Metals in Biology
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Lecture - 09
Hydrolytic Enzymes- Part II
Carbopeptidase

Hello welcome back to Hydrolytic Enzyme we are discussing Metals in Biology. In the last class we have seen two different hydrolytic enzyme which are carbonic anhydrase and liver alcohol dehydrogenase. In both the cases we have seen there is a zinc center which helps you in deprotonating either a water molecule or the alcohol molecule. And, we have also seen how enzyme backbone or protein backbone overall architecture of the protein has helped the active metal site to react and therefore, making the reaction perhaps best ever.

We have seen carbon dioxide is converted to carbonate or HCO3 bicarbonate and so, carbonic acid and then we are then we have seen here is alcohol which has been converted to aldehyde. And, these are all metalloenzyme not only metalloenzyme metal sites, but the protein sides chain has played a tricky role in providing an atmosphere so, that situation becomes much more exiting. Today will see yet another zinc enzyme that is carboxypeptidase. The role of this enzyme is hydrolyzing the peptide backbone or the protein residue right.

So, peptide backbone which are nothing, but consisting of the amide bonding that can be hydrolyzed; you know in any case these peptide backbone or peptide bones are quite strong they are not that easy to hydrolyze because these are amide. Amide hydrolysis is difficult because there is nitrogen lone pair which is delocalized into the carbonyl moiety of the amide right.

Now, such a seemingly difficult reaction is rather easy for an enzyme to cleave. There are many occasion where it is or manyness many steps where it is required to cleave a peptide bond and that is where carboxypepatidase comes into the picture.

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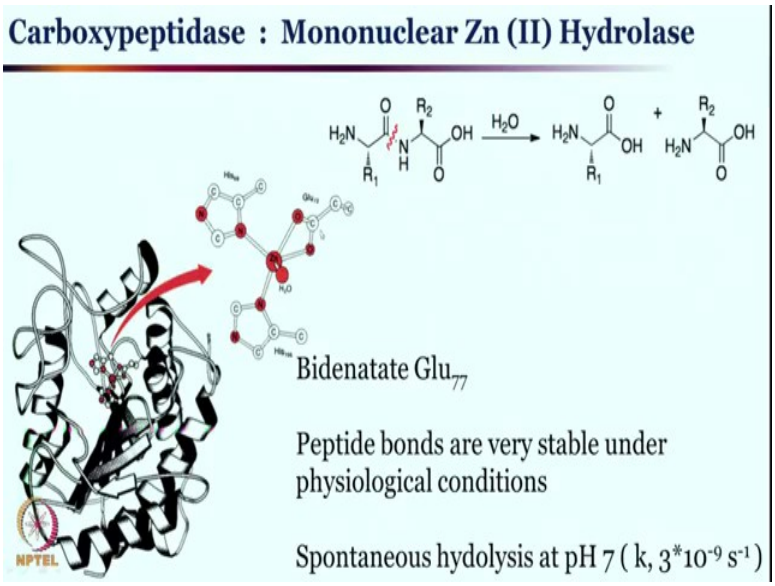

As we mention it is going to be a zinc enzyme 2 histidine and 1 glutamate is there. Quite interestingly this is not monodentate carboxylic acid binding to metal, it is a bidentate glutamate binding on the metal. So, these short of carboxyl acid aspartate glutamate its requires they can act as monodentate or in some talk cases just like as you have seen here it can act as a bidentate ligand. This switch of the denticity from monodentate to bidentate sometime is crucial to open up one coordination site for the substrate binding.

So, depending on the need this carboxylic acid side chain from the amino acid backbone or the peptide it can be really coordinated with the metal center in a monodentate or bidentate fashion. All this spontaneous hydrolysis of such peptide backbone is very slow right itself that is what I was mentioning that, this cleaving such bond is not that very easy. But this simple enzyme which is consist of the zinc and 2 histidine and 1 glutamate; now we can see that this reactions are very facile ok. In the last class you have seen 2 zinc based enzyme, but today we have just seen 1, but none of these three zinc sites you see are having exactly same coordination.

So, in each of the cases the complexes or the coordination complexes that nature has designed are completely different although metal is same all of them has one thing is common at least they have one histidine in majority of the cases that is what we will see, but more interestingly this ligand systems are different from different enzyme right.

Once again this carboxypeptide is very good in hydrolyzing these amide bond into acid and this the corresponding amine. well.

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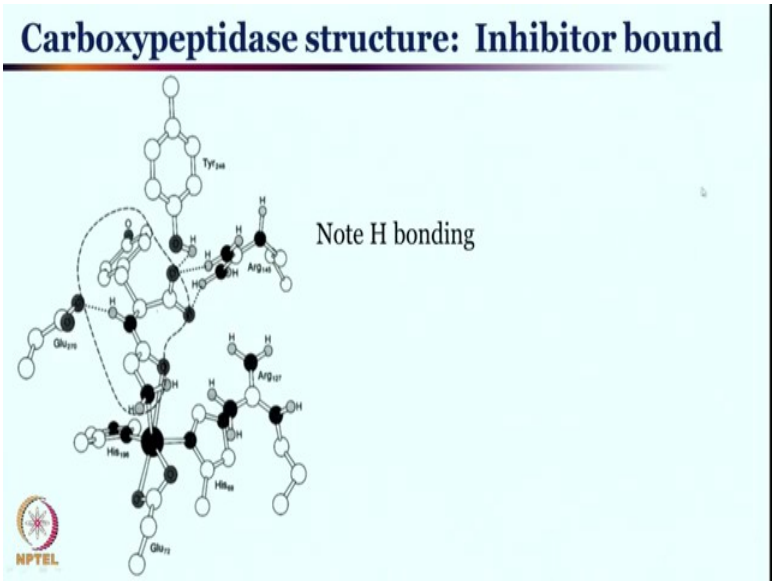

We were interested in seeing how this reaction is working right that is what one of our major goal; that can be done if we are trying to get the crystal structure of this compound right. So, obtaining crystal structure with a peptide backbone is not very easy, because the moment a peptide backbone is there it is going to be hydrolyzed because this is too good in hydrolyzing a peptide backbone and this is where a suitable inhibitor is needed to gain insides into the peptide binding pocket for the hydrolysis by this zinc enzyme right.

So, this inhibitor which inhibits its normal activity of peptide back bone is or has to be something special where the enzyme or the zinc site is not able to hydrolyze it very easily. It was glycyl-L-tyrosine that is used in this case as you can see over here this is glycyl-L-tyrosine that is used this is your zinc site this is histidine, this is histidine and that is glutamate of course other proteins backbone or the residue peptide residues are over there. So, number of side chains are coming into the picture and there is hydrogen bonding overall in helping docking the organic substrate right at the metal site.

So, this inhibitors which is otherwise not a good substrate for undergoing hydrolysis gives a glimpse of the potential substrate binding pocket. If you have a amide over here

which need to be hydrolyzed, where it can bind what are the potential hydrogen bonding site by which this organic substrate can be appended.

So, such a crystal structure is quite valuable of course, you can also see clearly the active site 2 histidine 1 glutamate and that other water molecule that was there is now replaced by this amine group from this inhibitor right. Now if you look at the mechanism of the reaction and the reaction we are talking about the peptide hydrolysis, this hydrogen bonding will also come into the picture and these residues mainly will be the one which will see in the next slide once again.

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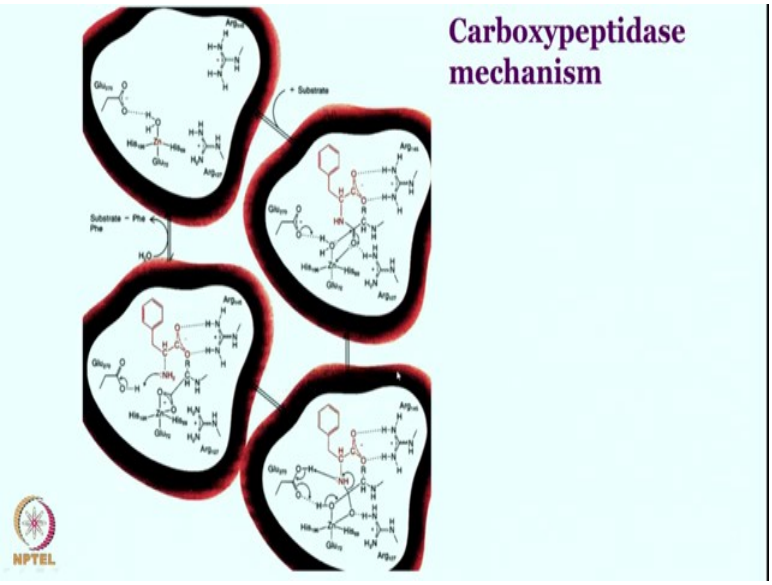

Let us look at it this is I understand little bit complex to look at, but if you zoom down to one structure at a time it is easy. So, these all are from Lippard book. Now, you can see that this is drawn as a zinc 3 2 histidine and 1 glutamate and 1 aqua molecule here no protein residue is there, there is one glutamate you need that is hydrogen bonded to the water molecule, there is a arginine sitting right over there and there is another is arginine in which is waiting or just positioned over there.

As you can see these are not playing too much role right now except this glutamate, now your main active site is over here this is where the chemistry will happen. But these side chains or protein residues are there present. Now, the moment the desired peptide that is shown over here it is little complex structure if you are not paying too much attention this is going to be little difficult to look at otherwise please pay little close attention, you

will see that this is the organic substrate or the peptide that is going to get hydrolyzed right.

Now, the positioning of this organic substrate is such that, the moment zinc hydroxo is generated and the substrate is there hydroxide is going to zinc hydroxide is going to attack on these amide carbonyl you need very effectively. To make it, make the substrate oriented right in front of the zinc hydroxo, you needed this arginine, that arginine of course, glutamate is still there which is helping in deprotonating these water molecules also. See all these side chain not only these are limited with these two arginine there are much more we did not show everything, but all this side chain of the protein of the architecture form your enzyme, will also be extremely crucial in hydrolyzing your peptide backbone as you can see over there.

This hydrogen bonding helps orient or dock the substrate in front of the enzyme perfectly as you can see here. This is the nucleophile that is getting ready; this is the electrophilic center that is absolutely ready. So, this orientation or the positioning perhaps one can never get in a synthetic setup or it is going to be extremely tedious to get such orientation and therefore, nature once again is always the winner to carry out a simple reaction as well as very complex reactions.

So, next step would be the deprotonation of water. So, now, you have a zinc hydroxide formation, you still have 2 histidine and 1 glutamate as soon as this zinc hydroxide is formed as you see it as attacked on the carbonyl site. Now, this sort of tetrahedral intermediate is formed over there, you have a hydrogen bonding from the arginine once again with the with your desired carbonyl or amide compound this hydrogen bonding is there, this is also definitely the whole peptide residue with a carboxylate you need appended from the peptide backbone is helped, docked or helped position perfectly by this another arginine which is arginine 145 right from the from the side chain 145 it is really really perfectly positioned ok.

Subsequently it is a hydrolysis mechanism like it is one of the easiest reaction, one can think of you just need to add water molecule across the amide all then subsequent (Refer Time: 11:48) or if you if you see by pushing arrows, you can see how things are going on over from here to there ok. I think I mean you know this is a very very simple reaction and this seems like a very complex reaction over there it is not.

Only take home message I would really like to have you is the enzyme did not leave even a simple reaction to be done in a lazy or very slow manner. It has evolved in such a way such as even a hydrolysis reaction can be done by the help of all the protein side residue and by minimizing the energy and perfecting the orientation, this reaction becomes extremely feasible. The rate of the reaction becomes 10 to the power 8, 10 to the power 10 times faster than the non catalyzed reaction.

So, that is once again the beauty of these of these of this enzyme or any metalloenzymes. So, these has to be appreciated the type of job that is done by enzyme is quite great, but then again all these understanding in the enzymatic setup are not always that very easy, lot of cases what has happened that that enzyme is capable of doing a reaction by no ideas we have as a reader, as a researcher we do not have any idea how nature is doing all these reaction in a in a stepwise manner, because studies on those enzymes are not always that very easy.

Getting crystal structure let say for each and every step is near impossible and this is where synthetic intuition or synthetic chemist inorganic chemist in particular comes into the picture right. They try to understand how things are going, if it is not feasible to do these to do these experiments in the enzyme because enzyme is a great catalyst. The definition of the great catalyst is it will not allow you to see any of the intermediates that very easily because the reaction is great right.

So, this is why synthetic chemist really try very hard understand this steps critically by synthesizing some of these related complex or exactly similar complex or same complex if possible in the synthetic setup and then try to see stepwise, try to see how it is happening, can we characterize these species crystallographically, can we characterize these intermediate by various spectroscopic technique. These all these things becomes quite handy in really understanding the enzyme right.

So, in a way the realization of the synthetic community or the scientific community, how nature works is quite phenomenal right and quite important as well because some of these studies can lead to discovery of new medicine for example. If some someone is suffering from a particular disease which has to do with the particular metalloenzyme by understanding that metalloenzyme will have tremendous effect in developing a new drug

or to provide a particular solution to solve the issue and to save peoples life in many a cases right.

So, will keep on understanding these mechanism in greater details, but let me then next discuss the other enzymes which are hydrolytic enzyme, but not really mononuclear. So, far we have seen only zinc and these are only one zinc present right, but there are other hydrolytic enzyme which is which can have dinuclear at center right. One of the reason why enzyme why nature has chosen this dinuclear site is simply nature wants better control on the reaction or nature wants the deprotonation of water let us say at physiological pH by every metal and by utilizing water molecule is not really always possible right.

Of course, you have to utilize water molecule to produce hydroxide in case of the biological condition or in our body or in everywhere, but it is always not possible to create a hydroxide under the physiological condition for a given metal ok.

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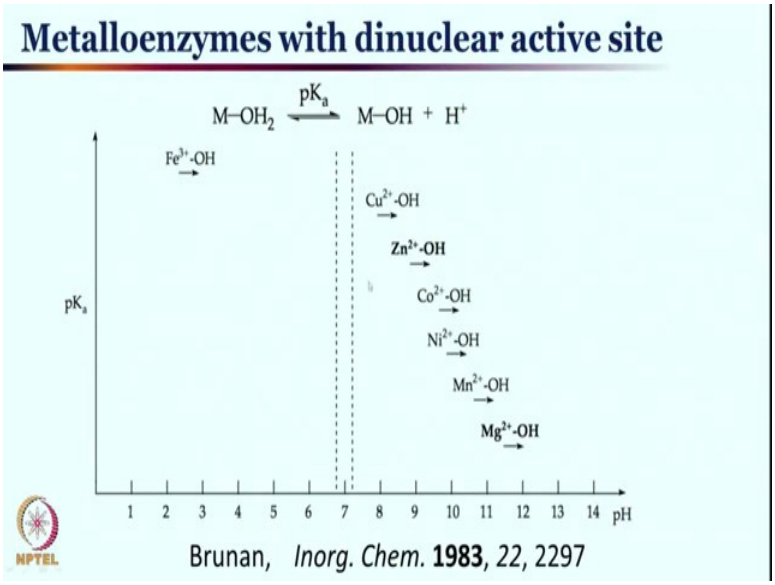

So, in those scenarios what has happen that this 2 metal centers are put together to make sure that this deprotonation has happening or the pKa value of OH is such that so, that it is getting deprotonated and providing hydroxide at pH 7 range ok. So, lot of metal centers, lot of enzyme centers are actually a dinuclear one as we have seen some of the mononuclear one, but there are many dinuclear active site exists which helps guide the

hydroxide or prepare the hydroxide at physiological pH which will otherwise be difficult. So, dinulear active site helps you to deliver the OH under the physiological condition.

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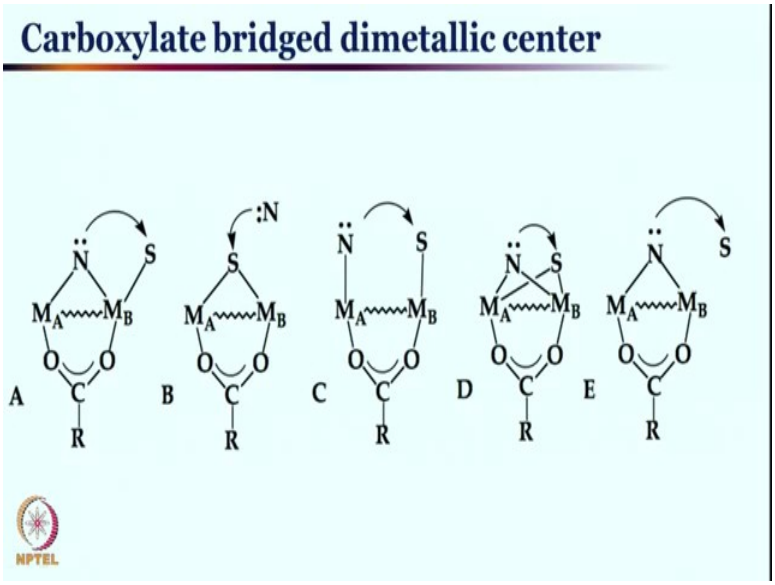

Now, the moment we are talking about this dimetallic center there is dock of obviously, how substrate and the nucleophiles are oriented. Because all essentially we are trying to do here is we are taking a nucleophile which is originating from the metal center and an electrophile which is placed right in front of the metal active sites so, that the nucleophile which is at the metal center can be attacking the electrophile. Now that sort of attack in presence of one metal it is very simple it is one metal center, but the moment you have more than one metal center let say dimetallic center, then what is the orientation of the nucleophile and what is the orientation of substrate that is also going to matter right. Of course, it matters also in the mononuclear sense or mononuclear side, but there is not much possibilities as you can have in the dimetallic cases.

For instance: this nucleophile which are generated can be bridged between the two metal center, in turn these two metal centers are also bridged by carboxylate that is quite common phenomenon. The moment you are dealing with two metal center as oppose to one to make the reaction facile to make the reaction even feasible, these two metal center usually will have communication and that communication is through the ligand and those ligands are going to be either glutamate, aspartate and that sort of carboxylate ligand. So,

you have a bridging ligand which bridges to metal center, you can have a bridging let say nucleophile which can be a hydroxide right.

Now, this bridging nucleophile can attack on the substrate or the electrophile which is bound only with one metal center that is one possibility. There could be another possibility where the metal two metals are bridged by the substrate or the substrate is bridged between the two metal center and the nucleophile is not bound with this metal centers and attacking from the outward side. All yet another fascinating option could be nucleophile is bound on one metal centers, substrate is bound on another metal center and then nucleophilic phile is attacking on the substrate.

So, that is also very very likely perhaps another little bit complex orientation could be that where both the nucleophile and the substrate which is nothing, but an electrophile let say nucleophile and substrates are coordinated both of them are coordinated with both of the metal center right. Alternatively you can have a metal nucleophile, nucleophile is bound between the two metal centers, but the substrate is not bound with any of these metal center it is hanging over there and then nucleophile is attacking on the substrate.

So, of course, these are the possibilities and one can think of what is happening in different type of this dinuclear heterolyte hydrolytic enzyme and how these reactions are happening ok.

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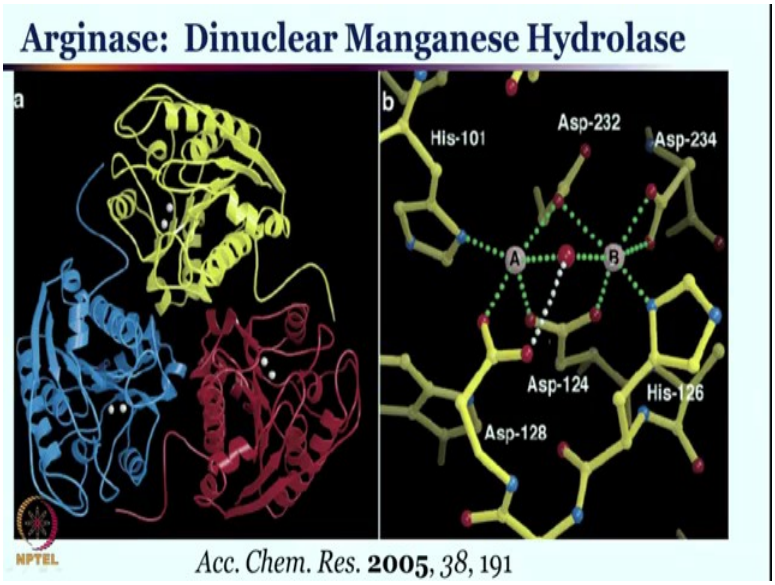

One such fascinating enzyme is arginase now, we are not talking any longer of the zinc enzyme, this is going to be dinuclear manganese ok. Can you identify these two metal centers over here this is obtained or this is reported before 2005, this is an accounts paper you can look at it. So, site A and site B there are two sites there it is bridged by this carboxylate right. So, two metal centers are bridged by the carboxylate and then there is a bridging by aspartate as well each of the metal center are having at least one histidine here another histidine here. So, this is connected each of them are connected with one of the histidine.

Now, there is other unit as you can see there is aspartate 128 this residue over here in yellow or dark yellow, this is this is aspartate 128 and that is bridged between A and this hydroxide which is over there also a bridging ligand between A and B. As you can see this sort of bridging ligand is not at the center B right. So, A and B are although they are as close as possible and they are as bridged as possible, but this is going to be not exactly same two center. So, this is manganese, this is manganese, this orient this geometry around this manganese is little different then than that we have seen in the case of B right ok. Let us look at the I chemdraw of it, this is a beautiful crystal structure and it clarifies the arginine structure quite clearly.

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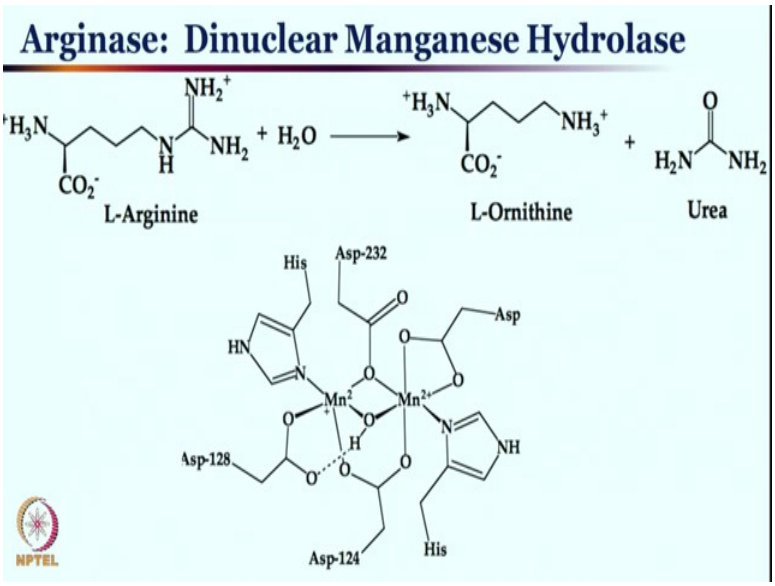

So, the activity these sort of enzyme or the arginase is able to carry out is hydrolysis of a of a urea unit. So, this is L-arginine, now as you can see if you want a hydrolysis over

there you need this metallloenzyme. You need this metalloenzyme to act as the nucleophile center so that the electrophilic center that is over here can be can be can be attacked and hydrolyzed in the process. So, you see the urea unit that is coming out of these L-arginine substrate upon hydrolysis.

This is a once again a very simple and clear reaction that can be done in a very very convenient manner by utilizing this arginase. The structure which you have seen in the last slide if you want to a simplify it, here is the two metal center bridged by hydroxo and bridged by carboxylate. So, this is aspartate 124, this is the structure you have seen in the last slide, this is aspartate 232 which is once again bridged between the two metal center another aspartate is there which is a bidentate coordination from this aspartate.

Now, aspartate 128 is mono coordinated with manganese and mono coordinated or mono mono or singly interacting with the hydroxide through hydrogen bonding right. In the overall you see the arginine is getting converted to L-ornithine and urea right. So, the hydroxy that is over there will be attacking on the arginase in the arginase active site on the arginine substrate.

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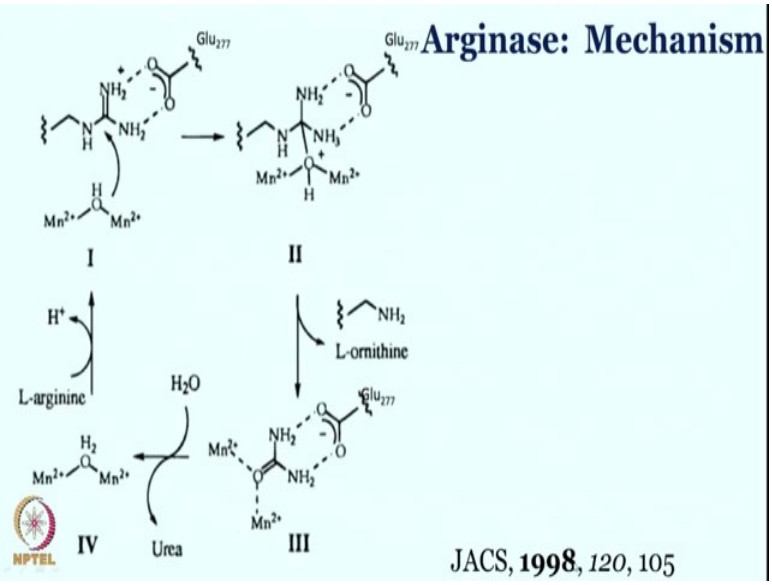

Let us look at the enzyme mechanism a little bit in detail. So, this is proposed in 1998 by Lippard group once again. So, we have a dimanganese site all other ligand residues are not drawn for clarity of course, they are all present over there.

So, the proposed mechanism goes in such a way where these bridged hydroxo that is the nucleophile bridged between the two manganese center this will now then attack at this electrophilic center you have generation of this tetrahedral intermediate. Still things are hydrogen bonded glutamate 277 is still there hydrogen bonded between these two NH2 group now and this is this is now a quite interesting tetrahedral intermediate right fantastic intermediate. Its a simple just a nucleophilic attacking on the electrophile.

From there on this L-ornithine comes out and it attacks it comes out over all your glutamate still there hanging on to the urea that is getting generated. So, rebound will happen or you just push the arrows, you will be able to see the urea is forming and the L ornithine coming out. Overall two manganese site is coordinated with the urea initially it was coordinated with urea this urea sorry hydroxo this is now coordinated by urea, you have double coordination from here and double support from the hydrogen bonding that is phenomenal. And this is once again I think it is very important to realize that designing such a simple yet effective catalyst in synthetic laboratory is not going to be easy, enzyme or the nature that is why it is so, brilliant and we have to just appreciate the nature that how it is really done so, effortlessly.

Now, the water molecule will come out will come in and urea will go out over all it will give rise to manganese manganese diaqua aqua complex, this aqua complex then will undergo the deprotonation to give the manganese manganese hydroxide intermediate. As you can see you know the redox state did not change throughout the catalytic cycle. So, that is one of the factor that remains constant in this hydrolytic enzyme these redox state of the metal remain constant. You do not see any advantageous reaction you do not see any oxidation reduction of the metal center that is essential because, our main reaction is the hydrolysis which is a simple reaction if it gets complicated by electron transfer I think that is not a desired one.

So, this is why once again nature as chosen the metal center, which are not too reactive in terms of the redox chemistry or even if they are little bit reactive you know nature has control its reactivity in such a way so, that no redox chemistry is needed to be discussed in the context of the hydrolytic enzyme. So, we have seen zinc which is redox inactive, we have now seeing the manganese also can do it in a dinuclear fashion and all those side chain is really placing things perfectly for the L-arginine to get it converted to L

ornithine and the urea; urea is moity is now really perfectly positioned it can be taken out and water can come in and things can go on.

So, in the next class we will we will try to see how synthetic efforts preliminary or first synthetic efforts are done to understand the mechanism of these of these enzyme. So, these are these are the proposed mechanism actually coming out of the synthetic studies, because this is once again this is where one can have some control on what thing how things are and how perhaps it is going, proposing everything and gathering some support or evidence in favor of the proposed intermediates are not really that easy in the enzyme.

So, this is where again the synthetic chemistry setup is required and that is what will see in the next class. Till then keep studying will will come back soon and if you have any queries, please feel free to post them.

Thank you very much.