Metals in Biology
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Lecture – 24
alpha-keto glutarate dependent halogenase

Hi, how are you all doing? Today, we are going to discuss general mechanism and some of the reactions of alpha-KG dependent or alpha-ketoglutarate dependent dioxygenases as well as halogenases. In the last class, we have seen the reaction mechanism for these dioxygenases right where we have seen that how the enzyme is dependent on alpha ketoglutarate and then how the high-valent iron oxo species is generated from this alpha ketoglutarate dependent enzymes.

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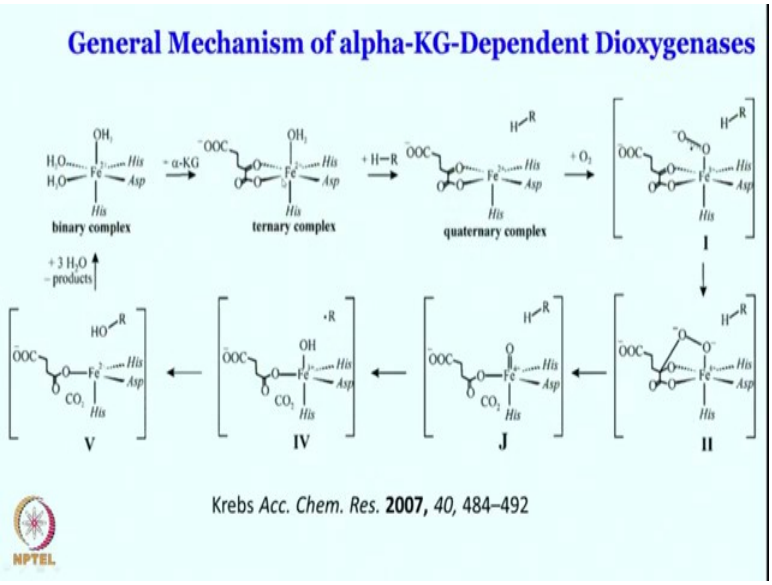

So, this is the alpha ketoglutarate and it is bound with the iron center then oxygen activation takes place and it attacks on the keto center. Overall alkyl peroxide type of intermediate and subsequently oxygen oxygen bond cleavage gives rise to the iron IV oxo intermediate which then can react with organic substrate right. So, we have seen that organic substrate can be hydroxylated by using this mechanism or by following this mechanism where R dot and OH react with each other to give R-OH right.

Now, let us look at little bit more on this we will see one crystal structure how these sort of intermediate looks like. Let us say one of these we will see how it is looking like when

alpha ketoglutarate is bound with iron and the organic substrate is also appended right next to or right at the active site ok.

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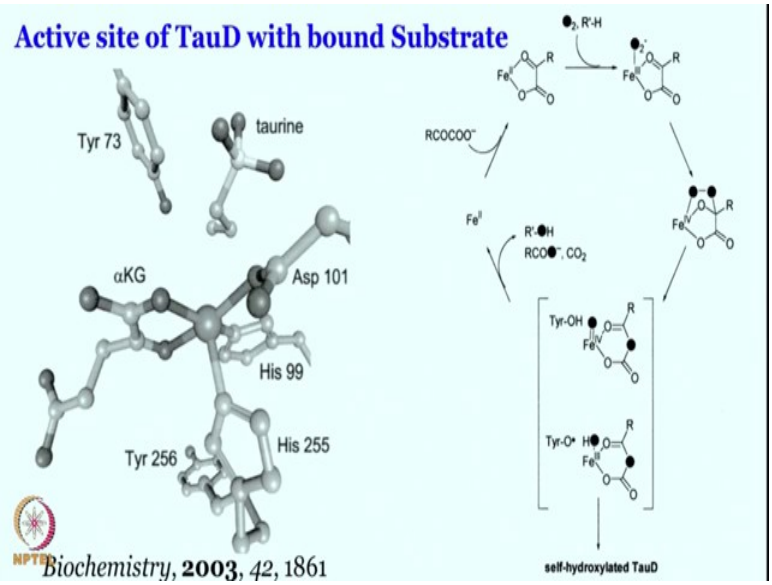

We will see TauD this is active site of TauD with bound substrate. So, this is the taurine that is the organic substrate and this is the aliphatic substrate that we want it to get hydroxylated right. As you can see this is the iron center and two of the histidine and another aspartate is right over there right. This is called facial triad motif. So, three of these substituents are right next to it. This is the alpha ketoglutarate as you can see it is bound and overall the one coordination is vacant over here where taurine is approaching of course, it is not coordinated. Now, the oxygen activation will takes place and the binding will takes place at this axial site right.

Now, the as you can see taurine is right next to the iron center; that means, it has the capability to reach out to the high valent iron oxo species that is getting generated right over there. One of the thing you must be noticing that in addition to taurine here is a tyrosine which is not a you know desired substrate to get hydroxylated because its job is to hydroxylate taurine, but when the taurine is not present then what happens we see quite a lot of reaction at tyrosine. We will come back to that later.

Let us see that what we can do. Well, this shorter mechanism is quite familiar when the taurine is not there actually tyrosine gets involved into the reaction right. So, that is quite unusual I would say, but what you can conclude is since the iron oxo species which is

getting generated over there. It is so reactive if the organic substrate that needs to be hydroxylated is not positioned perfectly or missing, then tyrosine is going to be the substrate and tyrosine to catechol formation can be done. We will see that in a separate slide and the mechanistic study on this we will discuss.

Well, this is also happening if these you know this sort of reaction also happens when you do not have alpha ketoglutarate present. If you have the succinate which is originating from alpha ketoglutarate upon decarboxylation and as you have seen in the last slide the succinate is there and then one can also get different type of mechanism ok.

Let us look at this mechanism one more time little quickly. So, this is the alpha ketoglutarate and if the organic substrate this one is not there this tyrosine will come into picture in absence of the RH or in presence of RH how things are going to be let us quickly look at. So, this superoxo intermediate is generated iron 2 reacts with oxygen, let us say this is a leveled oxygen iron 2 superoxo is getting generated then that attaches or attacks on the keto moiety right fantastic that attacks on the keto moiety and you have this you know beautiful ring compound.

From here on the rearrangement reaction gives rise to this intermediate where you have a iron IV oxo species generation. Now, this iron IV oxo species in absence of taurine or the suitable substrate it can abstract hydrogen atom from this tyrosine right tyrosine phenolic oh it becomes tyrosine radical and then hydroxylation can go on. We will discuss this again in few slide. Now, in presence of substrate like this R prime H, R prime H if it is sitting right next to it R-OH formation will be going on. Decarboxylation can lead to alpha ketoglutarate to succinate of course, carbon dioxide also will come out and the overall catalytic cycle can be completed by the regeneration of iron IV to iron II right so far so good.

Now, let us look at little bit related, but I mean much related lot different outcome reaction mechanism and the substrate halogenase and chemistry right. So, there is a series of enzyme or series of reaction that can happen if you have little bit twist into your active site; what is that?

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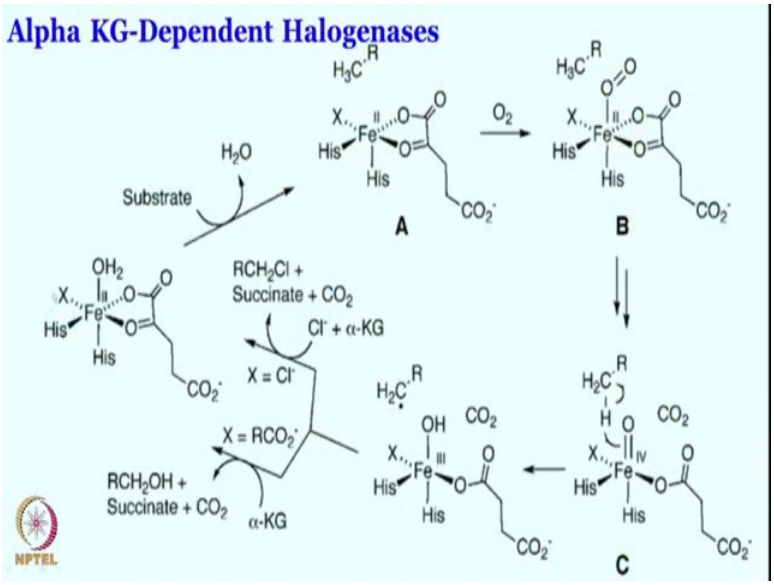

Well, you we were having 2 histidine and one aspartate right previously with the alpha ketoglutarate dependent hydroxylases right oxygenases I. So, we have 2 histidine and one 1 aspartate; if you remember 2 histidine and one aspartate was there. Now, what if this aspartate is removed and placed with another halide. So, this becomes a completely different enzyme now. This is called alpha KG dependent halogenases. So, that means, a halogen is sitting over there instead of aspartate and of course, you still need alpha ketoglutarate. This is alpha ketoglutarate dependent halogenase right.

So, the substrate just like previously for the oxygenation chemistry or the oxidases. You have seen previously this oxi dioxygenases, you have seen that substrate is coming on to the organic moiety or on to the metal centre. So, substrate is oriented. Similarly, over here also the substrate is going to be oriented or fixed right next to the iron centre, right. It is the exactly same thing what is happening previously to histidine one halide you have instead of this aspartate.

Reaction mechanism and things remain similar. We have seen the next step would be after substrate binding next step would be the oxygen activation or dioxygen activation, that is what happens. First of course, has to undergo binding, that is true in every case. You will have the bind bound oxygen intermediate iron-oxygen bound intermediate. This is fantastic 2 histidine, 1 halide and alpha ketoglutarate. Then one electron transform from iron II to this superoxo occurs the you get the iron III superoxo intermediate that

attacks on this center, not this center. This is the carbon dioxide releasing part. On this superoxo once it generate it will attack over there. As you have seen previously further cleavage will gives rise to the iron IV oxo intermediate.

Now, up to here exactly same actually up to next step is also exactly same as you have seen in the dioxygenase, but over here what you see that of course, iron IV oxo will abstract hydrogen atom from the aliphatic substrate that is all fantastic and we will give RCH 2 dot or the substrate radical intermediate and you have the hydroxo intermediate right if it abstract hydrogen atom you will get RCH 2 dot and OH.

Now, what happens in the halogenases enzyme, the halogen is sitting very close and nicely placed with respect to this radical even compared to the hydroxyl and therefore, quite excitingly for the halogenases you get exclusively halogenated product formation. Of course, succinate comes out, carbon dioxide comes out and then you delivers halide to regenerate the catalytic cycle. As you have seen in the earlier case when X is aspartate and that means, no halogenation business and hydroxo is close to that and then it can do the hydroxylation chemistry. Again succinate or a carbon dioxide comes out as the byproduct.

So, what you have just seen right now by changing just one ligand; of course, that is the iron halogen we are talking about. Iron X just one ligand nature can modify, control absolute control I think that is what is really amazing that nature can completely control the reactivity. Everything else is remain same only the aspartate is replaced by halogen. Now, all of a sudden no hydroxylation products are forming, but exclusively halogenation products are forming. I think that is quite phenomenon and that is why you see it is going to be sure difficult to compete or try to mimic what we have in nature right.

These efforts are going to be very tedious and lengthy. Indeed you would notice that that there exist mimics for the alpha ketoglutarate dependent dioxygenases, but there is no mimic so far on the structural plus functional together these halogenases one. Well, that is quite amazing how nature really does. Let us look at this one more time little bit.

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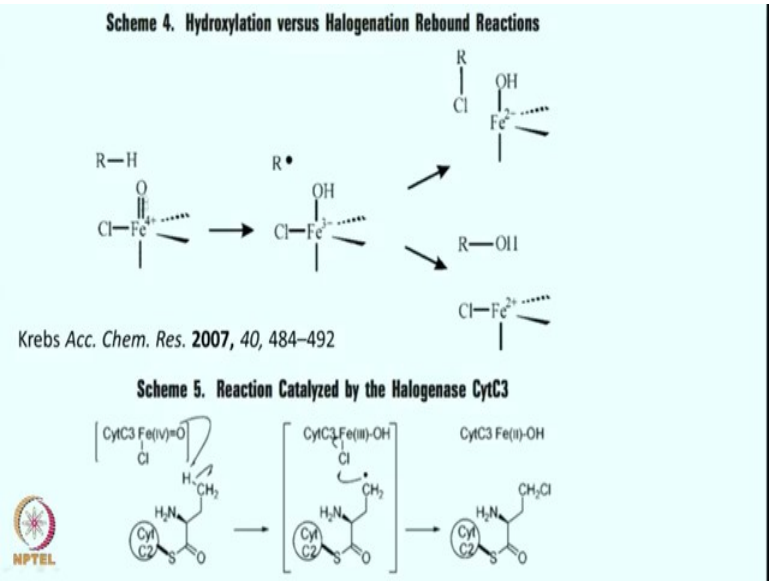

So, what we are saying that from the iron oxo this R-H under goes hydrogen atom abstraction to give you hydroxo and R dot. Now, this R dot is not going to the bound with the hydroxo, instead this R dot is going to bind with an react with halogen or halide over here to give sorry, halide over here to give the halogenated product.

Well, that is quite phenomenal I as I would say because this hydroxylation is quite challenging or in these cases, but overall you have seen exclusive halogenation was happening in the other case right. Now, no halogenations, only hydroxylation only halogenation is happening. This has to do in two count; one this is due to the fact that this radical R dot radical is positioned really perfectly to and very close to the halide over hydroxyl.

Another thing is the you know that reduction potential for the halogen is suited to transfer over there compared to hydroxo can transfer, but halogen transfer is much more facile. So, that is how nature has designed and decided to take advantage of this system right. So, one common intermediate almost throughout the catalytic cycle, one subtle change complete different product distribution complete exclusive product distribution. So, in one case you have seen the hydroxylation in the first cases and these halogenation enzyme can exclusively give the halogenation product.

Now, you can imagine when synthetic chemists are trying to promote some reaction it is actually always going to be the hydroxylated product that is going to be predominantly

forming ok. Almost 95% efforts that has gone in mimicking the halogenase activity actually ends up failing to mimic it, but it just gives the hydroxylation product. Almost in most of the cases no halogenation product whatsoever can be formed. In some cases at best mixture of halogenation and hydroxylation product can be formed so far.

Well, there exist alternate mimic which we may not be discussing too much, but there it is possible to promote halogenation reaction by utilizing high-valent iron oxo intermediate by taking a completely different route. In any case let us look at one of the practical example. This is for the organic substrate you see over here and this is in halogenase cytochrome C3 and you have a iron IV oxo chloro intermediate. Now, in these cases the CH_2 and Cl dot transfers and you see exclusively CH_2 Cl product along with the iron II hydroxy product formation. This is quite great.

Well, we will not be discussing way too much into detail. These are very very fascinating enzyme and lot is known lot of studies has been done on the enzyme. If you are further interested feel free to study.

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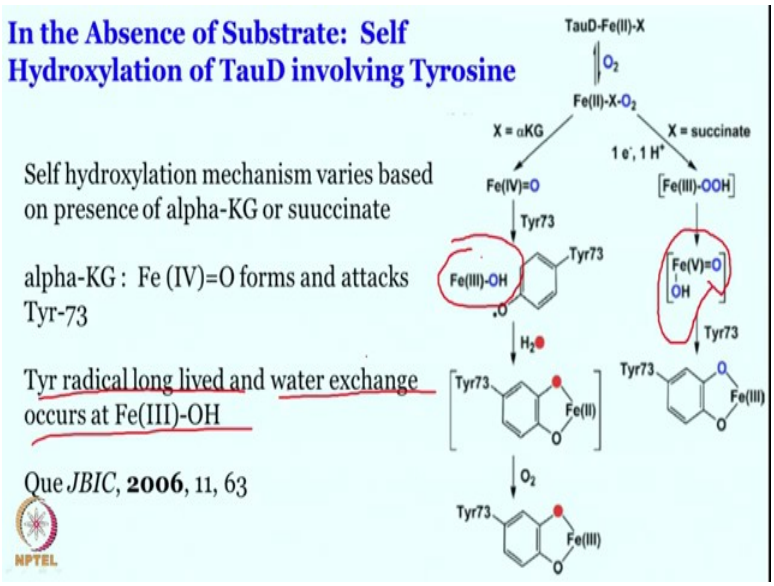

Now, we will try to see one of the other aspect which we are mentioning earlier and that is in the absence of organic substrate. For example that is in TauD taurine is absent what happens to the tyrosine because tyrosine as we have seen in the crystal structure is appended right close to the active site. Is the tyrosine going to be participating in the reaction in absence of the natural substrate that is a terrine for TauD right. Well, the

answer as we have briefly mentioned yes, phenol is going to be participating into the reaction, but what happens to phenol ok.

Before that let us look at once again the same reaction mechanism that we are discussing it forms a iron II oxo iron II oxygen intermediate subsequently with the help of alpha ketoglutarate we do see that this iron IV oxo intermediate is forming. Well, this iron IV oxo intermediate can then react with tyrosine if the organic aliphatic substrate that is required to be present is not there right well. So, you get phenoxy radical intermediate tyrosine radical intermediate along with iron III hydroxo formation right.

Now, you can imagine if this is the case, now this can go on and form a oxygenation or undergo oxygenation at the ortho position and that is what is exactly happening. This phenoxy radical then undergo oxygenation reaction to give you the catechol intermediate. Well, as you have perhaps noticed if you are using labeled water let us say O^{18} labeled water in these cases when tyrosine is reacting; that means, the organic substrate is absent then this water molecule oxygen. So, oxygen derived or oxygen of the water molecule can be incorporated in the phenol equivalent or phenol molecule right. So, this is going to be catechol now and you know that is fantastic if you under oxygen it can further get oxidized to iron III.

So, what you have just seen right now in the absence of the natural substrate organic substrate that it is supposed to be hydroxylated by alpha-keto dependent oxygenase you do not if you do not have such organic substrate, you end up getting a completely different reaction and that is the phenoxy radical generation because iron IV oxo is so reactive. It is not going to be sitting ideal just and you know just beside that it does not have the organic substrate. It is not going to be sit down over there it is going to react with anything that is available to it. In this case phenol is a easy substrate to react and it ends up reacting and giving us phenoxy radical subsequently the catechol moiety which is alright, right.

But, I think the most interesting part in this case is if you add O^{18} labeled water. As you have seen O^{18} labeled water is getting incorporated the O^{18} labeled oxygen is getting incorporated in the product. So, what water oxygen atom is incorporated into the product, this is a clear-cut evidence that something must be going on and that something is this iron III hydroxo and this water can exchange with each other. So, this hydroxo can

become water and this water becomes hydroxo and that hydroxo can be incorporated into here right.

Well, that also would mean that this stability of the phenoxy radical is quite high. Unless until this is stable or this has some lifetime you would not be able to see this sort of exchanges right. So, the reason or the moment you know that this exchange is happening then you are certain that the phenoxy radical is long lived enough so that it allows the exchange and then the reaction can also happen. Of course, it is not going to be exclusive formation of the O^{18} labeled water you still can get O^{16} water O^{16} oxygenation here. But, the fact that the labeling can be found is indicative of the two things as I said. One thing it is I exchanging this hydroxo and this water molecule are exchanging with each other, second thing is this phenoxy radical is long lived right.

So, that is what we have written over here and well, this is the self hydroxylation mechanism right. So, we see that self hydroxylation of the enzyme is happening in absence of organic substrate, but this mechanism will vary based on the presence of what you have. If you have alpha ketoglutarate this is the pathway, but it is not necessarily you have always the alpha ketoglutarate.

If you run out of alpha ketoglutarate you can essentially can have let us say succinate because succinate is the product derived from alpha ketoglutarate and as you have seen alpha ketoglutarate undergoing the reaction overall in these enzymes to give you succinate. So, succinate is going to replace alpha ketoglutarate if it is not present in enough amount right. So, in those cases succinate is definitely not alpha ketoglutarate it will act just as a monodentate ligand. As you have seen in the last slide alpha ketoglutarate is a bidentate ligand right.

So, succinate that is getting produced over here is going to be likely the monodentate ligand and of course, it has also an probability of bringing these two acid together. But, in any case this is almost going to be similar to let us say aspartate type of substrate and sorry aspartate type of ligand. In presence of such ligand or in absence of alpha ketoglutarate you end up providing one electron and one proton in the system. So, in the enzyme it ends up forming iron III hydroperoxo.

Now, it is not that something new in this. Yes, iron III hydro peroxo species can form. Essentially what is happening here iron II is reducing oxygen to give you iron III super

oxo, one electron transform from iron II into oxygen gives you iron III plus and oxygen 1 minus that in super oxo iron III superoxo is formed. You give one electron and one proton that superoxo radical, then it becomes iron III hydroperoxo. This is what you are seeing over here right. Iron III hydroperoxo you are getting and this iron III hydroperoxo can undergo further cleavage of the oxygen-oxygen bond to form iron V oxohydroxy species, if you break the oxygen-oxygen bond and then that radical will give you that iron V hydroxo.

Let me see if you can draw little bit over here and well, I would need a new page perhaps white screen.

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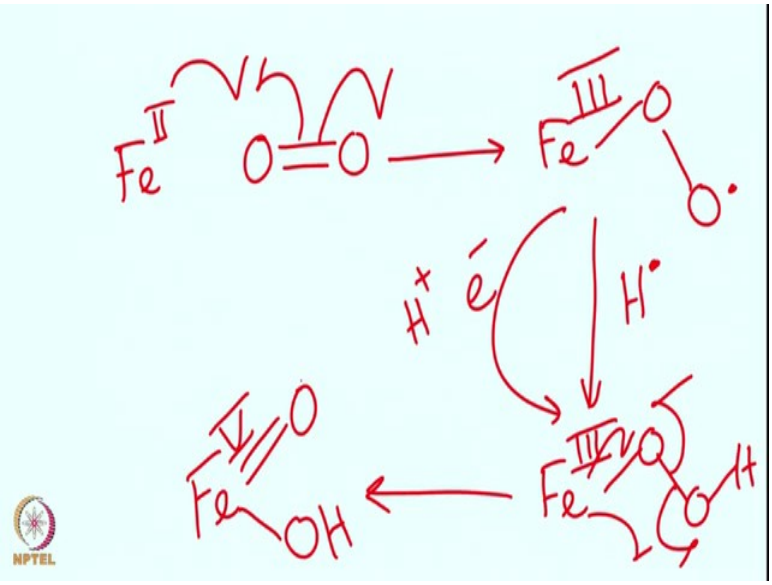

So, what we are trying to say is essentially if you have iron II right and reacting it with oxygen ok, now this is going to form iron one electron gives one electron gave one electron gives. So, you will get iron III superoxo radical right, that is fine and then you can give a hydrogen atom it could be one proton plus one electron overall a hydrogen atom if you are giving then you are going to get iron III hydroperoxo species right.

Now, if you have seen, if it is breaking oxygen-oxygen bond; that means, it give one electron here one electron there. Now, iron III to form a iron IV it will end up giving one other electron. So, double bond O will be forming right over here and this hydroxo radical if it has to bind with iron, iron will end up giving another electron. So, iron is

overall producing two electrons or giving two electron. So, iron III becomes iron IV and iron IV becomes iron V. So, overall you have iron V oxo hydroxy right.

So, iron V oxohydroxy intermediate is happening and I hope you understand how it is happening iron III gets oxidized once iron III get oxidized another time. So, the iron hydroxy and iron V oxo is forming in these cases. So, that is what and as you have seen that is what is happening over here in the right hand side ok. So, that is where you see in the right hand side in here and iron V oxo fascinating and it is happening right over there and you can get a clear idea from what we have drawn earlier. And from there on as you can see this iron V oxohydroxo can also give this you know catechol type of intermediate formation ok.

So, the conclusion from this reaction is tyrosine radical is long lived and water exchange occurs at iron III hydroxo as we you are mentioning over here and iron IV of course, iron IV oxo forms and attacks tyrosine 73. So, these are all we have discussed so far. So, what we have seen so far then in this class, two things I would say. First thing is the alpha ketoglutarate dependent halogenases which is a great enzyme right. You get exclusive halogenations, it is not that great. Aliphatic substrate halogenations, it has to be one of those base reaction ever I would say right.

Well, if you do not have the halogen you have seen that it could be hydroxylated, that is the normal enzyme or most of the things time that is what happens. But, special enzyme such as alpha-KG halogenases it can do helogenate the organic substrate that you have seen and the mechanism you have seen it is nothing different. It is almost exactly same as the oxygenases, but only varies in one of the ligand which is on the iron center.

On the other hand, the last part what we are discussing today contains what? Contains yes, thus does not contains the does not contain the substrate. So, it does not have the substrate the desired aliphatic substrate and therefore, we see that the reactive iron oxo intermediate is not going to sit idle. It is going to react with itself means its own environment and that is where tyrosine 73 which is right next to the active site will come into the picture and it gets hydroxylated right. Tyrosine getting hydroxylated means phenoxy radical; phenoxy radical leading to the catechol moiety ok.

Now, this mechanism or the you know the intermediates can vary. It could undergo a traditional oxo mechanism that we have seen in the oxygenase and halogenases, but that

is when we have the alpha ketoglutarate present. In absence of alpha ketoglutarate essentially you need to have something and that something is succinate because succinate is getting generated from alpha ketoglutarate in this enzyme.

So, when alpha ketoglutarate is running out or we ran out or of alpha ketoglutarate and that is when succinate comes into the picture. For the tyrosine in absence of TauD or organic substrate we are going to get once again the same product in terms of tyrosine because tyrosine is the substrate that is going to tyrosine will act as a substrate and will give you catechol, but more importantly the reaction mechanism is completely different here right.

As you have seen that reaction mechanism would required a iron oxo hydroxy intermediate formation right this iron oxo hydroxy intermediate formation ok. And I hope how it is forming you have seen it. Just practice it little bit. This is very simple and we will come back soon in the next class. Till then keep studying.

Thank you very much for listening; we will see you soon.