Metals in Biology
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Lecture – 10
Hydrolytic enzymes – Part III Arginase & Urease

Hello welcome to Metals in Biology, we are going to discuss Hydrolytic Enzyme. In the last two classes we were discussing the mononuclear hydrolytic enzymes and dinuclear hydrolytic enzymes; today we will continue discussing on that. Arginase is one of the very important metalloenzyme which converts L-arginine to L-ornithine and urea. You have seen that it has two metal centers; this is a homo metallic species two manganese centers are bridged by a carboxylate linkage as well as a hydroxide. Two type of carboxylate bridges are there: one is a you know the bi coordinated another is mono coordinated bridging right.

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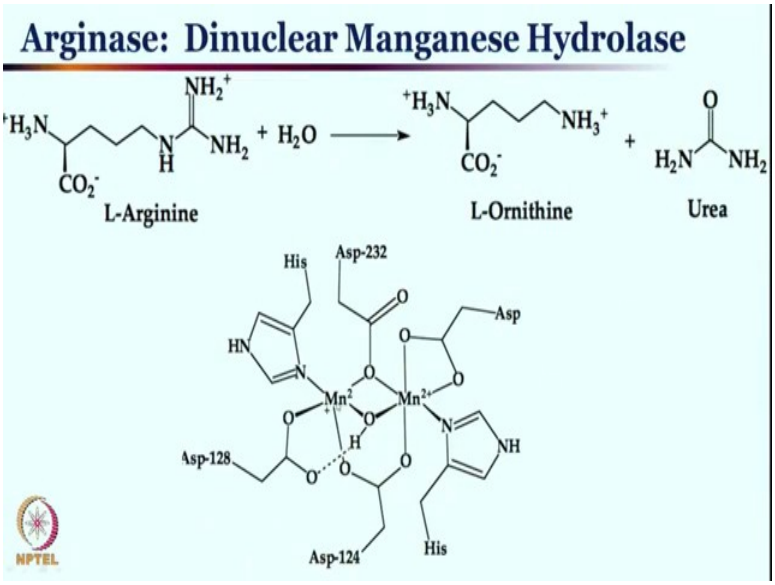

So, you have one manganese center another manganese center both of them are in plus 2 oxidation state you have this bridging aspartate between the 2 manganese center and you have another aspartate bridging through only one oxygen atom of the carboxylate.

So, the shift of or bridging shift can happen pretty frequently in fact, sometime this bi-coordination to mono coordination occurs in terms of a providing space for the incoming substrate, you have another histidine both of the manganese site. This aspartate is bi

coordinated with respect to manganese, but the other manganese center is having mono coordination as well as it is hydrogen bonded through this hydroxyl bond. Overall this CN bond getting cleaved by the water molecule to form L-ornithine and urea.

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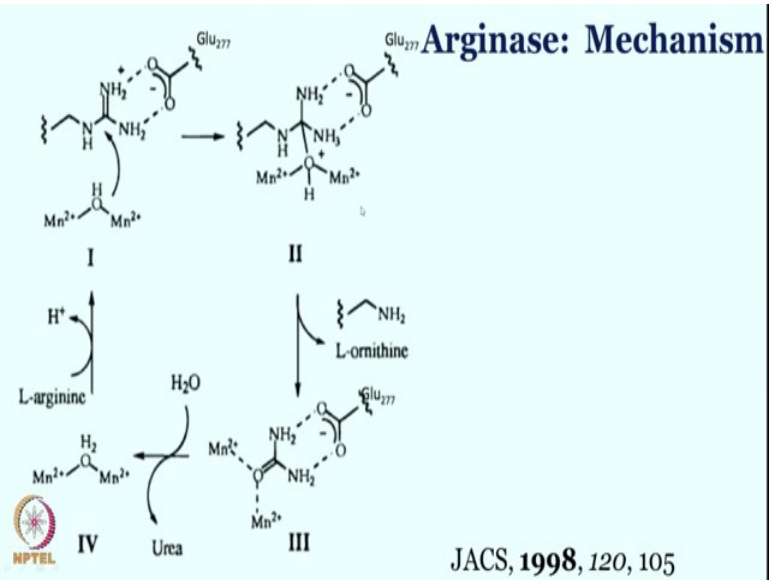

This reaction is quite interesting if one is looking for the overall reaction mechanism. Well as you have seen in earlier cases that not only the active site of the metallo enzyme active site mending that where the reaction is happening and in the primary coordination sphere what are the ligand involved this complete structure will be called the active site. But except active site the other residue other protein backbone that is over there the you know amino acid backbone the side chain that is appended from the nearby residue also plays a crucial role.

For instance in this case as you can see glutamate 277 participates in binding the organic substrate; binding the organic substrate for instance this is your L-arginine this is where we start manganese 2 plus manganese 2 plus bridged by hydroxo. This hydroxo is going to attack on this electrophilic center over here, but it is helped positioned perfectly with the sorry help, it is helped position perfectly with this glutamate 277.

Now, without this glutamate 277 over here we cannot have perfect positioning of this L arginine. So, not only the active site is important side chains are also critical in providing the catalytic activity mainly the rate of the reaction get enhanced significantly because of the substrate binding pocket multiple hydrogen bonding that is involved over there, but

overall all these effect put together we get a very functional and very effective catalytic cycle. Once you see over here that the you have this attack on the nucleophilic center and subsequently this rebound or subsequent rearrangement gives rise to the L ornithine and the urea production.

Now, urea is coordinated between the two manganese center and is supported by glutamate 277 subsequently one water molecule comes and produces urea. And then water molecule is bridged between the two manganese which can be deprotonated due to the dimanganese center, the pKa value of this water is such that that now at pH 7 you can deprotonate this water molecule to regenerate manganese 1 or manganese 2 plus manganese hydroxyl species.

One of the things that we were discussing in the last class as well that none of these metal center undergoing any additional oxidation state change; that means, wherever they are starting from its stays over there. For example, zinc 2 plus remained zinc 2 plus throughout the catalytic cycle that is quite a phenomenon or quite a standard phenomenon for the hydrolytic enzyme right.

In most of the other enzyme where you will see that there is a reaction involved in those cases oxidation state of the metal center will be changing therefore, redox active metal center will be chosen for those cases. And this is particularly why in some cases zinc is taken because mostly let say it is not required to do any oxidation state change. In other cases let us say iron is chosen because number of oxidation state change is required frequenting between those oxidation state becomes easier by having iron for example, present over there all or even copper center present over there.

Depending on the necessity and the availability and of course, hardness, softness labiality a number of factor that we tried to discuss earlier we will contribute to the fact that which metal center will be chosen for a given enzyme. As you have seen over here it is a dimanganese center bridged communicated between the two and simultaneously there is a role for this deprotonation as well as attack on the substrate to prepare the desired compound. Now, we will try to see if synthetic chemist bioinorganic chemist can mimic these activity. So, the objective here is we try to see how things are going on in nature and try to get inspired by nature.

For example, metalloenzyme in these cases and then try to recreate the magic that nature has created, but now in synthetic setup; that means, you will be able to take a reaction flask, star bar you know magnetic stirrer and add chemicals should be able to do these chemistry that in the laboratory other than without taking any help from the mother nature directly. Well sometime these activities most often are not actually that straightforward and that that rewarding because mimicking nature is always going to be difficult.

Well the good thing is you do not have to perhaps mimic one hundred percent all you want to, but it is very difficult that is why any mimic is the good enough. For example, there are two type of two types of mimic usually people talk about one is the structural mimic another is functional mimic right. So, structural mimic meaning whatever you see in the metalloenzyme you try to mimic or you try to create that compound synthesize that compound in laboratory. You do not have to really worry about the reaction; that means, whatever reaction is happening let say you do not have to do or your compound is not capable of doing that is called structural mimic right.

So, you try to let say you have three histidine therefore, you try to put three histidine or at least three nitrogen center on the metal center and a water molecule on the metal center you have same metal let say zinc. So, you end up doing exactly same thing, but in the effect you are really not able to provide the reaction that is the zinc enzyme was doing, well still it is a good mimic because it gives some spectro electronic spectral data, electronic data of the compound and that can be perhaps matched with the original enzyme.

So, these are structural motifsfor example, in the zinc cases you may end up getting into trihedral geometry and the zinc 2 plus is a d10 system, so not much spectroscopic data you can get maybe by getting a structure of the zinc enzyme will help you immensely right.

So, that is the structural motive, but more importantly if it is not really activity mimic, then it is not that a great thing to do, but anyway still you can do, but there is another mimic which is called functional mimic, wherein you may be not mimicking the enzyme perfectly in terms of its structure, but you are able to mimic the function let say reactivity of it, but all that is still good, but you may not have the structure.

So, this is also not perfect, but also this functional mimic can be of this type where you do not have to have perhaps the same metal as the nature is having you may lose out one or two binding mode what nature is having, but still without the right metal and perhaps without the right coordination let say nitrogen versus oxygen coordination. If there is a oxygen coordination you try to substitute by nitrogen coordination as long as the reactivity that enzyme was producing that can be recreated it can be called the functional mimic.

But as you have perhaps realized by now none of these are going to be that effective or any effective compare to any more effective compare to the natural design, that is why both the structural as well as functional mimic is necessary. Now, you have seen the manganese; manganese structure in the last slide its a you know it is over here two manganese center is coordinated by this by this aspartate and another aspartate and there is three at least aspartate where this is the fourth aspartate this is going to be very difficult to mimic for a synthetic chemist because this is completely unsymmetrical structure first of all.

And the complexity involve although to you may it look like its very simple, but if you want to implement or if you want to design a ligand which can; which can hold the manganese the way perfectly what nature is doing its going to be really really complicated right. It is exact same structural mimic actually does not exist so far.

So, you see the complexity over here there is bridging perfect bridging between two manganese. So, that perhaps is little bit easy to do if you have this, but how is one going to do exactly this mimic where one of the end is attached to it manganese another end is hydrogen bonded that is not going to be that easy also. Monodentate aspartate as opposed to one is having bidentate another is monodentate that is also going to be very very challenging and then on top of that you have a bidentate aspartate how exactly to mimic all the aspect that is there although it is very simple, but I would say this is going to be a one big challenge if exactly this going to be synthesized in the laboratory.

So, what alternatively people try to do? People try to simplify it and not even perhaps looking at manganese maybe cobalt can do the same thing as we have mentioned that this is essentially to deprotonate water to make it hydroxide other metal center will suffice right will be good enough. So, this is where people try to take advantage of

creating a dimetallic center even if possible with manganese if not it is getting characterized then other metal is also fine and try to design a ligand which will put these two metal center together let us look at one of those right.

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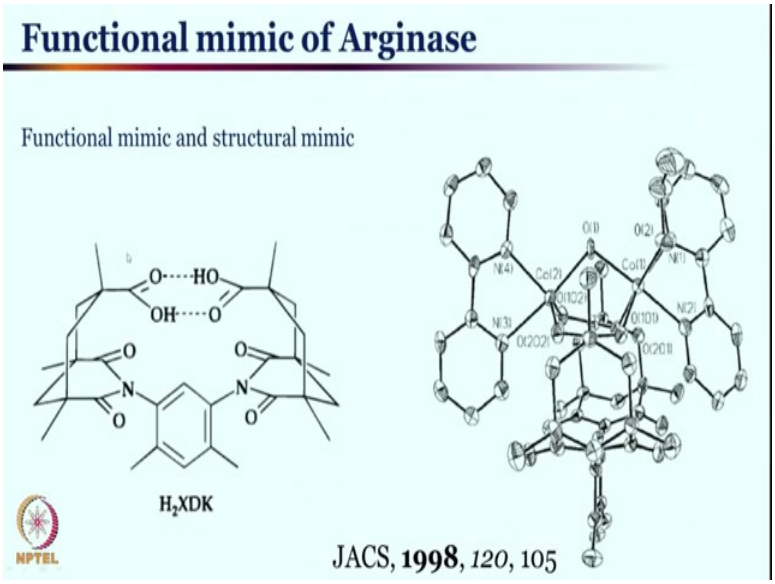

So, this is a gigantic ligand that has been designed and so these are the two carboxylic acid part which is potentially going to hold the two metal center together if you are looking at over here is cobalt here is cobalt and it is bridged by these two carboxylate unit. None of this architecture is going to be bound or binding the manganese center only it is going to bind over here.

So, these bridging manganese is over sorry bridging carboxylate is right over in between, but more importantly you see that there is a hydroxo bridge between these cobalt and there is this two nitrogen center bipyridine is right over there right this is going to be helpful in future when we see the reactivity, so let us look at. So, this is going to be a functional mimic as you see that manganese is no longer there even and of course, two histidine is there where sorry two pyridine is there; there was one histidine per metal center all those variations are there this is not perfect, but good thing what I can tell you that despite not having the perfect structure synthetic chemist were able to show that this is capable of doing similar reactivity as you have seen in the L-arginine cases.

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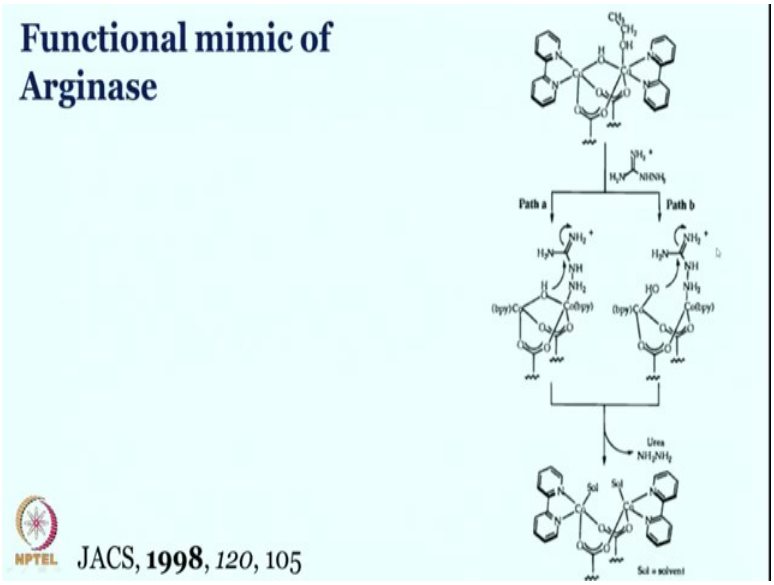

So, what is over here we have redrawn or this is redrawn and taken from here this is the dicobalt structure with the dicarboxylate bridge cobalt is having this bi coordination well if you; if you try to put this with respect to the manganese one I do not think this is matching perfectly, but what you see over here that there is a weekly coordinating ligand in this case ethanol that will be displaced by the mimic of the L-arginine.

So, this is the urea unit at least over there and that is now coordinated with one of the cobalt center. Upon binding on the cobalt center one of the cobalt center the bridging that was bridged hydroxy one of the end snap off. So, it comes out and this hydroxo now it is becoming a nucleophile.

So, this is where a nucleophile and a electrophile on two different metal center is there, but most importantly these are very close to each other right, these two both the nucleophile and the electrophile are very close to each other they are ready to react nucleophile is going to attack the electrophile and therefore, the hydrolysis of this of this compound is possible and C-N cleavage will lead to the urea formation as well as hydrazine in these cases.

So, this is one of the way other way could be the dicobalt is bridged although these hydroxo is new bridged um. So, both of them are attached the cobalt is attached with only one of the substrate and then this nucleophilic attack is happening. So, what we are trying to say here is this mechanism may or may not be 100 percent clear what exactly is

happening at this stage, but the fact that even by having cobalt in there in place of manganese and not having exact mimic of what these arginase were doing still we can promote Larginase type of chemistry hydrolysis of such a model substrate I think that is quite phenomenal.

So, this is showing that that this is what perhaps the mechanism were a hydroxy is bridged or it is on one of the cobalt center one of the metal center by having the electrophilic and electrophile and neclophile very close to each other these reaction can be made feasible and and these these are quite a quite an effective reaction right over there.

So, what we have seen right now an activity mimic functional mimic. So, the function that arginase is able to create that can be created in absence or in presence, it does not really matter you need a Lewis acid center such as over here is cobalt, but in original enzyme it is manganese, but nonetheless we are able to see that it is able to do the chemistry that one is required to do by arginase. So, these are not going to be a structural mimic, but a functional mimic let us look at another enzyme and that is urease.

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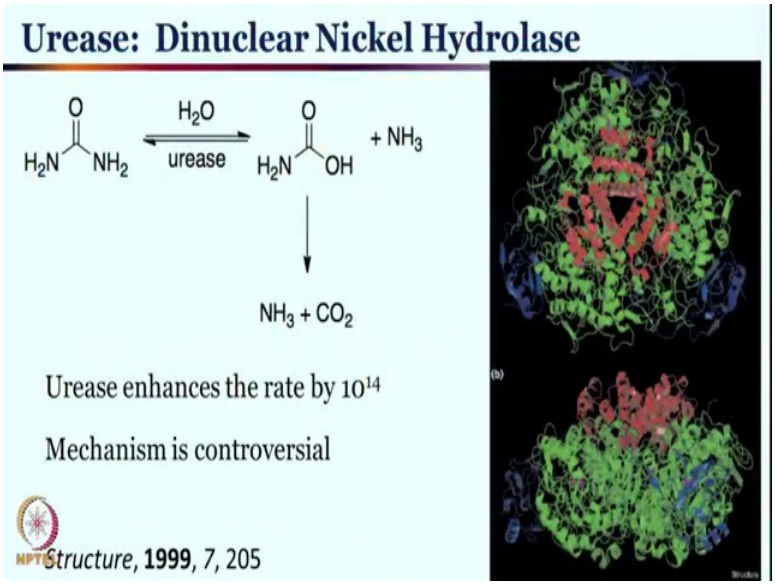

So, you have in the last case you have seen the urea formation in this case you see that urea is converted to ammonia and carbon dioxide right. This is also a fascinating enzyme quite a lot of crystal structure has been reported it has been well studied enzymatic structure and the reactivity is quite fascinating. Mechanism although remained

controversial and urease itself the enzyme can catalyze this reaction pretty efficiently without urease this reaction is very sluggish, but with urease you see that reaction is happening very fast and actually rate is enhanced by 10 to the power 14 4 just imagine that how capable the enzyme is to promote these reactions right.

So, let us take urea and try to make ammonia and carbon dioxide this is one of the crystal structure back in 1999, there are many crystal structure from many different sources, but all of them are telling one thing this is going to be a dinuclear nickel crystal or nickel compound ok.

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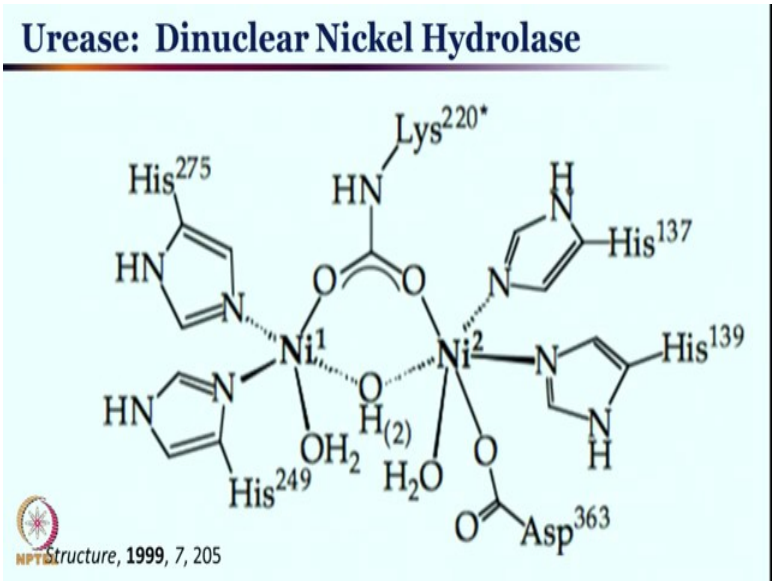

So, this is one of the crystal structure showing that its a dinuclear center, so dinickel is there of course, depending on how it is crystallize these ligand may vary, but I think this is one of the simpler one you can find in terms of crystal structure being reported. Now, each of the nickel as you can see is having two histidine ok, so two histidines are there and it is bridged by a bridged by this carboxylate which is quite exciting there is a hydroxo bridging also there is aqua; aqua molecule on both of them. So, that is I think it is quite simple yet beautiful active site right this is a dinickel previous case you have seen manganese dimanganese species now you are seeing dinickel.

Now, as we have discussed earlier the essentially these metal centers are Lewis acid. So, it is helping deprotonating water to make hydroxide under the physiological condition that is the first activity second thing is it will help activate the substrate directly by

making it coordinated with the metal center and during these process other side chain will be also playing a role not only these side chain, but the other side chains that is present nearby will also be helping in carrying out the reactivity quite effectively right.

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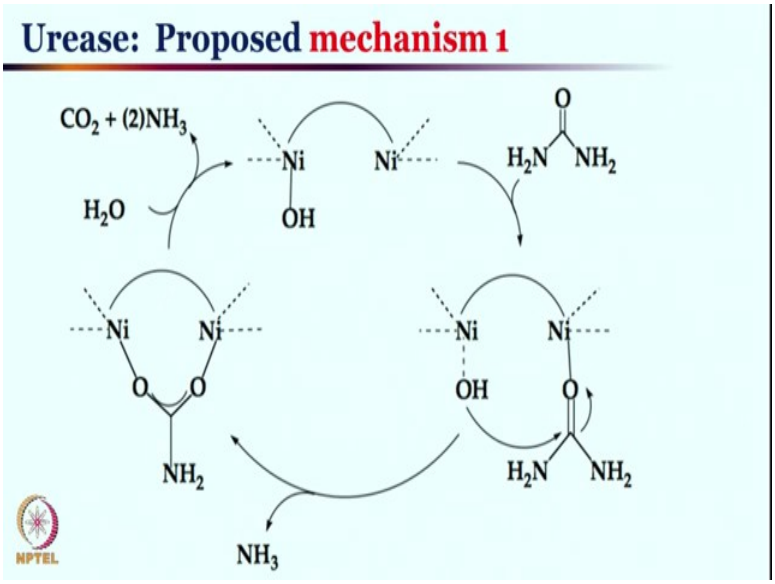

Let us look at the mechanism and just to tell you that mechanism is still controversial it is not a solved till, but let us try to understand the complexity in it. So, we will start over here as you can see a water will come in and it is getting deprotonated to give nickel hydroxide urea as you would imagine urea will be binding with the nickel center in a way that is shown over here.

So, urea is bound and nucleophile is (Refer Time: 21:39) urea this carbonyl center is the electrophilic center it is going to be attacked by this hydroxy and you can see and overall in this process a nucleophile attack and this ammonia goes out of the system to give rise to this you know intermediate which can be displaced by which the bridge can be displaced by water molecule again carbon dioxide this can be broken down into carbon dioxide and ammonia 2 equivalent of ammonia is getting generated from one equivalent of urea right. Well that is I would say quite simple and interesting urea plus water giving you carbon dioxide plus 2 ammonia.

So, this seems a quite logical mechanism and simple mechanism isn't it, but the problem is there is another effective mechanism that cannot be ruled out let us look at that mechanism ok, over here what you see is a subtle difference maybe you have not noticed

yet over here. So, we start once again over here nickel hydroxide, urea binds with the nickel, so you have a urea bound nickel center then the hydroxy attacking on the carbonyl center that was happening in the last case, in these case urea sort it itself if you push the arrows this lone pair goes on there and then that goes outside, so, over all cyanate formation happen right.

A cyanate formation happen and then hydrolysis of this cyanate by this water molecule gives rise to the carbon dioxide and ammonia right. So, almost it is like self reaction of urea to form the cyanate and then that can be hydrolyzed to give carbon dioxide and ammonia right another ammonia is over here. So, overall the reaction remains same water plus urea giving rise to carbon dioxide and to ammonia.

Now, quite a lot of studies been done particularly in these cases computational studies will be quite useful and the bad news is even the computational studies cannot differentiate between the mechanism 1 and mechanism 2; that means, either this intramolecular hydroxo attacking on urea or urea is self sorting as it mechanism 2 both of them remained feasible; that means, it is very difficult to distinguish one versus another right.

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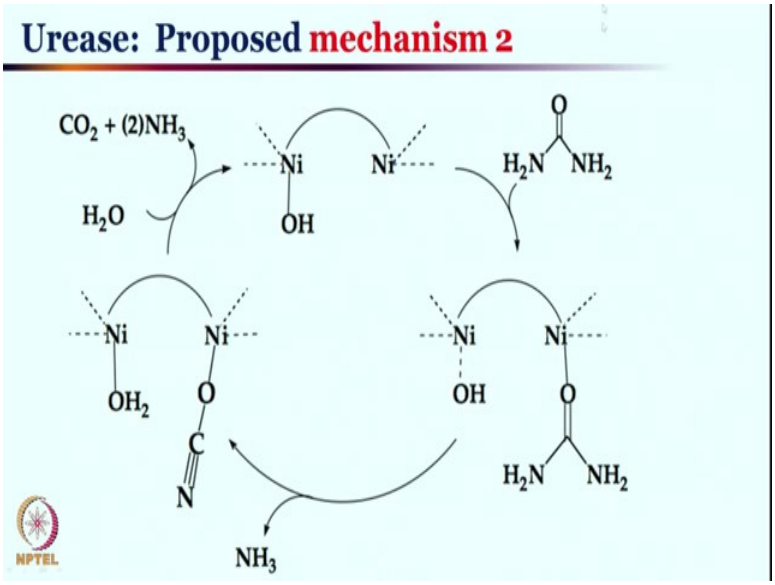

Well, having said that when we will see in the next slide that urea is you this synthetic model system has been has been created and then it is been studied what has been found that the cyanate could be detected in some of the cases. And therefore, it seems signifies

that perhaps this is going to be likely mechanism right you cannot still conclude. I think this is this is the one thing about mechanism you can never conclude about a mechanism right whether this mechanism is correct or that mechanism is correct that is not the right way to learn about any system.

I think the best way is to keep an option open because you never know what exactly the mechanism is even if you have crystal structure intermediate trapped you have done many many things to understand the mechanism still you can never be completely sure that that is or one particular mechanism is operating because there is always a chance to switch between the mechanism right.

So, whenever you guys all of us write that you know this proves that this mechanism is correct I think that is a very dangerous statement to make, if one should write down this suggests rather than this proves you cannot prove a reaction mechanism right you can gather information in support of your of your proposition right. So, this is what we should we should be talking that mechanism remain controversial, but this suggest that this is what is likely mechanism I think particularly in these cases that is going to be important.

So, the suggestion of a mechanism is accepted or welcomed, but, but complete proving of mechanism I think if one should stay away even no matter what how sure one becomes still it cannot be 100 percent guaranteed that this is what or that is what is happening.

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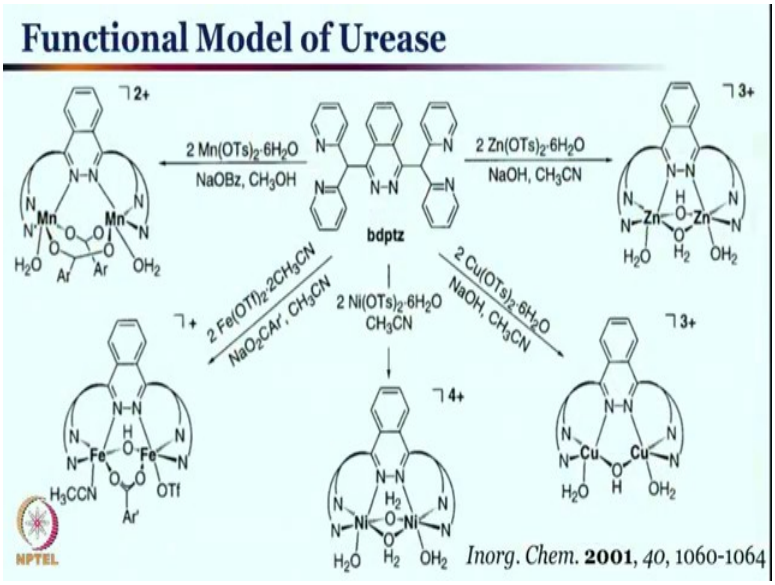

Let us look at the synthetic efforts that has been done to mimic the urease all this remain to be quite interesting I would say many models exist, but essentially what we are looking at is a bridging center right the center which can bridge between the two metal center this ligand is designed and that is quite turn out to be quite exciting in terms of functional mimic these are again not again going to be the structural mimic just more of a functional mimic.

So, this center can bridge between the two metal center, the way it is shown over there let us say these are the two nickel center this is closest one can get in terms of that enzyme. So, two nickel center bridged by a ligand and you have water molecule bridging between the two metal center and just like just like in the enzyme there are; there are histidine center here you have the pyridine center once again mimicking; mimicking the aspect of nitrogen binding. And, what is interesting is such a molecule can indeed convert urea into carbon dioxide and ammonia that is functional mimic once again these functional mimics are quite important and remained quite valid and you have seen other metal can be synthesize and also can be started.

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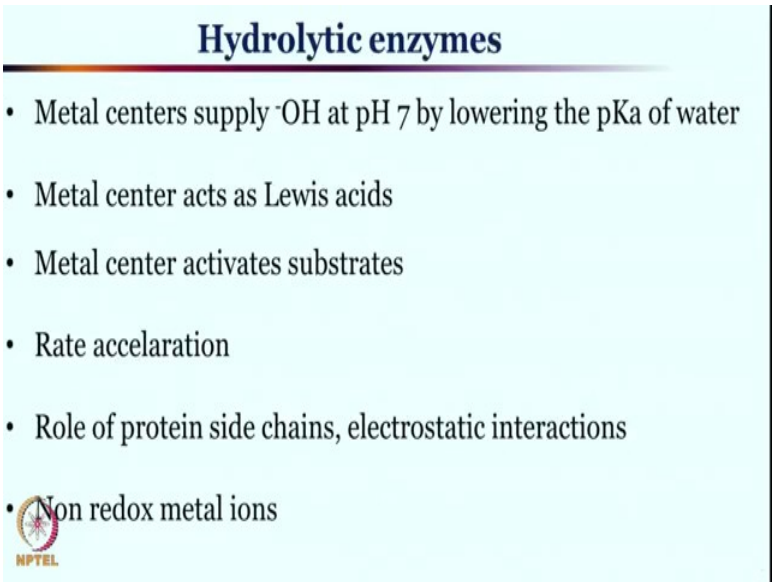

So, to sum up what we have learned in the hydrolytic enzyme is it does not matter whether it is a mononuclear center or dinuclear center that role of metal centre is precisely to be Lewis acid ok. By being Lewis acid it allows the water molecule to be deprotonated to provide the hydroxide in the solution or during the reaction. Also this metal center will help bind the organic substrate that is going to be hydrolyzed that is going to be attacked by the nucleophile right.

In addition to those you know to those ligand for the metal center the protein side chain also helps you in docking or orienting the organic substrate in front of the metal site So, that so that these nucleophile electrophile business can go on very easily right. So, the pre orientation of the organic substrate is crucial for rate enhancement and for overall the activity as well. These are the I guess the main take home message I must mention that in the last case where you have seen that urease is convert into convert a into a converting urea into ammonia and carbon dioxide by doing so you can you can basically neutralize the acid right you are generating ammonia in the process you can neutralize the acid.

So, these in the liver this enzyme actually provides the neutralization of the HCL and thereby some good a you know bacteria such as H pylori can be can be colonized right. So, and therefore a lot of diseases can be can be prevented by having this urea to ammonia and carbon dioxide prevention and if these are not properly functioning.

So, H pylori it helps it grow H pylori that is what neutralization of the ammonia to HCL does neutralizes and helps H pyroli to grow inside the inside our liver, but not in liver its I guess in the in our body in our abdomen. Now, if things are not great, then it can have effects such as such as you know it can provide the atmosphere, so that ulcer is happening or even deadly cancer could be there. So, this ammonia production from urea is quite helpful for our body.

It has also long standing long standing implication in agriculture for example, by producing these ammonia it directly relates to crop production and overall as we have seen role of protein side chain, electrostatic interaction hydrogen bonding are important and non redox metal ions are used in all of these cases or most of the cases we or all of the cases we do not see any oxidation state change, with this we will come back soon keep studying.