Bioinorganic Chemistry
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Lecture - 16
Manganese Enzymes

Welcome. We are still continuing with manganese.

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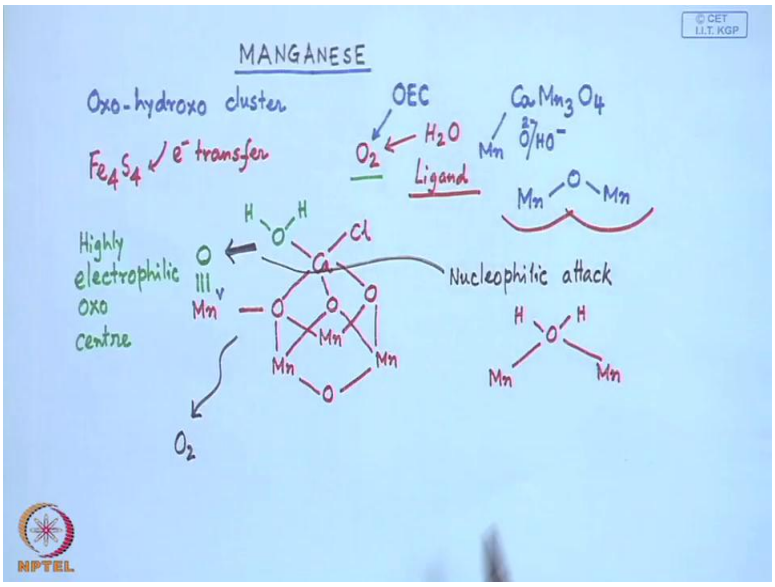

Today, we will see some more examples. This manganese – we have identified, when it is present as an oxo-hydroxo cluster. And, in photo system, it is involved in the center, which is responsible for oxygen evolution. And, we have also identified there that, not only manganese, but we have also calcium. So, we have calcium; we have manganese intimately connected to one calcium; and also, we have one more extra manganese. And, here we have for completion of the cubin structure we have 4. And, sometimes this O and OH is connected to that.

So, we are just looking for the evolution of O 2. So, it is a completely different structure and the reactivity. Compared to the well-known system, what we know as is the ferridoxin system. In case of ferridoxin molecules, they know they serve the purpose of only electron transfer. In this particular case, it has some other roles to play, because to get this dioxygen, we have to use water. And, this water should be a very good ligand for this cluster system.

If we just think of the basic structure; we just look at the basic structure only. You have calcium. And, calcium – what we have seen last time that, it is also connected to the manganese. So, if we have some connectivity pattern like this; and, this calcium is highly distorted in nature. And, if we have two more manganese at these two points; sometimes, we call it as a distorted adamantine type structure. And, this particular site is important; and also, this particular site, where you have the separate manganese.

So, like all other catalysis system and some other cases, people have still propose, is not yet settled; that you can have a double bonded oxo structure or a triple bonded oxo structure from this manganese. And, this calcium is bound to one single water molecule. So, these are the very important structures related to this cluster; that you have the ligand in your hand; and, this ligand as well as some of these form, because we have identified that, these groups – that O 2 minus or hydroxide groups – they are always present in form of manganese oxo manganese basic unit.

When we have this oxo-hydroxo cluster, sometimes, we also can get in a manganese oxo center like the vanadyl, molybdenum and uranine system. So, if you have a highly oxidized form; if you just… manganese oxo in this form – it can be manganese five. So, what we are basically proposing there; is a basic mechanism for that is that, this oxo centre is important for this O 2 evolution. So, we can generate some highly electrophilic oxo centre. So, generation of that is very important on manganese. And, this particular one will go for this water molecule. Then, if you have adjacent, we have this entire cluster arrangement. If we have this particular water molecule close to this oxo center, it can go for nucleophilic attack to that oxo centre. So, we just allow nucleophilic attack from this water molecule.

And, from there, we get this O 2 molecule. As the very basic oxygen evolving center, this particular manganese motive, that means, this entity. So, everywhere, you will find this manganese oxo manganese, manganese oxo manganese. So, this particular one – when we find that… We are talking about this calcium attached with water molecule. So, in some other form, you can get this particular basic unit as well. So, water bridging, hydroxide bridging and oxo bridging center. Whenever we have these two manganese adjacent to it; here also, it is not that some isolated manganese centre like cytochrome P450 or something; that means which is not that it is a porphyrin bond manganese centre, mononuclear manganese center. Then, it is attracting the water molecule. This – the

cluster arrangement is required; that means you need some adjacent manganese site, which is bound to this manganese, which is the catalytic site through some oxo-hydroxo or water bridging. So, will see some more manganese centers, where you can put more than one manganese centre.

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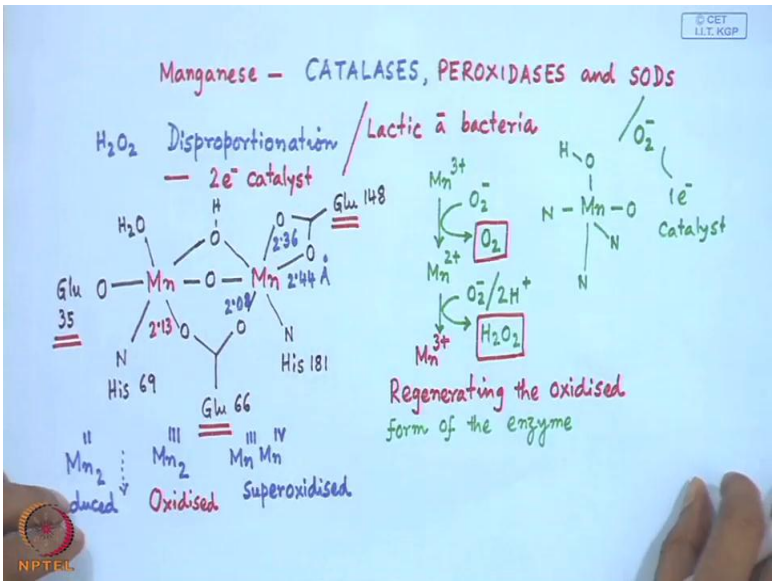

These manganese have been identified in several other molecules. Some of we already know and we call them as they are present as cofactor in catalases. The most important catalases we have seen, they are heam proteins. So, iron porphyrin unit; but, there are some other species which is available for their reactivity like that. Again, the disproportionation of hydrogen peroxide, which is well-known to us from the different E 0 values that, it can go for its disproportionation reaction, because it is thermodynamically favorable. If you have some reaction thermodynamically favorable; and, we want to just change the corresponding rate of the reaction. So, rapid reaction requires… What we require? We require a 2 electron from catalyst. So, once we know that for disproportionation reaction, we need a 2 electron catalyst… We can have a manganese site; that means you can have a 2 manganese site. So, it is again a dimanganese catalyst. So, the manganese bearing catalases; it is not that the iron bearing catalases, that means, the haem catalases.

Manganese bearing catalases – there are also like that haem proteins; you have the peroxidases also. But, the mechanism of action is completely different and super oxide

dismutases. So, catalases, peroxidases and superoxide dismutases. And, in all these cases, we have manganese. So, in case of… Side by side if we see that, in case of hydrogen peroxide, we need a 2 electron catalyst, which can increase the rate of the reaction. Similarly, for super oxide. So, we can have a single electron transfer from the superoxide anion. So, this particular one is very much useful if we can have a distorted trigonal bipyramidal arrangement on a mononuclear manganese. And, mononuclear manganese initially can bound to water molecule and then to hydroxo function. So, the corresponding reactivity with the superoxide anion can be taken care of by using a mononuclear centre. But, in case of this disproportionation of hydrogen peroxide, we definitely need the binuclear one.

Here if we just generate simple for iron-based manganese system; that means if we have a manganese 3 plus centre, we go for reacting this O 2 minus; a superoxide anion is reacting with it liberating O 2. And, this center can be reduced to manganese 2 plus. Then, in the second stage also, we can have the corresponding reaction of O 2 minus. But, now, two protons will also react. And, reaction of these two protons with manganese 2 plus in presence of the superoxide will generate now the hydrogen peroxide. So, the superoxide – we break into O 2 and H 2 O 2 regenerating the centre again as manganese 3 plus. So, at this point, we are regenerating the oxidized form of the enzyme. So, we are regenerating the oxidized form of the enzyme.

Considering that number of electron transfer; we say 1 electron catalyst in a stepwise manner; we can consume two molecules of superoxide anion to produce oxygen and hydrogen peroxide. But, for this disproportionation reaction based on manganese, it should have a corresponding dimanganese system. And, this dimanganese – all the time, like the photosynthesis center, that it can be connected with an oxo bridge and one carboxylate bridge. And, this carboxylate bridge is coming from glutamate 66. This has nitrogen coordination from histidine 181. And interestingly, some distorted coordination, which is not very common for first transaction series elements like manganese; that it can go for bidentate carboxylate coordination from another glutamate residue, which is glutamate 148. And, the third bridging is from hydroxide function. So, that gives us some idea that, both the two manganese sites, this particular part can be close to an octahedral geometry; this can also be close to octahedral geometry, because we are

accepting three bridging units. So, one particular face of the octahedron is shared between these two manganese sites.

We have other three coordination sites. This is again like this nitrogen is histidine 69, this oxygen from the carboxyl end; but, this carboxyl end is functioning as a monodentate one; this is glutamate 35 and this is water. This is a more or less symmetrical arrangement; only thing that you have two nitrogen (( )) carboxylate bridging; then, oxo-hydroxo carboxylate bridging. Any dimanganese system like this – we have manganese site; and, we have two such manganese unit. And, if both the two sites are closed, two octahedral geometry… because this particular part is highly distorted one. And, if we look at the bond distances, this is 2.44, which is quite weak and this is 2.36 – it is well above the corresponding manganese oxidase distance, because this particular one we get in a very distorted way. And, this carboxylate function is little bit weakly bound compared to this particular bridging carboxylate unit. This is in a short range, which is 2.08 Armstrong and the other one is 2.13.

Why we are looking at all these bond distances? Because sometime, we find that, these bridging groups like the simple copper acetate – these two bridging groups are not symmetrical. So, asymmetric bridging groups are there. And, identification of this carboxyl end, even it is true for isolated metal salts; that is, if you are talking about manganese acetate; if you are talking about iron acetate; or, if you are talking about some other metal acetate; how these acetate groups are attaching to the metal center? Here we will find; which is also true for different types of iron enzymes like hemerythrin and ibonic glutate deductases; that you have one such carboxylate unit, which is glutamate. So, you have three such glutamate ligand: one is this; another is this; and. another is this one. Depending upon their positioning within the long protein chain, one is functioning as the bridging unit; another is functioning as a bidentate one; and, another is functioning as a monodentate one.

Still we have some other possibility, because of this charge neutralization from the acetate anion, because these are negatively charged. Sometimes, this hydroxo function can also be replaced by some acetate function, where one single oxygen of the acetate group can be used for bridging to manganese site. This hydroxy function can be equivalent to that of the acetate function coming from the glutamate residues. In all most all these cases, whether we can have two different types of these catalases; sometimes we

find that, in these catalases are present; one such example is in lactic acid bacteria in different lactobacilluses.

So, in lactic acid bacteria, they are present. And, almost in a very low oxygen environment; almost, oxygen free environment, they are surviving. So, it is not that you have water or aqueous medium and you have the dioxygen molecule and which can give rise to the corresponding oxidation of the manganese site from manganese 2 to manganese 3 and then again 3 to 4. So, if this particular system is such that you can move from manganese 2 to manganese 4; then, one such mononuclear manganese was sufficient to get 2 electrons out of that.

But, in this particular case, like all other binuclear systems, it can go for one single electron transfer from one site and another single electron transfer from the other site. So, if we have the enzyme that dimanganese enzyme for catalyst, which is in the oxidized form, we can consider that, both the two manganese sites are in the trivalent state. This trivalent manganese can go for that; then, the reduced form – if both the two centers can be reduced, we can have a manganese 2, 2 site. And, at the intermediate position, which should be the mixed-valent one. So, one manganese site can be oxidized and equate manganese 2, 3 compound. So, manganese 2, 3. Then, other one also – that if we are able to oxidize one such manganese site to its plus 4 oxidation state, we get manganese 3 manganese 4 compound. So, these are the different possibilities. And, some of them are only observed, because you need two electron only for the catalytic site. So, all these things – you have the oxidized form; you have the reduced form.

And, when we consider this is oxidized; and sometimes, if this manganese 4 is not so common in other cases, we call them as the superoxidized form. So, the basic unit for this corresponding oxidation reaction – that if we have this triply bridging groups; and, if two of them; that means this particular one; when one single oxygen atom is utilized; that means when all three bridging groups are present, you can have some control over the manganese-manganese distance, because this manganese-manganese distance is very important, because some other reactions are also taking place during electron transfer.

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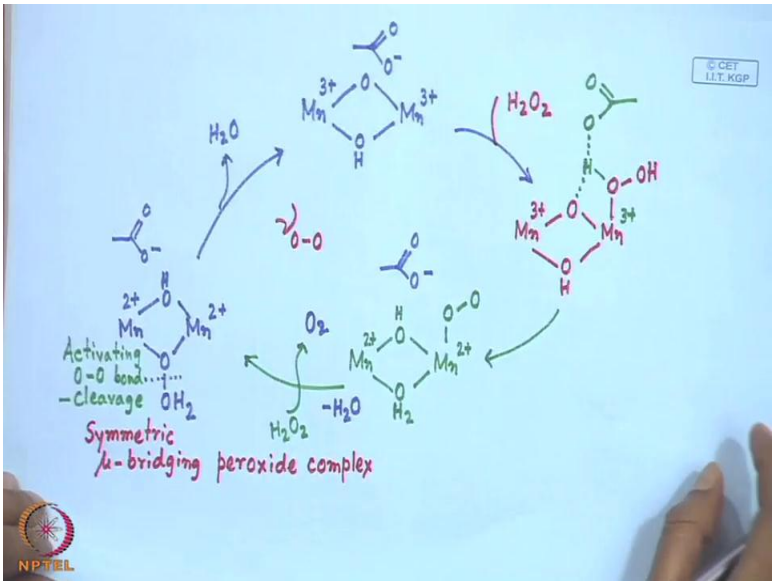

Briefly, we will see how the catalytic cycle is operating. Initially, if we have the manganese site, the most common one, that means, the oxidized one – the trivalent manganese, trivalent manganese – both of them are trivalent. And, one is bridged by hydroxo and another part is bridged by oxo. So, what happens, if you just… This particular motive; that means if we consider the corresponding shape as a corresponding diamond shape; if we bring the third bridging unit – acetate unit, how it goes to bind these manganese sites? Because these all four atoms will be in the plane of the paper. So, if you have acetate, it will be above the plane of the paper or below the plane of the paper; you cannot have these things. So, this will be a three-dimensional structure. When you talk about the sharing of one particular phase of an octahedron; these all four groups in one plane and you have the acetate group in the other or the other group below the acetate function.

But, the catalytic site – that means when you have the oxo-hydroxo connectivity, which is basically controlling the manganese-manganese distance as well. And, this particular case, these four atoms basically play that most characteristic role for its corresponding reactivity with hydrogen peroxide. And sometimes, we see that, we can have this group – the carboxylate function. This carboxylite function – if it is without the substrate molecule or without the species on which it is reacting is there; the entire substrate, that means, the carboxylate N, which is connected to manganese, can go and remain as the free carboxylate unit, because sometimes, we consider that, they are not bound to

manganese, but they are some distal groups; distal histidine, distal acetate, distal glutamate unit we call, because they have some role to play for interactions, which we consider as some hydrogen bonding interactions. These hydrogen bonding interactions are sometimes very much needed for proper positioning of the different substrate molecules. So, we need to react with hydrogen peroxides.

We bring hydrogen peroxide over there. And, whatever reaction we are thinking of here; that can be on these diamond shaped M 2 O 2 unit. That is sufficient for our catalytic cycle. So, one is oxo and another is the hydroxo one. And, this particular manganese – if they are not completely fulfilled by the donor groups; what we seen on the right-hand side? The manganese was loosely bound to the glutamate unit, which is highly distorted. This particular site – the glutamate unit was using for the corresponding distorted coordination. So, this distorted coordination is now moved. So, you can release one such oxygen connectivity from this manganese; and, the hydrogen peroxide can go and bind to this particular manganese. And, these sort of binding and coordinations are well established now when we study hemocyanin and all these. So, binding of all forms of peroxide linkages starting from the oxygen, superoxide and peroxide groups to the manganese site is well-known now. So, you have this particular positioning of the hydrogen peroxide. So, this is the hydrogen peroxide.

Why it is binding in this form? Why it is not going to bridge with this bind to this oxygen? Because this particular hydrogen can interact with this carboxylate unit. So, we have this oxygen of this carboxylate unit; that means not only the manganese; manganese is responsible for the proper coordination from the hydrogen peroxide with one of the oxygen atom on it, because the leveling experiment can also establish that which particular oxygen is responsible for this binding. But, this corresponding hydrogen bonding interactions with some terminal – carboxyl end; can also go for the proper positioning of this hydrogen peroxide group. And, not only these two interactions, this is one – the coordination; the second one is the hydrogen bonding interactions of these hydrogen with this oxygen. Also, you can have these two groups: one is OH and another is O 2 minus. So, this O when bound to the manganese site; and, this 2 manganese; this is also plus 3. So, manganese compared to the plus two oxidation state. So, this oxygen is again nucleophilic in nature. So, this will also show some interaction with this hydrogen.

These are the very well-known interactions, because we will see for the other example for this dimanganese compound that, whenever we have these oxo and hydroxo function; and, when these hydroxo function is available and which bound to such manganese 3, 3 center; this oxygen can always have some tendency, because some little bit polarization is always there. So, this hydrogen can go for hydrogen bonding interactions with other carboxyl end or any other group. And ultimately, it can be removed from there. So, the interconversion of these groups; that means always depending upon the coordination on the manganese site, is very difficult to predict for all these cases; whether you have a bound or bridged water molecule or a hydroxido function or an oxido function, because you cannot control the corresponding pKa values of the groups or the protons attached to these oxygen atoms.

Once this connectivity is established; in the third step, what we get that, this particular oxo function can now take up this hydrogen. So, that interconversion… What I am talking about that, interconversion of oxo and hydroxo function. So, this will become hydroxido function. And, this hydrogen can also after bound. So, this hydrogen can also be released. So, hydrogen peroxide is basically giving you two protons together. So, this one will be – it was OH; this one will be OH 2. And, the corresponding oxidation state of manganese will also change. And, like that of the binding of myoglobin or hemoglobin molecule, this is… Now, we can write it as a dioxygen coordination to that. So, that is the thing. So, in one case, we have seen that, in case of haemocyinin type of system, that two centers are utilized; where, initially, in the low oxidation state of copper, dioxygen is bound to the copper sites; then, electrons are transferred to dioxygen in its antibonding orbitals. We move from dioxygen to superoxide; then, to peroxide.

Now, the reverse thing is happening. You have added hydrogen peroxide. So, hydrogen peroxide is now slowly moving towards a dioxygen molecule. So, that is the thing, what we are getting for the catalase reactivity. You have the bound dioxygen molecule now. You put now, the second molecule of hydrogen peroxide. When we bring second molecule of hydrogen peroxide, this O 2 molecule as dioxygen is released from there. And, one water molecule is also released; that means this water molecule. So, these are weakly bound now. Originally, it was hydroxide; it is now weakly coordinating to these two manganese sites. So, it will be released as water. And, these two oxygen atom is like

oxygen molecule is also released. So, what we will get at this point that, you have manganese; both the two manganese sites you have.

And now, a peculiar arrangement from this particular unit is operating and sometimes is attached to edge also. So, this H 2 O 2 – this when it is terminally connected to the oxygen; that means you have initially O O H. So, hydroperoxide is forming. Then, that hydroperoxide is interacting; that means this oxygen is interacting little bit with one such hydrogen atom. So, this – still it is OH. So, you have manganese 2 and this is also manganese 2 plus. And, from that particular unit, you have this free carboxyl end and you have to write. So, at this point, you need the elimination of water molecule now. So, two water molecules and one dioxygen from two molecules of hydrogen peroxide. So, this particular thing has been proposed from there that, you have OH 2 is released and this will remain as oxo function. So, is a direct oxo transfer agent. So, hydrogen peroxide is functioning as a direct oxo transfer agent for this manganese. Already, you have O H. So, this O H is there and this is giving the corresponding oxo site. So, at this point, the situation is such that you have the corresponding… This O O H 2 is releasing. And simultaneously, both the manganese sites are available to activate the hydrogen peroxide molecule.

And, this particular coordination, that means, one such oxygen; that means you have this O O stretching frequency; you can monitor. So, it is a symmetric bridging, because the corresponding Raman and IR stretching frequency is very much characteristic. All the time, if you just look at these values; for maintaining this, whether you have bound dioxygen molecule or whether you have some hydroperoxide interaction or it has been transferred to peroxide interaction. So, symmetrical mu bridging is possible there. And, we have mu bridging peroxide complex. And, at this point, when it is terminally bound to both the manganese, we are activating the O-O bond. So, at this point, we are activating the O-O bond for cleavage. So, we can generate the original catalyst and that can again continue the cycle. So, basic idea behind that, the different types of substrate that, peroxide groups we know; that this can be formed when we talk about the dicopper system for hemocyanin. Similarly, the manganese based system. So, manganese based system based on manganese 2 and 3; that these two are immediately reacting with these manganese sites; and, here we are not forming any manganese oxo species. So, in a different sorts of binding of these hydrogen peroxide, can ultimately lead to the

corresponding reaction for catalase activity. So, this is one part; that means the corresponding redox reactions we are talking about.

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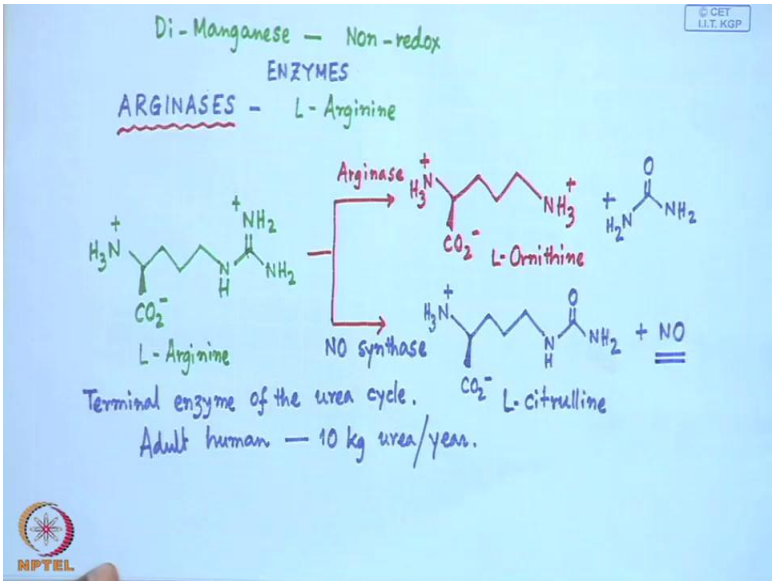

Again, manganese – this dimanganese system – dimanganese system can function as a very good lewis acid. So, we do not have any electron transfer catalases. So, it is basically a non-redox dimanganese enzyme. They are known as non-redox dimanganese enzymes. And, one such enzyme group is arginases, which is responsible for the hydrolysis of L-Arginine. What is L-Arginine? Amino acid having gaunidinium group. And, this is a very important molecule, because this leads us to give urea. So, (( )) in our system in any living organism, wherever we assimilate the protein molecules, the nitrogen assimilation; and ultimately, we remove those molecules as urea. So, this is the… And, they always present as different zwitterionic forms depending upon the pH of the medium. So, this is the corresponding L-Arginine. This substrate molecule can go for the corresponding hydrolysis reaction. So, these arginases will be responsible for hydrolysis of L-Arginine function. So, you have the cation-dependent hydrolysis.

And, there are two competitive pathways: one by using arginase to provide L-Ornithine and urea; that means this part will go as urea. So, this gaunidinium cation – this particular substrate is you see that, you have zwitterionic form. So, these charges are there. So, binding of these within the enzyme molecule is sometime facilitated, because if the enzyme functions have some different carboxylic ends C O 2 minus end. Suitable

positioning of these groups – the jwitterions – you do not need any hydrogen bonding interactions or any other non-covalent interactions. So, the part on the right-hand side will be cleaved, which is known as L-Ornithine plus (( )) urea. So, this particular part – how we cleave using this important manganese-based enzyme.

And, another interesting molecule – we will not study here, but we should know that, because these are the competitive pathways, where the very interesting molecule… Inorganic chemist always claim that this is our molecule NO – nitric oxide synthase. So, if you look at the substrate – same L arginine; you have to take out NO from this molecule. So, NO from this molecule when you take out, here we are getting this particular part as urea. But, here this part still remains with the original molecule. This is L-citrulline – c i t r u l l i n e plus NO.

So, these two enzymes basically go for competition. Why we are talking so? This is one pathway, where you can produce urea; this is another pathway, where you produce the NO molecule. So, if these two enzymes can compete for this particular substrate, you never know how much urea you will have, because you want to release these urea; because in mammals, one such case, that is, the arginine is the terminal enzyme of the urea cycle; and then… because we always need an adult human being release or excretes some 10 kg urea per year from our body. So, we basically remove this much amount of urea. So, this thing is therefore, important that, proper positioning of these particular substrate on again a dimanganese type of the catalyst type of enzyme, will go for this particular reactivity.

And, here if the hydroxo function can attack over here and it can transform to this and that cleavage of this particular group can lead to the corresponding urea molecule. But, this particular case – this simply goes. So, this particular NH 2 plus; that means what is happening? this is the carbonyl function. And, this carbonyl function, when reacting with some ammonia molecule say; it also gives the amine function – double bond NH. And, that NH – instead of attacking this particular carbon, if the nitrogen is attacked by this hydroxo function, you will get double bond N O H – the oxime function. So, that oxime – if once you can make, that oxime function can ultimately release that NO function. So, this particular – the NO synthesizes as that particular interesting function that it can release from there from the oxime function for this NO.

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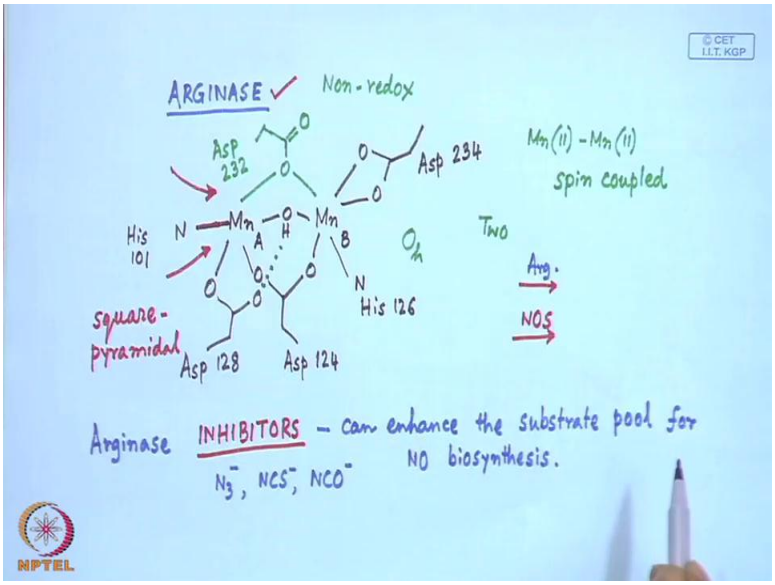

Let us see for this arginase – the basic active site structure; only we will see the arginase molecule, because once you know the arginase, we will have some good idea that, how the NO synthase can also work. So, you have two manganese very much similar to that the catalyst site. And, we simply sometime level this as the corresponding Mn A and Mn B. And now, you have OH bridging. And, this we already know that, this immediately we can go for carboxylate is now aspartate; that residues may be different. This is similar to that catalase; this is nitrogen from histidine 126. Instead of glutamate residues there, we have now aspartate. This is also another aspartate.

Now, this is much more distorted compared to the catalase active site. Now, you have this doubly bridging unit. And, the triply bridging motive is completed, but we were just telling you that is a single oxygen carboxylate bridging. So, now, you have single oxidate carboxylate bridging again from aspartate residue of different number, is aspartate 232. So, this triply bridging unit is there. And, this oxygen is basically functioning to coordinate to this manganese. So, 1, 2, 3, 4, 5, 6 – this manganese is close to the preferred octahedral geometry.

But, this manganese is not. So, you have 1, 2, 3, 4, 5. So, it is still five coordination; it can also be six-coordinated; and, in octahedral geometry, if we bring this oxygen coordinated to this manganese. But, this oxygen when it is pointing towards this particular region; that means it is a different one and this oxygen is not available for

coordination to manganese A; instead, this can show… This is close to this group basically. So, it can show some hydrogen bonding interaction with the bridging OH group. So, that gives some very important idea that, we have the two spin-coupled manganese sites, because these both the manganese because, it will not show any kind of redox reactivity; arginase is a non-redox one.

Both the manganese two will be there. So, is a dimmer of manganese 2 and manganese 2. And, since these donor groups are mostly oxygen-based, you see only you have 2 nitrogen; in catalases also, you have 2 nitrogen from the histidine residues. So, they are weak in nature. So, you have the high spin system. So, is a 3D 5 system, but there are interactions. So, there we call they are spin coupled; that means interactions are there. So, it is a spin coupled dimanganese system having manganese oxidation state on one as plus 2, another one – plus 2. And, this spin couple system will tell you that, there are interactions through its magnetic orbitals bearing the unpaired electron density and the corresponding manganese-manganese distance, which is close or around 3.3 armstrong. So, when these oxygen is not available for coordination to manganese A; rather it is engaged in hydrogen bonding interactions with the bridging hydroxido function. So, this particular one will be square-pyramidal – close to a square-pyramidal arrangement.

And, these interactions will see that, when we see the corresponding catalytic cycle, we will find that, how this particular OH group will come and will interact with this manganese show. When it is square pyramidal and it is not a trigonal bipyramidal arrangement, because this particular face is the corresponding octahedral face. And, once you have this trigonal face and one such oxygen from there; so, it is basically giving a square-planar arrangement and you have the histidine as the lone coordination site from the other site giving the corresponding square-pyramidal arrangement. So, if you have some positions like this, that immediately tells us that, the substrate is the L-Arginine and binding of this substrate to the active site is very important; how close you can push the arginase molecule to these manganese sites.

Initially, if some donor groups like the binding of catechol unit or any other phenol unit, the tyrosinase activity, what we have seen; that you have the phenol groups; and, those phenol groups are going and attaching directly to the manganese site. But, if these both the two manganese sites are surrounded by all these donor groups, we will find that, positioning of this arginase would be difficult. And, if we get some manganese – one

particular manganese site, which is square pyramidal; that means you have sudden space; the vacant sites are available. So, those vacant sites are there, which can be approached by the substrate molecule for corresponding reactivity. And, we are talking about some two catalytic sites: one is the arginase activity and another is NOS – the nitric oxide synthase. And, they – both of them will compete for this particular substrate; that means the arginase. So, whether you are producing urea molecule or you are producing the nitric oxide molecule; that will be dependent on the level in our body, which can vary from one person to other – our level of this enzyme concentration. So, one is the substrate concentration; another is the enzyme concentration. So, those enzyme concentrations for this particular arginase catalyst will tell you that, we can also design something, where you can little bit disturb the corresponding activity for NOS. So, that is one such mechanism we call, when you have some manganese with some vacant site or vacant space or some exposed area, which are designing some inhibitors called…

Next day, when will see this actual catalytic cycle, we will find that these inhibitors… These inhibitors – we will straight away call them as arginase inhibitors. So, inhibitors are some group, which will make the catalyst inactive for the arginase substrate. Designing for these inhibitors is not that all groups like the very small groups; sometimes, we even test the inhibition of catalyst inhibition by some small inorganic anions like azide, thiocyanate, cyanate, etcetera – these small groups, which can have some affinity for manganese binding. So, once you put that, there goes and bind to that manganese site. And, that manganese site will not be available for any kind of reaction with the substrate, which is arginase.

So, when we put these arginase inhibitors, what will happen? It can enhance the substrate pool; that means the concentration of the available substrate, which is there in the central pole; that means it is available for arginase activity as well as NOS activity. So, we can enhance the substrate pool for nitric oxide biosynthesis. So, that gives us some clue that, how inhibitors design sometimes can help us; that means designing these inhibitors, we do not want to see much activity from these arginases. So, we want to increase the corresponding production of NO. These are very good neurotransmitter molecules. So, they can activate the neuron and other positions. So, they are very much affective in that way. How you can increase the nitric oxide biosynthesis? You reduce the corresponding

activity of these arginase molecules such that the NO biosynthetic pathway will be activated and you can able to produce more and more NO molecules.

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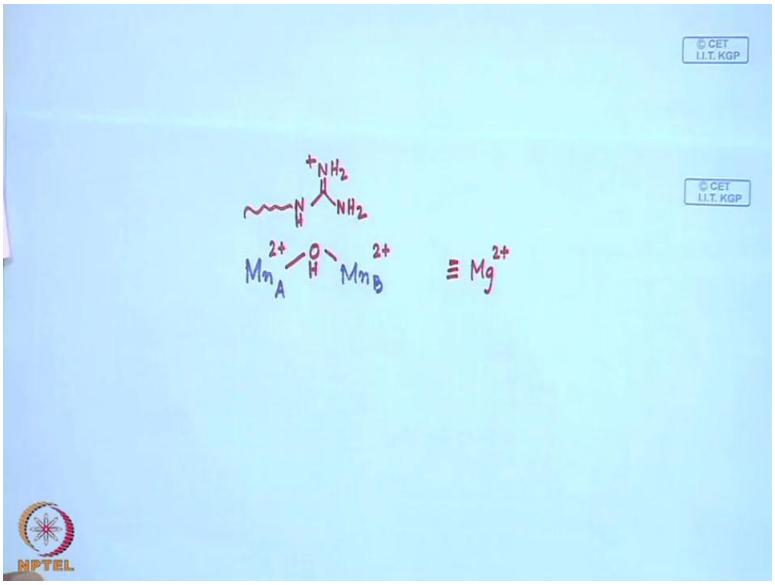

Next day, we will see the entire catalytic cycle, where the binding of the substrate would be important. And, we will have manganese A as well as manganese B for this binding. And, both these two sites are in plus 2 oxidation state. And, already, we know that, we have this particular OH function – OH group. Apart from this catalase, where we go for the redox; now, we have no liability for changing the oxidation state of manganese. So, manganese can remain as manganese 2 plus. And, which is very much similar – the atomic size and all these, because sometimes we can compare this as the charge and all other thing, is very much close to the magnesium, what is present in the photo system. So, this hydroxide function and that tail of the substrate – the gaunidinium function. So, this tail of this function, which is it is NH 2 plus. So, tail of these functions. So, it is bound.

Now, you know what will happen. It is nothing but the attack by these bound OH function to produce the urea molecule. So, very simple strategy for that. Nature has also devised for this simple strategy. First, you have the binding of the substrate, because this particular part, which is a charged one and also positively charged; you remember that, this is the beauty of this particular enzyme; that is, positively charged. You cannot consider this particular group or this unit as a ligand; otherwise, it will be negatively

charged; it will have the lone pair of electrons; it will not go. So, this sort of binding will see how it is positioning and how OH is attacking that particular end of the arginine.

Thank you.