Metals in Biology
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Lecture – 23
Mononuclear nonheme Iron (NHI) enzymes

Hello. Welcome back again. So, we are discussing metals in biology right. I think you got a sense that metal has a role in biology right. Well, just to remind you more than 50 percent of our body weight is metal. So, I think it is not just there just to be there, it is playing a role right. Let us see some more exciting chemistry.

So, today we will discuss mono nuclear non-heme iron enzymes. You have seen some of it before, but let us see a larger picture. Larger picture than what we have seen before.

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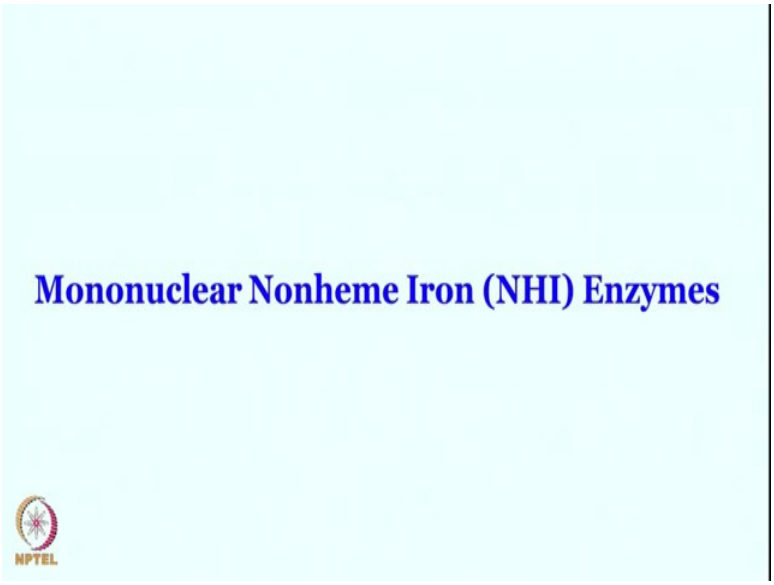

Well, as the name suggests this is mono nuclear; that means, one nucleus I guess, one center, one iron center, one nuclear. Non-heme means there is no heme involved, no porphyrin involved; iron is iron NHI. non-heme iron enzymes. So, the enzymes where you have one iron center and non-heme one which whatever is not porphyrin we usually call that as non-heme porphyrin iron is not there. So, it would be called non-heme iron. And, it is mono nuclear; one metal center would be there or one heme non-heme iron center will be there fine. Well, the role of these enzymes are very simple or rather complex or rather interesting ok.

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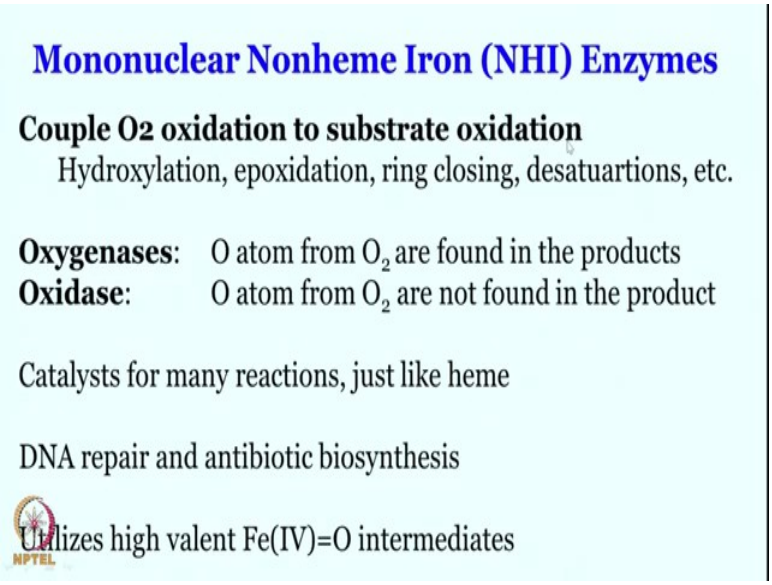

And, that is it can couple oxygen oxidation to substrate oxidation. So, that is the organic chemistry, synthetic chemistry absolutely synthetic chemistry. It is nature is a I know I think there is nothing stopping in saying that it is the best chemist ever. Nature is ever best in everything and that can do nature can do substrate oxidation better than any synthetic chemist can imagine. Hydroxylation – precisely done wherever you want done. Epoxidation – effectively whatever all of you need to be epoxide you know form epoxide can be done. Ring closing you asked nature did it. Desaturation absolutely perfect.

So, all I mean there are many other reactions this is just a short list. You know there are many reaction in the alkylation done, sulphoxidation done. Whatever you need I mean of course, you may not need it, but if you want to do it by using enzyme it can be done ok. So, these non-heme iron enzymes are coupling oxygen oxidation to substrate; that means, substrate is also getting oxidized. Well, not necessarily always oxygen has to get in ok. Some cases oxygen is getting in some cases, there is no oxygen involved into the substrate right. Well, in the mechanism yes, but in the final product there is no oxygen is getting incorporated.

So, there are two different type of non-heme iron enzyme one can think of. One is oxygenases which will end up incorporating oxygen atom from oxygen into the product. So, you start with a organic substrate, you get a product oxygen atom is inserted into that product n number of. It could be 1 oxygen atom, 2 oxygen atoms and so on substrate to

product will have at least one more oxygen atom incorporated. On the other hand, these are of course, these are oxygenases enzyme; on the other hand, there are oxidases where you have a substrate, but in the product there is no oxygen atom incorporated ok.

So, these are called oxidases, but of course, still oxidation is going on in the product. It could be ring closing, desaturation and some other things happening, but overall as you will see some of it in a moment that these are catalysts. These are non-heme iron enzymes are catalysts for many reactions just like what we have seen in case of the heme. It can participate in DNA repair by involving themselves in these oxidation processes and antibiotic biosynthesis collagen and many other things ok. Their reach is really far and also it utilizes quite interestingly high-valent iron oxo intermediate.

Of course, the nature of these species may vary, the ligand may vary, but overall it is a high-valent iron oxo species that is at play which is a very reactive intermediate and that is why perhaps nature has chosen high-valent iron oxo intermediate to be the reactive intermediate forming a number of oxidation reactions right alright.

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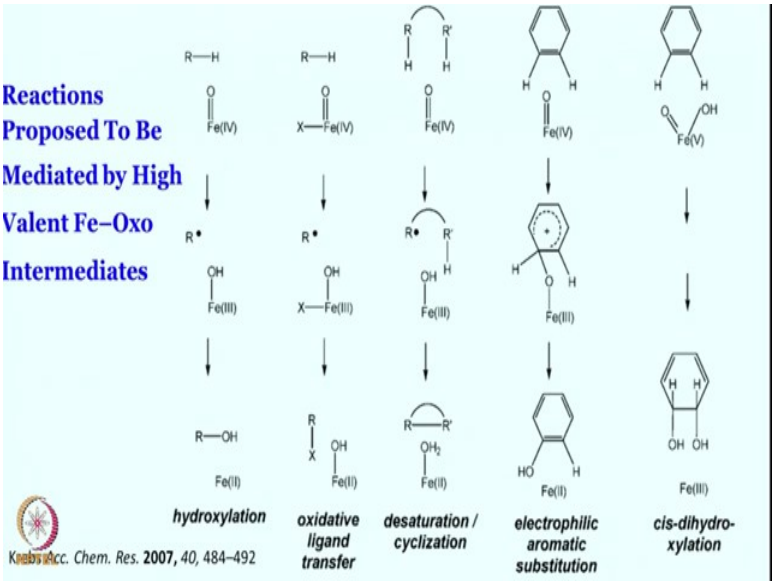

Let us try to see a little bit of the chemistry that these guys are capable of doing. So, we are trying to see the reaction that is proposed or believed or now kind of proved to be mediated by high-valent iron oxo intermediate. High-valent means usually it is iron 4 oxo if not iron 5 oxo, iron 4 and iron 5 oxo.

So, as you can see over here let us say this is a iron 4 oxo, this is a representative example you can read really this nice accounts. So, iron 4 oxo with some other ligand attached with it; iron 4 oxo, iron 4 oxo, iron 5 oxo hydroxo. So, these are the different types of species one can think of forming in the context of non-heme iron enzyme ok. These are the reactive species. These are the truly reactive species. So, for instance if you are looking for a hydroxylation reaction, it could be aliphatic substrate right. So, these are the dream reaction we have seen this right.

So, these are the dream reaction in a sense synthetic chemist cannot really do these reaction that very efficiently I mean little bit, but not really that great at all I mean you know where close to be great. So, these hydroxylation reaction by nature can be done in a predictably selective manner in an efficient manner. Things are really excellent and it is going on beautifully right.

So, we will have these iron 3 hydroxo species formation and from there I will of course, you start with iron 4 oxo react with R-H to abstract hydrogen atom from the R-H to give you R dot and iron 3 hydroxo intermediate. Now, this hydroxo can undergo rebound to facilitate this R dot to R-OH formation. So, overall you started with a high valent iron oxo intermediate reacted it with a organic substrates such as aliphatic substrate aliphatic sp3 CH bond can be hydroxylated through this mechanism.

Of course, there is a twist very interesting twist in this mechanism and that is this iron 4 oxo. If there is a another ligand such as halide let us say chloride. Now, these halogen can also participate provided this R-H is perfectly positioned well this is a chemistry which is quite fascinating I would say still, it utilizes iron 4 oxo to abstract a hydrogen atom to give R dot and OH iron 3 OH is formed along with R dot. This R dot and this OH will combine with each other in other scenario as you have seen over there, but in this case if this R dot is positioned very closely with respect to this halogen or halide chlorine let us say chloride. Now, this chloride will end up reacting with the with this R dot of course, in a radical fashion to give the R-X species.

So, this is actually a page out of this chemistry, but then there is an up twist into it to provide further this R-X and iron 2 hydroxo. So, that is I think quite phenomenal no. So, this is oxidative ligand transfer. There is going to be another interesting avenue where you will see that this R-H which is a part of the substrate can be abstracted or this CH

bond can be abstracted with iron 4 oxo to give R dot radical. Now, this R dot radical and this R-H which is again inside the ligand backbone CH bond can be cleaved to undergo CC bond formation; that means, a cyclization reaction can occur.

Of course, you know you can have desaturation another you know adjacent bond or carbon hydrogen bond can undergo radical formation and a double bond olefin formation can be can be possible. So, these chemistry either cyclization and or desaturation will be possible. So, as you see that the same species iron 4 oxo, it is taking part in all these beautiful chemistry and simple yet effective chemistry right.

So, you see substrate hydroxylation; in this case substrate hydroxylation is absolutely prevented; only selectively halogenation or ligand transfer is happening. So, hydroxylation is a possibility still it is not happening. So, the design is such that strategy is such that it is selectively of course, the oxidation potential will also play a role, but still most importantly the positioning of the substrate is the key which is essentially pushing for the halogenation reaction ok.

In this case, the substrate hydroxylation has to be prevented you know R dot is forming of course, you can immediately think of R-OH formation just like here, but that is not happening you see a cyclization which is perhaps a perhaps more likely to be happening rather than the hydroxylation reaction. So, diverting this pathway is always going to be difficult, right. So, always there is a tendency for this hydroxylated product formation, but as you have seen in this case as also in this place, it is possible with a suitable substrate and the right orientation. It is possible to undergo or force them indirectly to do pickup pathways to pick up some other possible product formation ok. So, it could be desaturation or cyclization.

Another fascinating aspects of this high-valent iron oxo intermediate is these are these are electrophilic in nature. So, this is a nucleophile a benzene ring and nucleophile can attack on this. So, electrophilic aromatic substitution type of reaction can occur as you have seen the wheeler type of intermediate is forming and you can end up getting the hydroxylated form of this benzene ring right. So, essentially benzene to phenol is forming. So, these are electrophilic aromatic substitution type of reaction.

Well, this is not all. There is yet another fascinating aspect that the cisdihydroxylation chemistry if you take even a benzene ring, it is possible to have this non-heme iron

enzymes set up where it is a iron oxo hydroxo species is the key intermediate, where you see the cisdihydroxylation of this olefinic double bond is taking place.

Well, that is getting quite exciting right. Are you not excited? I am quite excited right. This is like absolutely complete range of synthetic chemistry you can see right. You ask a synthetic chemist anyone. Anyone like us to do this chemistry in a very effective manner I think it is going to be challenging. I mean you know in synthetic setup we are not that smart yet right. What nature has done is unbelievable I mean complete control, no problem whatsoever, do the chemistry that is required or thought of absolutely perfectly. The catalyst turnover number is absolutely brilliant, selectivity is perfect and it is like whatever you want. It is like toying with the substrate you see. All of them are having similar substrate, but the outcome is completely different right.

So, I think we need to go long another way. We synthetic chemist has to has to learn quick or has to fight for decades and centuries, if ever we get anywhere closer to what nature does and how beautifully it is done. I think one way to perhaps do this chemistry is to do bio-catalysis or enzyme catalysis or try to do synthetic chemistry which is as closely mimicking those enzyme as possible, but that is going to be extremely expensive and extremely time consuming to design what nature does in the catalytic domain or in the metalloenzyme cases right. These are beautiful metalloenzyme.

So, the fight will still be on fight will always be there to get closer to the nature right, to do what nature does right. Let us move on and see one of these cases, I think it is a fascinating case.

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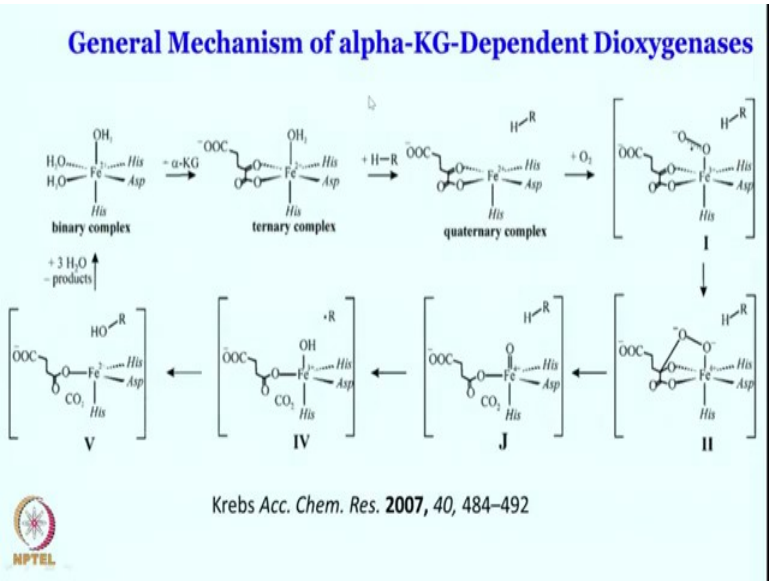

And, I think this is something you may not have seen something before and that is this alpha-KG-dependent dioxygenases. So, there is a organic substrate which is utilized in the process to extract out substrate hydroxylation chemistry or other ligand ligand transfer chemistry such as halogenation reaction.

This is a very I think exciting enzyme. These are having 2 histidine and one aspartate a facial triad motif. As you see 2 histidine and 1 aspartame and 3 water molecules are there. So, these are quite interesting compound this alpha-KG-alpha ketoglutarate. So, this is alpha ketoglutarate you know the glutamic acid. So, alpha position of it is keto. So, this is whole is alpha ketoglutarate. This alpha ketoglutarate is bound with iron centers in a bidentate fashion. This is now a bidentate ligand 2 of the water molecule has gone out from this coordination sphere of iron and you have seen that alpha ketoglutarate has come in fantastic. It is a iron 2 plus.

What do you expect that is going to be even beautiful because now you have a organic substrate attached to it in 2 histidine, 1 aspartame and 1 water molecule and then the real substrate; this is the dummy substrate. This is required for its activity right absolutely essential, but this is not our target substrate. Target substrate is something else, it could be completely aliphatic C-H bond containing substrate. It is Asp3 C-H bond containing substrate. These are the most difficult substrate usually bond dissociation energy for these are 104 or 100 plus K cal per mole.

So, this is pretty difficult bond to break and nature has mastered it like completely got it right absolutely got it right. So, that is you will see in a moment that this R-H which is appended right in front of this active site right. So, do you have 2 histidine, 1 aspartate and alpha ketoglutarate is appended beautifully right over there and R-H is just hanging on that it is on a support. There is a substrate binding pocket that is holding it right in front of it. Now, you have oxygen it gets activated ok. Iron 3+ is forming and this you have the superoxo right. One of the electron from iron 2+ is given or delivered to the oxygen to give you iron 3 superoxo.

Well, fantastic, right over here reduced species oxygen iron 3+ superoxo; iron 3 superoxo is forming. Now, this iron 3 superoxo can react with this alpha ketoglutarate because this is an intramolecular substrate already ligated with iron perfectly placed. It cannot easily react with this one because you know this is an easy substrate for the superoxo to attack. So, this iron 3 superoxo will now then attack to this alpha keto center forming a paraxol like intermediate, beautiful intermediate as you see this is a 5-membered ring formation is happening and this is very much fast.

This is very fast compared to the hydrogen atom abstraction from the aliphatic substrate. That is not going to happen that easily anyway this is very difficult. Now, so superoxo may not be that active to pick up the C-H bond off of an aliphatic substrate. Subsequently as you see the oxygen bond cleavage of these so called alkyl peroxo; so, this is the now whole alkyl group and this is now a peroxo group if you may wish to call it peroxo.

Now, of course, in the process when it is attacking it is going to be one electron oxidase and further to make it to make it a O- so, iron 3 is now becoming iron 4 iron has given 1 electron to the oxygen so, upon abstracting. So, if you are thinking a radical mechanism it is a radical O dot over here, it attacks over there. So, it forms a bond homolytic cleavage of this CO bond, if you are just following stepwise. So, a this form a bond is formed O dot is formed O dot need to be O- that electron is coming from iron and that is why it is iron 4 right.

So, this species is forming really beautifully and simply subsequently you will see that oxygen-oxygen bond cleavage to give you this succinate upon removal of carbon dioxide. So, removal of carbon dioxide happen over here. You end up leaving this

oxygen-oxygen bond and you form a CO double bond here CO double bond and this O is remained bound with the iron. Nothing happened to the oxidation states of the iron. It this remains 4+ from here. This oxygen-oxygen bond cleavage happening. Overall this iron 4 oxo then is formed.

Now, this iron 4 oxo is capable of abstracting hydrogen atom from the substrate more so because the substrate it is positioned right over there right in front of the iron 4 oxo species right, that is fascinating right. So, you have an iron 4 oxo or sitting and you have a substrate sitting. This is a highly reactive intermediate. Now, they have almost no chance of doing some other you know adventurous thing. It was alpha ketoglutarate which was ready to be there and very active substrate it was therefore, and therefore, attacking that. But, in presence of organic substrate at this point when you have a iron 4 oxo, it is not going to pick up on anything else at this point since the substrate is there. If the substrate is not there that is a different ballgame we will discuss sometime later.

Now, this since the substrate is there it perfectly matches everything so, it will go on in abstracting hydrogen atom from the CH bond and then iron 3 hydroxo. If you are cleaving it homolytically this become iron 3 and O dot; O dot picks up H. So, H-OH is formed and then this cleavage one electron here another electron there. So, one electron H dot comes over here and then R dot goes right over there in the binding pocket to the close vicinity of OH with the you know with not too much released from this you can say that it is solvent case.

Now, immediately it reverts back. This is what is known as rebound mechanism hydroxo rebinds with this R dot to give you R-OH right. So, this iron 2+ is regenerated if you are looking at if you are once again cleaving homolytically iron 2 dot and OH dot. This OH dot and this R dot combines to give you R-OH and the catalytic cycle goes on beautifully.

So, what you have seen so far? You have seen that it is possible to manipulate I would say the chemistry. You have seen in the last slide manipulation can happen based on what type of organic substrate is there. Of course, also what type of iron oxo species is there it is most often it is a iron 4 oxo species, but in some cases it could be iron 4 oxo appended or attached with some other ligand right. That is going to be quite exciting right.

In the other cases it could be iron 4 oxo instead of iron 4 oxo it is a iron 5 oxo hydroxy. Now, these are the chemistry happening. A moment ago we were just discussing this chemistry substrate hydroxylation chemistry done by a very effective you know set of that is alpha ketoglutarate dependent enzyme right. We did not discuss much of this yet. We will discuss this in the next class. Let us look at this chemistry hydroxylation chemistry once again very quickly.

So, this is alpha ketoglutarate dependent chemistry. Without alpha ketoglutarate this enzyme does not work really well. So, we have to have these alpha ketoglutarate over there and that is because it facilitates overall these iron 4 oxo formation rather easily without anything else from outside right. So, alpha ketoglutarate is a sacrificial substrate you can say. In this case it forms alpha ketoglutarate to succinate. Alpha ketoglutarate is overall forming succinic acid or succinate as you can see over there and in each and every step it has complete control right.

So, initially it is a iron 2 to iron 2 formation and then substrate orientation. Oxygen reacts with iron 2 to give one electron transfer to form iron 3+ and superoxo. This superoxo radical, then attack on the alpha ketoglutarate to give you iron 4 peroxo alkyl peroxo intermediate which undergoes cleavage to give you iron 4 oxo. It is a radical I mean it is one way to do is think of it as a radical mechanism then things becomes much clearer to understand.

So, it will abstract a hydrogen atom from here. So, R dot remained, hydrogen atom comes in. So, that leads to the oxygen-oxygen bond cleavage, iron 3 hydroxo and then OH and R dot combines to give you to give you R dot iron 3 hydroxo and hydroxo radical transfer and iron 2 deforms.

So, this is how things are happening and that is beautiful. In the next class will be discussing the alpha ketoglutarate dependent halogenase ok. These are hydroxynases or hydroxylation chemistry you have seen. In the next class we will discuss almost same mechanism, but with a twist ok. Twist is in the ligand that is associated with the iron center instead of these 2 histidine 1 asparted one of it will go out ok.

We will see that in the next class. Keep studying alpha ketoglutarate dependent dioxygenase and other enzymes and the beautiful oxygenation and oxidases chemistry by the non-heme iron enzymes ok. See you next time, till then bye.