals in Biology
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Lecture - 08
Hydrolytic enzymes - Part I Carbonic anhydrase & liver alcohol dehydrogenase

Hello, today we will discuss some of the reactions right that enzymes are carrying out quite naturally and quite efficiently. How they work? How the metalloenzyme works and how a simple reaction can be converted into product and the efficiency goes up multiple times? We will discuss today the Hydrolytic enzymes.

That the as the name says it will mainly involve the hydrolysis, right

to book to follow would be that by Principle of Bioinorganic Chemistry by Lippard Berg as I said, earlier also this is the book mainly we are following, but there are a number of other books you can read. Some of the slides are prepared from this book and also the materials available online and materials available from MITs lecture series and talks by professor Lippard and others are included over here in different slides throughout these courses ok.

So, some of the references are given, if you have any queries please let me know.

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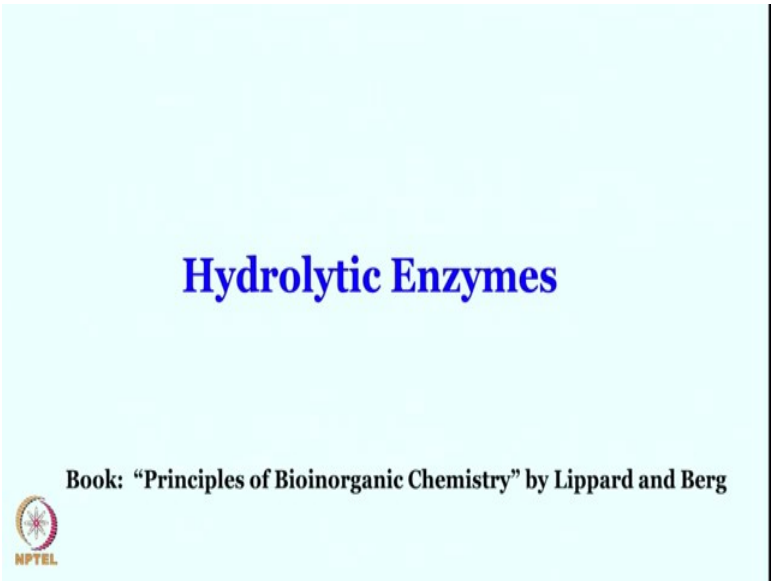

So, let us get into the hydrolytic enzymes well.

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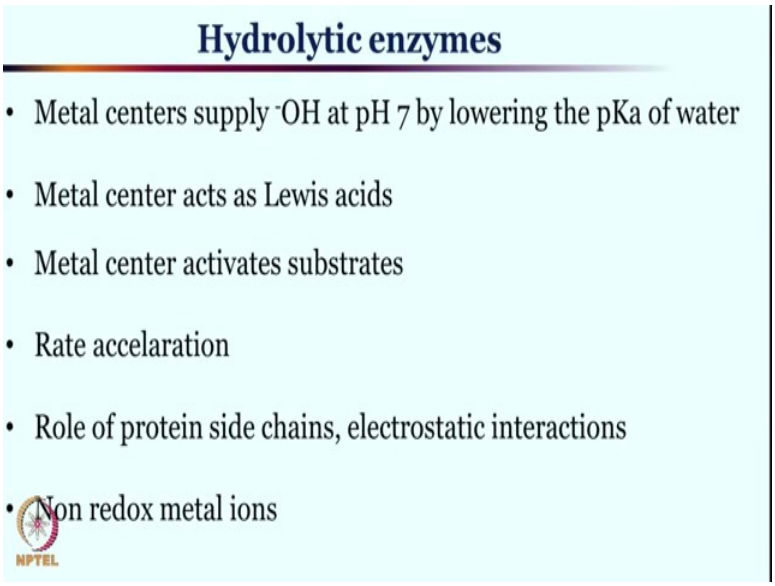

Well, the role of these metalloenzymes simply is to promote hydrolysis or similar reactions. I think the main important things to understand in these reactions or in these cases that over the time nature has evolved in such a way so that metalloenzymes are found to be the best we can have in transforming a simple reaction and making it extremely beautiful.

A simple reactions, for example, conversion of carbon dioxide to carbonic acid can be made faster and extremely efficient by the enzymes. These hydrolytic enzymes is a group of metalloenzyme basically of similar activity or similar kind of activity are having a metal center at its core. Of course, there are protein residue, protein backbone, from those protein backbone the side chains of amino acid side chains are appended some of those amino acid are binding with the metal and acting as the ligand for the metal center. By doing so, metal is now really feeling powerful and can do a metal chemistry that would otherwise not happen, that easily or the rate of the reaction is very slow otherwise.

Now, these metal centers which are chosen for the hydrolytic enzymes, essentially does one thing very efficiently and that is their Lewis acid. Metal center is acting as Lewis acid and therefore, the substrate that organic substrate that we are interested in looking at or the enzyme is interested in reacting at will become more powerful and more reactive.

Well the role of the metal is essentially to see that we will get an supply of hydroxide from the water molecule at physiological pH and that is pH 7. The way metal does it binds with water lowers down the pKa of water.

So, that at pH 7 hydroxide can be delivered. Metal center as I said will act as a Lewis acid metal center activate substrate both it does show it acts as a substrate it acts as a Lewis acid as well as it activates substrate during the process. It enhances the reaction rate and more of a role of protein side chains and electrostatic interactions. We will see in a moment that how they facilitate these hydrolytic enzyme.

Of course often non radox metal ions are used that means, other side reaction remain constant.

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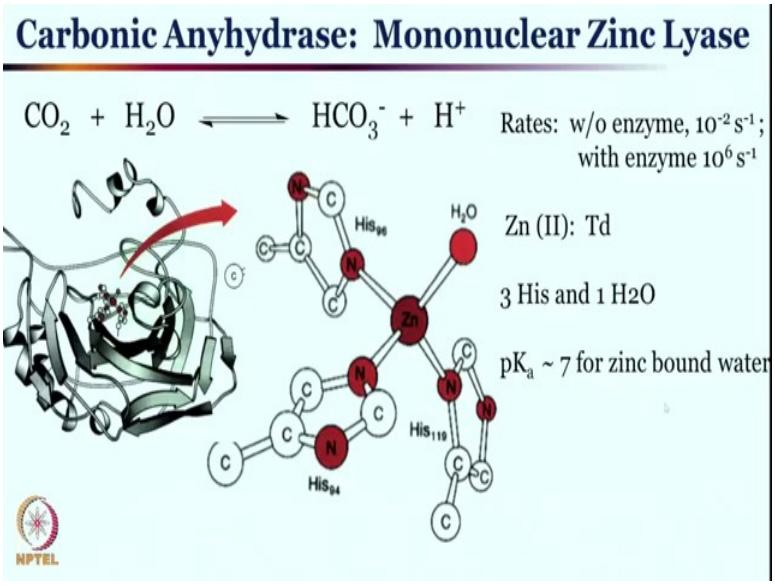

So, we will see these examples very soon; let us look at the carbonic anhydrase. It's a simple enzyme, it is a mononuclear zinc enzyme as you can see in the active site this is the active site ok. So, if you look at the whole protein, this is a very complex structure as you can see lot of protein backbone and residue, but overall you see that in the middle there is this active site which is acting quite beautifully in converting carbon dioxide and water into carbonic acid. Overall so, zinc is supported by 3 histidine, histidine 96, histidine 94 and histidine 119; what that means, is this protein backbone these are the sequence that is participating in holding the metal ion and there is of course, a water molecule; this is the same water molecule that is bound over there.

So, what you have just seen that, protein is very complex, but if you zoom down to a center where reaction is happening, we find that this is the coordination compound it is a simple metal complex supported by 3 nitrogen containing ligand and then there is a water molecule. The geometry around this zinc center is tetrahedral as I said this has 3 histidine and 1 water molecule and the pKa of this water is nearly 7 for zinc bound state.

Rate of this reaction without enzyme is very slow, this reaction carbon dioxide converted to carbonic acid is very slow, but with enzyme as you can see there is a 10 to the power 8 fold increase in reaction rate. That means, the enzyme has a role this metal center has a role and that role is essentially nothing but promoting this reaction efficiently by acting as Lewis acid so, that water molecule combined and this deprotonation water to hydroxide formation occurs where in a very facile manner right. Let us look at this reaction mechanism as you could imagine perhaps, this reaction is going to be very simple and simple perhaps anyone can think of.

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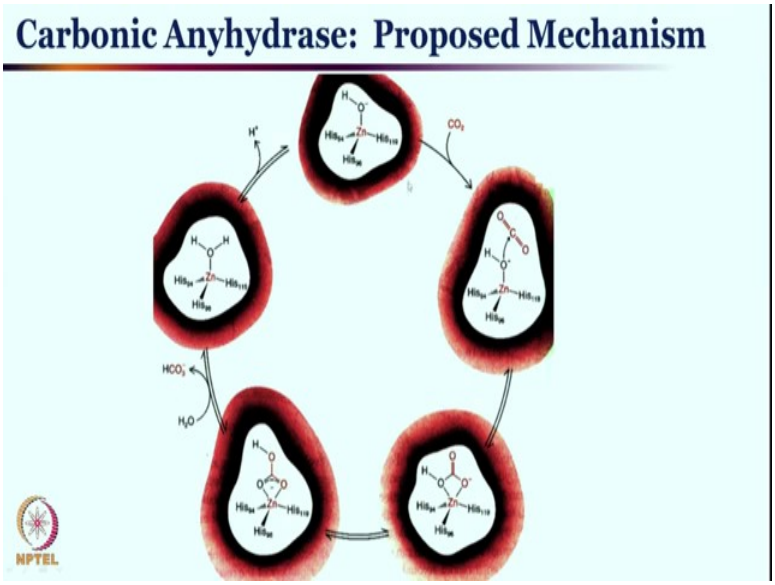

This is one of the simplest reaction but of course, this represents this is these pictures are all from Lippard's book. This represents the active site structure.

So, of course, we can start from this zinc site as we were seeing in the last case there are this 3 histidine bound with water deprotonation of the water is happening quite easily because it is bound with Lewis acid zinc. So, the deprotonation is facile at pH 7 under physiological condition, this deprotonation is now feasible of course, this is a reversible

step, but we managed to then form zinc hydroxide. This is only possible because of this metal center and also you see that there is this you know protein backbone, protein residue that is making it perfect for giving this atmosphere around the zinc center and more importantly it is also allowing carbon dioxide to preposition itself or the positioning of carbon dioxide is perfected in front of the metal site right.

So, this is what we see, zinc hydroxide is there which is a nucleophilic site this hydroxide which is a nucleophile will now then attack on the carbon dioxide. Carbon dioxide is almost fitted in a cavity where this organic substrate binding pocket is there. So, carbon dioxide is feeling welcome over there and then hydroxo is attacking over there ok. Once a nucleophilic attack is occurring you generate the intermediate which is which is leading to further product formation and water molecule comes out and releases the product.

So, what you have seen so far is quite interesting reaction it is although a seemingly very simple reaction carbon dioxide reacting with water but the orientation the fact that there is a zinc metal which is a non redox metal. The fact that it is a tetrahedral center and it is supported by this peptide backbone very beautifully. It allows also the carbon dioxide the whole architecture of the protein or the metalloenzyme allows the carbon dioxide to orient in such a way so, that the hydroxyo and carbon dioxide is very close to each other right and therefore, this attack become very facile.

So, this short of reaction if you want to do in synthetic setup; that means, in your laboratory the rate of the reaction will not be that fast. Let us say you managed to synthesize a zinc complex which is similar to this, you managed to get this complex also, but this orientation of carbon dioxide in front of this active site that is not easy to manage as it is managed in enzyme. Because enzyme is really perfect; it has perfected this skill over long time right and in now it is in such a situation that everything is perfect this is the perfect you can get, but creating that perfect atmosphere in synthetic laboratory will not be that easy and that is why it is perhaps never possible to have a system which works better than nature, but if we can anyway get this sort of reaction that would be great in terms of synthetic catalysts right.

So, this is really one of the benefit of studying this sort of chemistry so, that you start understanding nature. The way nature does you can get inspired by that and then design

your catalyst the way nature is doing and by doing so, perhaps you will be able to manage to synthesize a catalytic reaction or a catalyst which will do this reaction exactly same reaction. Because if you can convert let us say carbon dioxide to this bicarbonate or carbonic acid that is a fantastic reaction a lot of industry will be interested in finding out if it is the best reaction ever right. So, this sort of catalyst development and understanding of the enzyme is crucial if really we have to take our synthetic scale skill to the next level. Let us look little bit more on the some other hydrolytic enzyme for example, liver alcohol dehydrogenase ok.

Well this is the enzyme that all those alcohol that is produced in our body or any consumes consumption is there.

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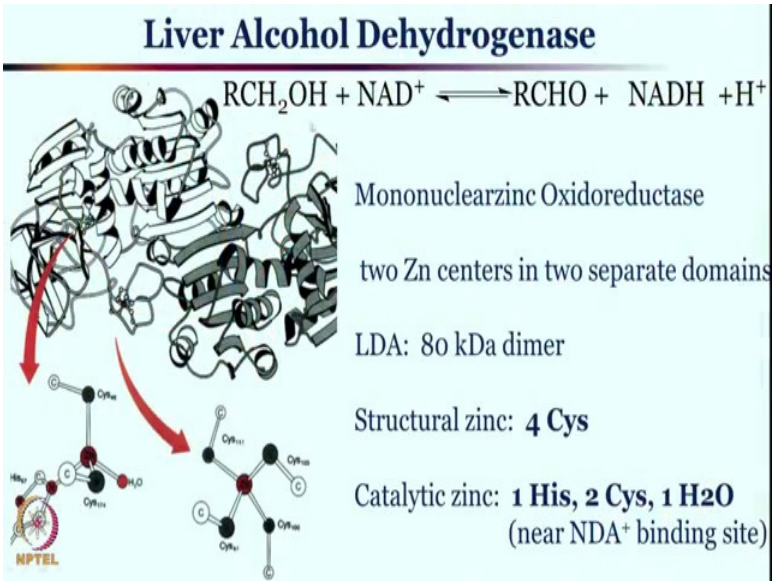

This can be converted the alcohol can be converted to aldehyde by this enzyme, liver alcohol dehydrogenase. This is in our liver, it converts let us say ethanol into acetaldehyde. Of course, this subsequent acetaldehyde can be picked up by other enzyme to convert further further oxidized product. But today's discussion is mainly focused on how alcohol is converted to aldehyde and in an very very efficient manner ok.

Now let me tell you that these are these are once again zinc active site. There are two zinc sites one of the zinc is not participating in this reaction directly. This is providing basically the structural or the architectural architectural context for this liver alcohol dehydrogenase. This is not the active site, but nonetheless this is present this gives the

structure to this to this it helps in giving the proper structure for the liver alcohol dehydrogenase. This is a dimeric compound. So, 2 monomer units are there each of the monomer will have 40 kilo Dalton molecular weight right.

Now, the structural zinc as you see has 4 cysteine 1 2 3 4 four cysteine units are there as you can see this numbering represents the side chain from where it is originating. For example, 111 and 97, 100 and 103; these are the side chain residue from which they are arising and binding with zinc ok. The main active site as you can see is over here. So, this whole structure is for liver alcohol dehydrogenase and this is the side, a little bit center a small center where you have the real active site ok.

So, all these architecture of the enzyme actually first of all prevents the active site from any damage. In addition it also ensures that substrate orientation is perfect, substrate approach is perfect, substrate its getting diffused or channeling through these metalloenzyme pockets and reaches in right in front of the active site right.

So, that is the role of well of course, among others those are the crucial role of this huge architecture. That substrate will be welcome and substrate will find its way to come to this active site or towards the active site and it will be positioned perfectly in front of this let us say zinc active site so, that any other site reaction does not happen. Any other residues that is required for this reaction from this protein backbone will also orient throughout these orientation. You will see many electrostatic interaction hydrogen bonding interaction that are playing key role in making this reaction extremely facile. Once again these reactions may be very simple looking, but the type of speed or the rate of the reaction that you see is phenomenal. Something of that significant I think is very difficult to achieve other than those in enzyme.

So, the catalytic site the zinc you have 1 histidine, 2 cysteine and 1 water molecule. So, this is your histidine molecule, this is your cysteine molecule, another cysteine molecule is there another water molecule is there. So, this is the four coordinated zinc; remember in the previous case carbonic anhydrides you have 3 histidine. Now you do not have 3 histidine, you only have 1 histidine and 2 cysteine right.

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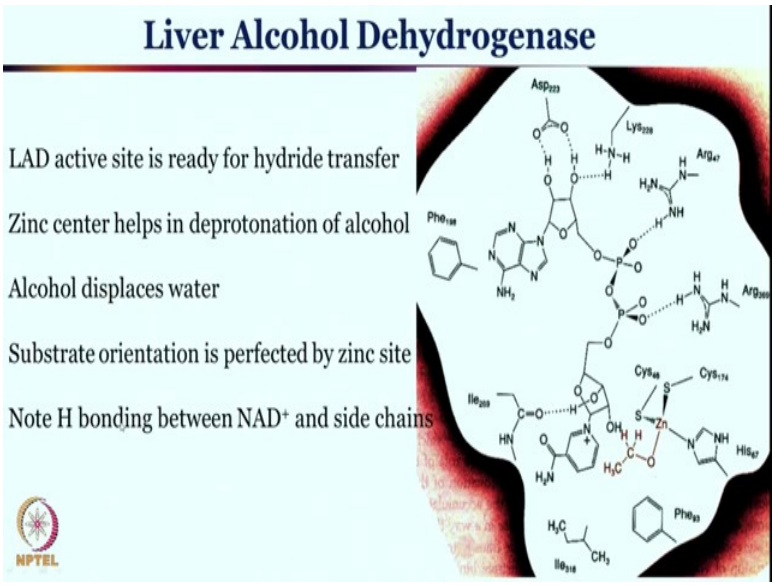

Now, if you look at the reaction overall what is happening here that as you have seen the major reaction, the alcohol is converted to aldehyde right. So, in the with the help of NAD plus, NAD plus is converted to NADH and a proton is released.

Now, how it overall this reaction occurring? Now before that let us try to locate our active site. So, this is the active site that we were mentioning in the last slide. So, the zinc site is there, S cysteine is there, cysteine is there, histidine is there. So, two residues this is the third residue and this is the site where water binds the moment, it senses alcohol presents. So, the water goes out and alcohol is replaced replacing the water upon binding to zinc the deprotonation of O H from the alcohol are CH2OH or acid this is ethanol this OH gets deprotonated to make alkoxide complex.

So, this is a zinc alkoxide complex, but I think the most important part in addition to deprotonating this alcohol is the positioning of the NAD plus. So, this whole architecture is NAD plus right this whole compound is NAD plus, now this NAD plus is positioned perfectly I think this is this is what is phenomenal that it is right over here it is not over here it is not over there or anywhere it is right where this CH bond is present right. So, if this hydride has to attack over here it is really perfectly positioned nothing is stopping it. So, the rate of the reaction will be the best ever, I would say that is why this liver alcohol dehydrogenase are so, effective right.

So, this is the part where we will see the oxidation overall oxidation of alcohol to aldehyde will happen and that is essentially triggered or started with the formation of zinc alkoxide by deprotonating the alcohol. Well you notice that NAD plus is not alone there is many side chain also from other protein residue or protein backbone these are coming and the hydrogen bonding. This is helping over all see this is also hydrogen bonded these are hydrogen bonded. Overall all these hydrogen bonding is helping the NAD plus to be positioned right in front of the metal active site. I think realizing that and realizing many mainly the power of it is quite essential and important.

So, LAD active site is ready for hydride transfer as you can see this hydride if it has to transfer it will transfer right over here at the center right. So, in it is essential to understand in the next slide we will also discuss actually this drawing is little bit not perfect; this hydride will be transferring over there. So, that is how the orientation is LAD active site is ready for hydride transfer, zinc center helps in deprotonation of alcohol right that we have discussed.

Alcohol displaces water that is also we discussed, substrate orientation is perfected by zinc site right. So, this is the substrate and this is also another substrate both the substrate orientations are perfected by the zinc site and we have also seen that many different hydrogen bonding, that is present over there that is helping in bringing the LAD plus where it should be right in front of the active site.

Let us look at let us zoom down this center little bit details if you are looking at let us look at it little bit more carefully.

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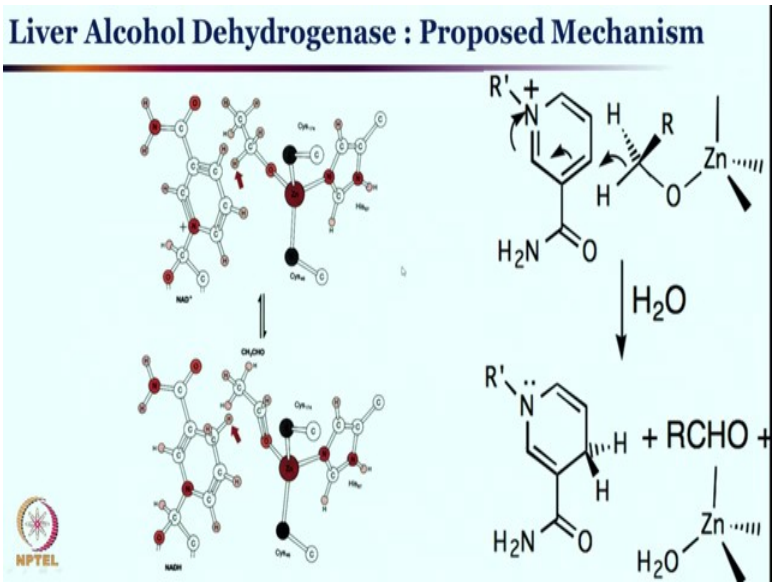

What we find over here the same thing we are drawing here. So, zinc alkoxide is over here, this is now O minus CH2CH3 and as you have noticed this is the pyridine nitrogen, the para-position with respect to this pyridine nitrogen that one this is where the hydride transfer is happening. So, overall it is a double bond formation from here from this O minus double bond formation and then hydride comes out and attack on the this fourth carbon center. If you are looking a double bond for here hydride is attacking on this carbon center to make it NADH.

So, overall then if you are getting it correct now so, alcohol is converted into aldehyde into this process and zinc gets back its water because the aldehyde that is forming over there is not a great ligand compared to even let us say water. So, water will come in and bind with the zinc site and NADH is produced over here right.

So, what you have seen simply then, the protein architecture is protecting the metalloenzyme active site and orienting the NAD plus and the alcohol substrate in such a way so, that alcohol upon binding with the metal center can be deprotonated and right over there when it is or where it is deprotonated there is NAD plus waiting for it to react with it and therefore, alcohol is converted to aldehyde in the process upon deprotonation and the hydride transfer hydride transfer to the NAD plus that is a very powerful reaction. Without these enzymes or without these active sites this reaction may be possible, but those reactions are not that facile not that effective right.

In synthetic chemistry these reactions are not unknown, many reactions are known which can convert alcohol into aldehyde its a simple oxidation process, but those reactions in synthetic setup is nowhere in competition with the enzyme setup because as you have seen enzyme have taken advantage of electrostatic interaction, hydrogen bonding interaction NAD plus, zinc active site, deprotonation and everything right. So, this reaction is quite beautiful on its own right and mainly because this is so, effortlessly done so, precisely done ok. Now we will move to a new enzyme and that is carboxy peptidase.

Once again you will see as you have seen now that this is a zinc enzyme. So, in the first 3 enzyme we are trying to discuss our zinc enzyme, we have discussed carbonic anhydrase. We have just discussed liver alcohol dehydrogenase and we are discussing carboxypeptidase now.

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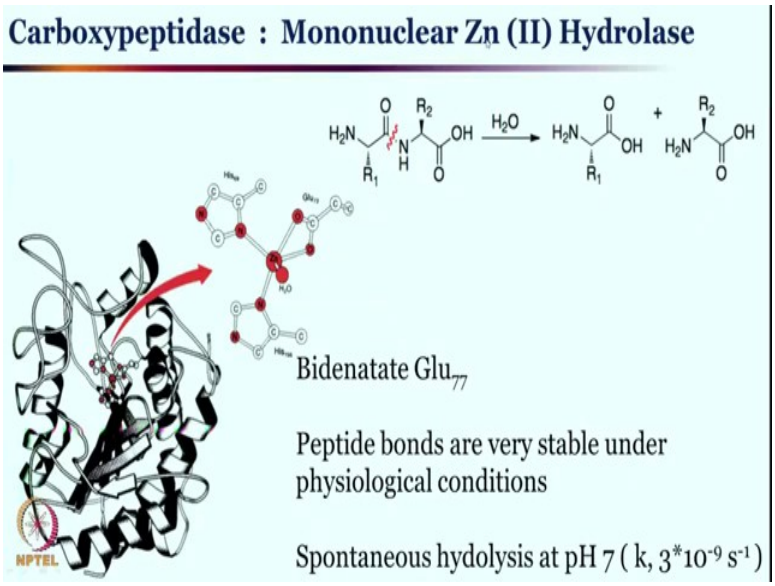

Before discussing carboxypeptidase let me go back once more to this liver alcohol dehydrogenase and let me tell you that this is the reason why or you know why this alcohol consumption is not a great idea in too much quantity. Because whatever alcohol we are consuming or drinking those are going to be taken care by this liver alcohol dehydrogenase to convert it to aldehyde and then further oxidized to carbon dioxide and water and all the fragmentation.

Of course, some of the amount can go out by urine, by sweat, but still a large majority of the alcohol can stay in our body. Those need to be taken care if it is not getting out it need to be taken care by this LAD Liver Alcohol Dehydrogenase and although these are great enzymes can do reaction excellently, but the amount of alcohol makes it sure a large difference. If it is too high slowly, but steadily your liver gets damaged because it cannot handle this much of alcohol anymore. It can convert some, but not like what you know person can maximum consume day after day.

So, this is why ones really need to take care of the drinking habit, this can lead to the permanent liver damage this is one of the reason why right. All we you have seen different you know you know throughout the society, you have seen different bad effect of the alcohol that that one can one can pretty easily identify right. So, by knowing these and understanding this enzyme indirectly I am sure will make you aware that we need to take care of our body Because no matter how good this machinery jar we have excellent machineries the best in the world, best in the you know in the universe perhaps, but nonetheless these need to be taken care. Just because you have an enzyme does not mean that everything is all right.

Well of course, there are other enzymes that is participating in the whole process of converting the alcohol into something that is no longer danger to us, but you know some of these enzymes are not present in large amount or the quantity that we should have in every human. So, that is why in some human we see that alcohol intolerance is there.

So, some of the people you may have seen or might will encounter just when they drink even a drop of alcohol, they becomes completely red right. That is because perhaps I mean mostly because this enzyme or one of those supplementary enzyme that that we should have or they should have in their body is are not there in adequate amount and cannot manage the any alcohol efficiently.

So, there are many side effects like these skin rash lot of people have alcohol intolerance right. So, they can have skin rash, redness of the skin due to the malfunctioning or or not enough not having enough of this liver alcohol dehydrogenase. It is of course, in a way it is genetic also, it depends on the region let us say let us say some of the Asian people are having such problem. Of course, it can occur in anywhere in the world, but it is in impart also genetical right.

So, so I mean there is nothing to really think too much here I think most important thing is we have to really take care of the alcohol consumption, and not everybody is going to react to alcohol equally, but it is bad in large quantity for everybody ok. With this in the next class, we will discuss carboxypeptidase where we will see once again another mononuclear zinc hydrology enzyme ok.

Thank you very much. See you in the next class soon.