Metals in Biology

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Lecture – 17
Iron catalyzed oxidation of unactivated sp3 C – H bond Part I

Hello welcome back to today's class on Metals in Biology. So, hopefully you guys are doing good, right. I hope you have seen in the last class what we were discussing and that is copper oxygen chemistry right. Today we are going to discuss some iron chemistry is that ok.

So, the today's topic is selective hydroxylation of aliphatic C-H bond by iron chemistry ok. Now before getting into that topic let me tell you that the iron chemistry and the copper chemistry are similar indeed a lot of metal oxygen chemistry and you know metal oxygenation type of chemistry are similar in nature if you want to compare and contrast.

But every species every metal species are different and therefore, stabilizing them and making them reactive is also different or difficult at points right. Well, today we will discuss how the reactive iron species are generated and how they can functionalize substrates such as hydroxylation of organic substrates and if there is any reactivity pattern that is coming out of this iron chemistry that we would also like to see.

So, reactivity with the pattern study will be the major focus, but getting into that will require us to synthesize these complex and understand the reaction mechanism right let us get into that.

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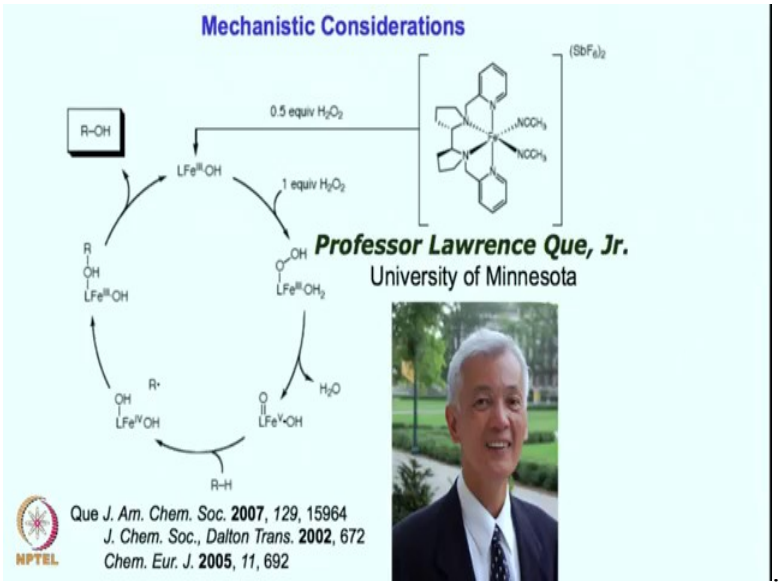

Now, we can get fast into the species formation. So, this iron active species in intermediate can be generated by different ways, one of the way today we will discuss where iron II species is a reacted with hydrogen peroxide. First of all this let us say any ligand this is a particularly ligand used by Cristiana Whites group, but this ligand iron unsupported this is a tetra dentate ligand as you can see ligand supported iron species is capable of reacting with hydrogen peroxide half a equivalent to give iron III hydroxo intermediate, such a iron III hydroxo intermediate can react with hydrogen peroxide to give you the iron III hydroperoxo intermediate.

So, this is nothing, but an acid base chemistry, here homolytic cleavage of hydrogen peroxide gives rise to the hydroxo radical and two of these molecule will react one of this hydrogen peroxide to give the iron III hydroxo compound, fine. Now once you are able to generate these iron III hydro peroxo species, it can undergo oxygen-oxygen bond cleavage by homolytic or heterolytic a mechanism these mechanistic details can vary, but let us say the one of the electron from iron III will be donated over here and this hydroxo radical or hydroxide minus that generates can now add to this.

So, whatever way you want to see it overall iron is getting oxidized doubly. So, if it is a radical mechanism you are looking at O dot OH dot forms iron III gives another electron iron IV oxo OH dot react with iron IV oxo to give iron V hydroxo to form this bond. So,

it is a iron V oxo hydroxo intermediate that is generated once a iron II intermediate is reacted with hydrogen peroxide.

Of course one and half equivalent total is required. Now these intermediates are fully active and very reactive as you can imagine this iron V oxo hydroxy intermediate can abstract hydrogen atom from the organic substrate to give you R dot radical and iron IV hydroxy intermediate. This hydroxo intermediate can then react with R dot to give you ROH which can go out.

Essentially what we have seen just right now then, we are able to synthesize high valent iron intermediate a specific intermediate in this case iron V oxo hydroxo intermediate, it is capable of reacting with organic substrate to give the hydroxylated product. Now a number of studies from early days over decades has been done by Professor Lawrence Que group in this field and a number of ligand has also been utilized by using this bethought, but before getting into that let me discuss other possibilities that one can think of if some other ligand or other type of chemistry is explored.

For example if let us say porphyrin type of ligand is used it could also be a non heme tetradentate ligand or other ligands.

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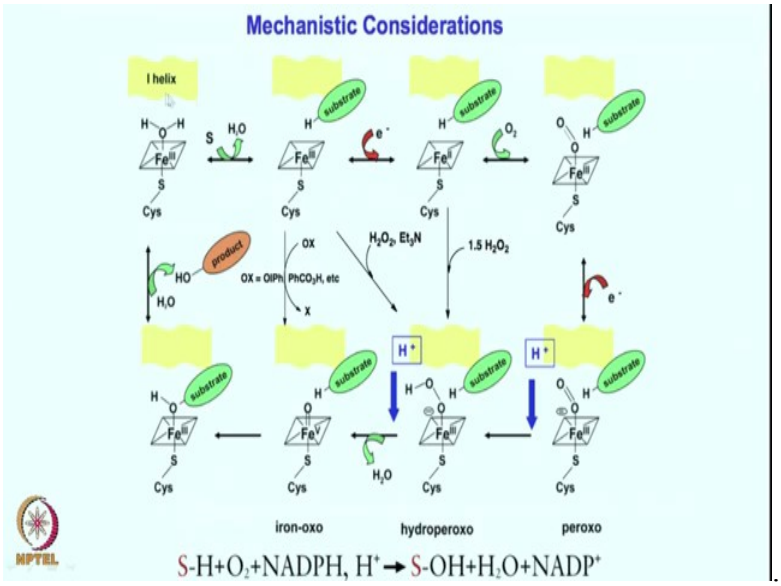

But let us just to keep it generic we tried to put here a iron III aqua (Refer Time: 06:22) complex bonds with a cysteine these are biologically relevant that these are active

intermediate in quite a number of the enzyme metalloenzyme as you have seen earlier. Now this iron III species will react so, this is a iron III porphyrin species and then there is a cysteine ligation from the axial position ok.

Now, this iron III species can react with a substrate this is not a reaction as per say this is just a substrate orientation, where substrate is oriented right in front of the active site. Then what we see is this electron transfer happening to this center to reduce it a iron II (Refer time: 07:11) thiolate bound intermediate ok.

So, iron II ligand intermediate is generated you can synthesize of course, from different other species synthetically, but overall this is a how the enzyme activation takes place. So, now, it was not that reactive substrate binds also electron transfer occurs, now it is iron II and a very reactive intermediate.

From there on previously we were discussing that is the hydrogen peroxo chemistry if a similar hydrogen peroxo chemistry is happening, we can get iron III hydroxy and then finally iron III hydroperoxo which can undergo oxygen-oxygen cleavage ok and then can overall give a iron V oxo intermediate which can abstract hydrogen atom to give the hydroxo bound substrate intermediate which can then be released and the substrate gets hydroxylated.

So, what we have seen so far then starting with iron III these aqua complexes can be reduced to overall iron II intermediate and from there on this intermediate can react for example, with hydrogen peroxide to give the hydro peroxo intermediate and oxygen-oxygen cleavage can give you the iron V oxo intermediate, which can then abstract hydrogen atom from the substrate which is placed right close to this active site and substrate oxygenation chemistry takes place and therefore, overall substrate hydroxylated product is generated ok.

This is an alternative pathway so, called peroxide shunt mechanism we will have seen in different other places. Now the usually the enzyme can also react with iron II and oxygen to give the iron III superoxo intermediate, this is the iron III oxygen is reduced by one electron iron III superoxo intermediate, one electron from iron goes into oxygen to form the iron III superoxo intermediate, from there one electron transfer gives rise to iron III peroxo intermediate, protonation can gives rise to iron III hydro peroxo intermediate rest of the chemistry is same.

So, what we have seen in these mechanism is iron II is reacting with oxygen superoxo, peroxo, hydroperoxo, oxo hydroxy bound substrate and the product formation catalytic cycle goes on. Well, you must be noticing that there is similarities with copper chemistry right, if it was proper it would it was copper II reacting with oxygen copper superoxo, copper II superoxo, reacting with one electron copper II peroxo, proton copper II hydro peroxo and so on.

So, as you see the iron chemistry and copper chemistry parallel to each other and more importantly all of them are capable of doing chemistry of course, not all of them will be equally reactive. These comparisons are very very sensitive and difficult to make, but overall as you can see iron III going reduced to iron II and reacting with oxygen to give you the substrate hydroxylation product.

Yet another alternative method could be starting with iron III is reacting with high valent Oxyl intermediate PhIO which forms iron V oxo directly. So, this is the smallest possible catalytic cycle probably over here without hampering anything this is the reactive intermediate and these perhaps also can be an alternate mechanism for different substrate hydroxylation chemistry.

In synthetic setup all these 3 pathways that we have shown of course, there is you know if you are counting this other one hydrogen peroxide there is another pathway reacting with iron III hydrogen peroxide and base. So, all these possible pathways can be seen in different system or in our synthetic studies, but enzymatically we are limited in the number of paths so far known, mainly a number of reactions are happening by this mechanism and also the hydrogen peroxide pathways can also be utilized in few cases.

Now, for our purpose of discussing substrate hydroxylation chemistry with non heme iron setup this is heme, the last slide we were discussing mainly non heme right. This is heme chemistry this is also similar to what can happen in non heme chemistry we have seen part of it like we have seen this part is happening exactly same as non heme, actually other part and can also happen in non heme chemistry as well.

Let us look at of what we are going to see in the non heme chemistry where porphyrin is not there ligand is non porphyrin based ligand.

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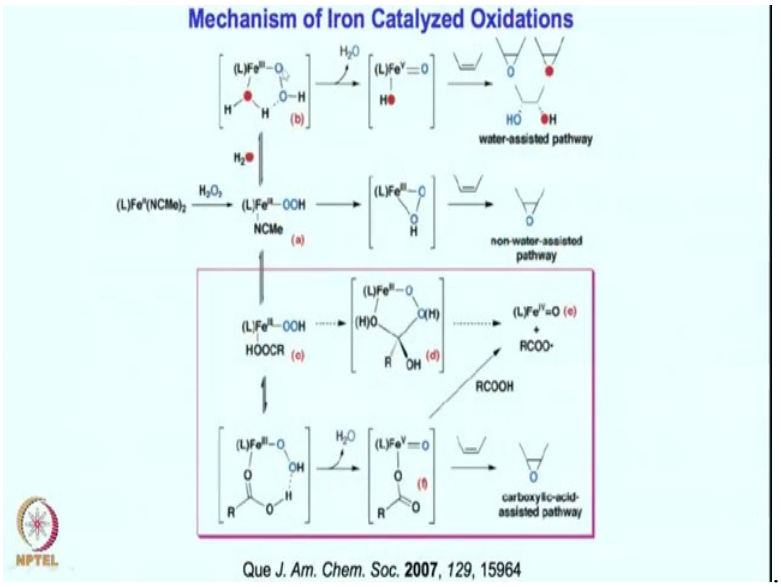

Now, we have seen how in non heme or heme system iron III hydroperoxo can be generated right. Also there is a aqua molecule attached over here this aqua molecule along with this hydro peroxo can form a hydrogen bond and over all this oxygen-oxygen cleavage can go on and this water molecule can come out and you can imagine that this if you are looking for a homolytic cleavage one electron they are, another electron there comes in and then iron III goes to Iron IV and this water goes out this water OH radical that gets generated, then would require another electron from metal overall iron V oxo hydroxo species is generated.

So, if you want to see homolytic cleavage cleave homolytically all the bond, then water goes down hydroxyl radical here, O radical there, hydroxyl radical will form iron hydroxide. So, we will take one electron from there, these iron O dot radical will pick up another electron from their this iron V oxo hydroxo form, such an intermediate can react with substrate organic substrates such as olefin to give the epoxide.

Now, depending on the labeled oxygen or unlabeled oxygen, if you have labeled this water molecule for example, then we will end up seeing both the oxygen in the epoxide is forming so or coming. So, you can imagine that there is an equilibrium between this oxo and this hydroxo this can be oxo this can be hydroxo also overall the oxo transfer or cis hydroxylation chemistry can happen where both the unlabeled are labeled oxygen can come in if water is labeled with let us say O^{18} ok.

So, what we have seen then it is possible to react iron hydro peroxo species with organic substrates such as olefin to give the either olefin epoxidation product or the cis hydroxylation product. But more importantly we understand that this ligand can be labile because this water molecule, it can be replaced with acetononitrile or other nitrile solvent or it can be replaced replaced by this acetic acid or any other acid right, where acetic acid can be bound with the iron center in this fashion. So, what we have in the hand is iron III hydro peroxo intermediate, this iron III hydro peroxo intermediate is a hydrogen bonded with the system right.

Then over all what is happening is similar a oxygen oxygen bond cleavage can go on to give this you know carboxylate bound intermediate instead of this hydroxo bound intermediate and from there on we see that iron V oxo carboxylate just like iron V oxo hydroxo intermediate is generated and this can then do the oxygen transfer or other substrate hydroxylation chemistry.

So, by this way what we have seen that non heme iron species of course, heme iron species are also very very reactive, but we are trying to focus on the non heme iron oxygen chemistry we try to see that it can you know in this case hydrogen peroxide is reacting and giving a reactive intermediate which can then be forming our desired product or the substrate oxygenation product. So, a number of ligand system that can be used or here indeed there are hundreds of ligands perhaps.

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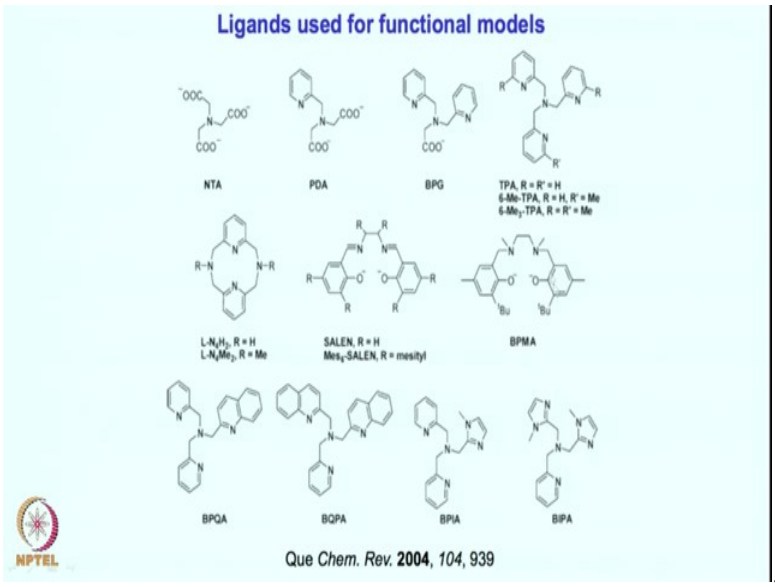

And this is a good review to study the iron reactive intermediate chemistry feel free to study if you are very much interested this is a very very well developed field and lot of things are known you should be familiar with this topic.

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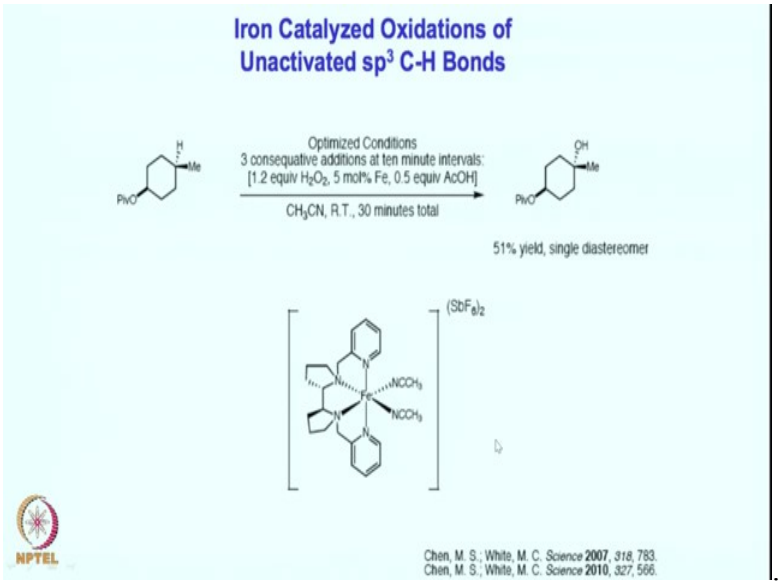

Now, let us get back to what we were done trying to mainly discuss or trying to focus on and that focus is about the substrate hydroxylation chemistry of organic substrate right. Well, we essentially what we are trying to see is by understanding the enzyme mechanism intercepting the intermediate of the enzyme we are trying to utilize those understanding in synthetic setup and after synthesizing this iron suitable reactive intermediate we are trying to see if we can do a synthetic transformation such as hydroxylation selectively right. While of course, there is not much reliable method known that can do the hydroxylation chemistry of the alkane which is a very very difficult reaction because bond dissociation energy of these aliphatic C- H bonds are quite high.

And therefore, we wanted to focus and see how these hydroxylation chemistry happen ok, these are the two research work for this predictably selective hydroxylation chemistry. This is the ligand being used for this iron center as you can see these will be perhaps the site for this iron oxo carboxylate or oxo hydroxo intermediate.

As you can see hydrogen peroxide and acetic acid is used therefore, you can think of having the chemistry similar to what we were seeing over here right. So, oxo carboxylate

intermediate will be generated, this intermediate will then abstract hydrogen atom from the organic substrate right let us go on. So, this oxo intermediate that we were talking is the one which is doing the chemistry.

Now, if you take this substrate for example, there are many different type of carbon hydrogen bond present as you know this is sp3 centre all of these are sp3 center. This is primary sp3 C-H center, this is a tertiary center, these are secondary centres and this is of course, a tertiary, but it is attached to which an electron withdrawing moiety. Overall what has been found despite having this 1 2 3 and 4 type of carbon hydrogen bond I would say 1 2 3 4 5 maybe you can say 5 different type of carbon hydrogen bond. One can still see that selectively this carbon hydrogen bond which is tertiary in nature is getting reacted which is quite fascinating ok.

So, what we are then trying to see is the reactivity pattern of these iron oxo intermediate iron V valent 5 oxo intermediate and try to see if a complex organic molecule like this is there can we predict the site of hydroxylation. Once again this is a primary, this is a tertiary, this is a tertiary centre, this is a secondary centre, this is a secondary centre selectively a tertiary C- H bond is getting hydroxylated right.

Let us look back some more example before that let me try to discuss that in epoxidation chemistry some predictably selective thing can be done for example, these olefin epoxidation.

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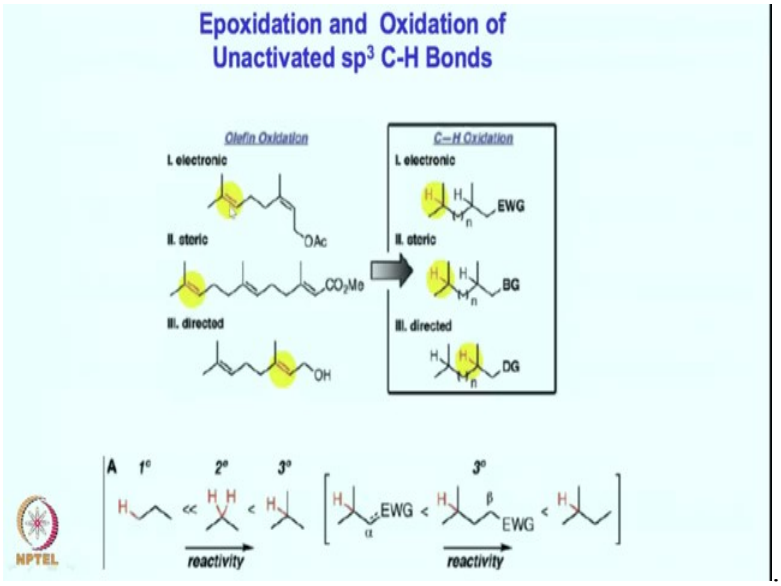

If you look at if you have a olefin which is like over here and there this olefin is deactivated. So, less electron rich, this is more electron rich right, both of them are triply substituted 1 2 3 triply substituted 1 2 3 triply substituted olefin, but since this olefin is right next to an electron withdrawing group electrophilic aromatic type of substitution or the electrophile will add epoxide will add at this center. So, the more electron rich center will attack on the iron oxo intermediate to give the epoxidation chemistry over here.

If you look at a related, but a different substrate if you are looking at this is deactivated as we mentioned this is right next to an electron withdrawing group, one needs electron rich organic substrate olefin in this cases to attack an electrophile efficiently and selectively since this is deactivated by having this electron withdrawing group, this electron richness is not too much and therefore, this is not too much reactive.

This is the internal olefin which is little bit more sterically crowded over there, but this is the terminal olefin as well as this is present from the far away position from this olefin. Of course, this olefin can impart be deactivated by this electron withdrawing group of course, the effect is not too much, but one can still think of having it as a completely internal substrate which is sterically crowded as well as little bit deactivated by this ester group.

Now, overall this olefin is the one which is most reactive for if oxidation chemistry now and this is perhaps the most electron rich also and less sterically crowded compared to these. So, then therefore, we can see that selectively and let us say exclusively this olefin is getting reacted and epoxide is forming over here without touching these olefin centre that is quite amazing that we know. And so, you can actually override the formation of epoxide at the centre by making it a directing group. So, you put a directing group to direct the epoxidation formation over there. In this case this is directing group these are not that good a directing group very weakly coordinating or weak directing group. This is a good directing group it can override the preference or for this center reaction it can selectively react over there that is quite interesting.

Quite similarly then, one can think of reacting these aliphatic substrate and get their hydroxylated product in a similar fashion. For example, if an electron withdrawing group is there and a tertiary versus tertiary is there, this tertiary is not that much deactivated by this electron withdrawing group this is more electron rich carbon hydrogen bond and

therefore, this is going to be reacted over here this one is right next to the electron withdrawing group although between this center and that center very little difference is there, except this electron withdrawing group and therefore, this is deactivated compared to this one because most reactive carbon hydrogen center reacts faster.

Over there if you have a bulky group also steric crowding can influence the C-H bond reaction and therefore, less sterically crowded one gets reacted faster and so, of course, ever hear this one sterically crowded part of the reason was that is why this did not react that efficiently right ok. As you have seen it is also possible to bias the substrate or included directing group such as this hydroxo which can now direct over there the direct the metal center to react over there not over there.

So, the reactivity pattern that we see over here can be overridden in all those cases both those cases this was a bulky group, this was an electron withdrawing group, but if you have a suitable directing group it can direct the metal center to react over here by overriding the preference for the center. So, overall what you have seen then is primary is going to be much more reactive because this is most electron rich 3 alkyl groups are there then secondary secondary is also of course, reactive, but not that much compared to primary and the secondary center will then react. So, most reactive is this one, then this one and then that one tertiary C-H is most reactive secondary C-H is then reactive and primary C-H is then reactive.

So if you have an organic molecule with tertiary secondary and primary center being present and not too much other biasing elements are there. So, it is perhaps possible to selectively react only the tertiary center. If no tertiary center is there perhaps it is possible to selectively react the secondary center without touching perhaps the primary center.

Similarly, if you are comparing apple versus apple where nothing is there except the tertiary C-H there are primary and primary as well as secondary C-H selectively tertiary will work. If you are biasing it with an electron withdrawing group these centers are getting biased they are deactivated still selectively this tertiary center will work that is fine, but this is going to be most reactive then this one because some biasing or electron withdrawing a group is there. If electron-withdrawing group is very close to this tertiary center again it is reactivity drops significantly.

So, the C-H bond has to be electron rich right so, the tertiary will react predominantly over secondary, then primary and if you are introducing an electron withdrawing group into the organic substrate. The substrate becomes less reactive compared to the non-electron withdrawing substitute and one as you have seen over here no substituent bias in terms of electron withdrawing group or steric crowding. So, this tertiary selectively work there is secondary, there is primary, primary, primary, none of them are reacting selectively this is working.

In this case as you have seen once you put electron withdrawing group reactivity drops, but nothing else happens if electron withdrawing group is very close to this reactivity further drops. So, in this class will we have seen little bit of the example, but we will discuss in the next class mainly this reactivity of these iron oxo intermediate iron V oxo intermediates, but sum up today's class I hope what you have seen today is it is possible to generate different reactive intermediate either in heme system or in non heme system no problem what is ever in there.

But most importantly we are trying to see how this hydroxylation chemistry is varying with respect to the organic substrate and a particular organic substrate and we have established some sort of reactivity pattern for this substrate. Tertiary is more reactive than secondary than primary aliphatic sp3 C- H bond, similar to what we have seen that epoxidation chemistry similar sort of guiding principle perhaps can be there for aliphatic C-H hydroxylation chemistry, electron withdrawing group of course, makes it makes the things little difficult that we have discussed.

In the next class we will discuss the same principle with much more examples and hope that will be crystal clear to you all right, keep studying this these are very interesting result these doing this reaction by any other synthetic procedure in our synthetic toolbox it is not that going to be easy. So, these are going to be quite exciting chemistry and one must try to understand them in greater detail, see you in the next class keep study.