Metals in Biology
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Lecture - 27
Cytochrome P450 Part III-Mechanism

Hello welcome back to the discussion of Cytochrome P450.

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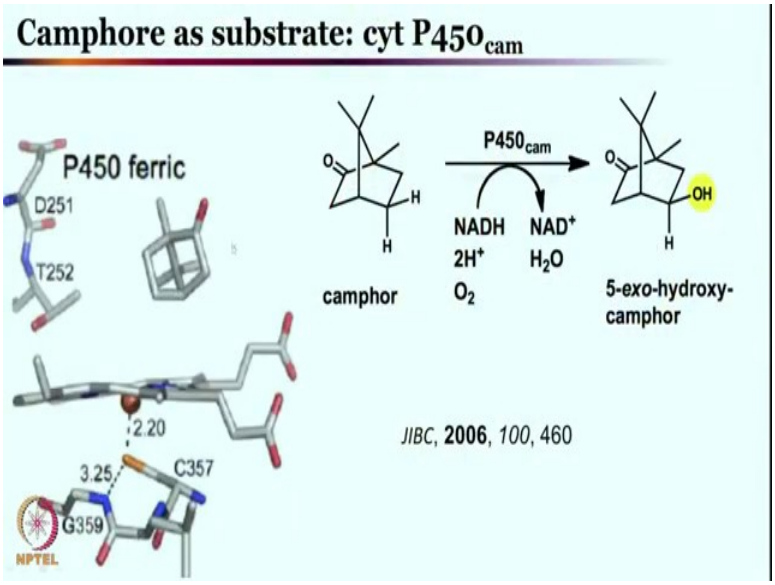

Today we will discuss the substrate camphor which is right over here this is a crystal structure of P 450cam, this is one of the really cool crystal structure you can ever come across that mainly due to the fact that the organic substrate is crystallized with the heme iron centre.

As you can see heme iron center is over there, this is the iron side well this is still outside the plane right. So, this is not really in the plane yet, it has to bind with oxygen get ready or get the oxygen reduction going and then only this iron will come inside the plane ok.

But the fascinating factor is substrate is right over there of course, there are protein side chain which are omitted for here for clarity. But you note also this binding of the axial ligand; all of the proximal ligand this is the proximal side, this is the distal side once again. So, the proximal side of the that is the axial centre we have the sulphur coordination from the (Refer Time: 01:38) which is fantastic; see that also interaction of

the other side chain with the sulphurs or S- right. So, this is from journal of inorganic biochemistry and the differences there in and you see that this camphor substrate overall which is over here; it is redrawn over here.

And selectively this C-H which is the exo one, exo C-H is getting abstracted and then subsequently hydroxylated. So, C-H bond abstraction of H dot abstraction and then hydroxylation occurs to gives rise to the 5 exo hydroxy camphor in it. So, which is very very fascinating right camphor as a substrate for cytochrome P450 is quite exciting. As you can see over there you need still the overall process with need 2 electron because we are starting from the resting state of the enzyme which is iron III plus 2 electron each of those are supplied by the NADH in that pool of electron pushing or gushing towards the towards the cytochrome P450 sides.

You still need also 2 proton and nothing will happen with in absence of oxygen. So, oxygen 2 proton 2 electron gives rise to this water and the substrate hydroxylated product.

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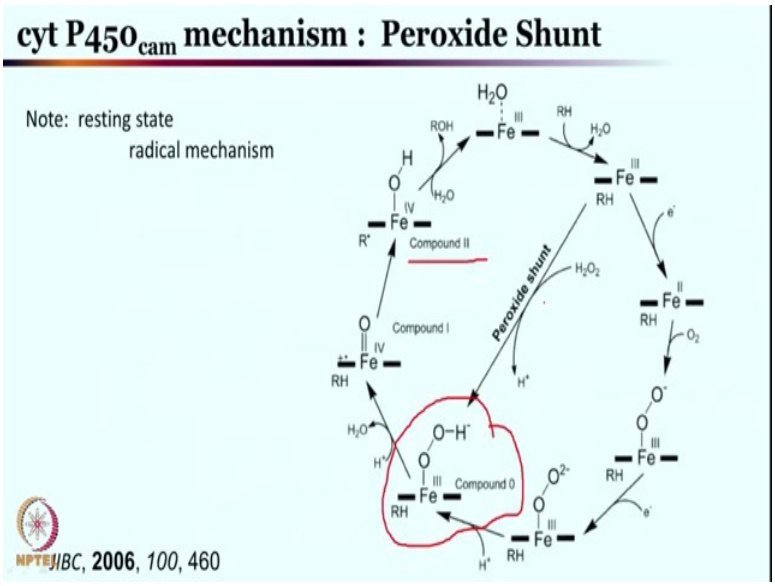

As we have seen the mechanism before; we will discuss one more time very very quickly here. So, this is where we start this is the iron III complex right; this iron III aqua complex also known as the resting state of the enzyme.

It reacts with the organic substrate; I will not say it is a reaction, it is just a displacement of water molecule in presence of the substrate when the substrate comes in water molecule goes out. So, substrates sits very close to the active site and then this iron III sides gets reduced to iron II plus in presence of the substrate over there.

Still I would say these centers are outside the porphyrin ring. Now once this iron II is formed still although outside the porphyrin ring oxygen will then react with it; react with this iron II which is fully capable of doing the reaction with oxygen to form the iron III superoxo; iron II note here is getting oxidized to iron III plus.

The electron from this is reducing the oxygen; this electron is coming from NADPH, as you have seen through the iron sulphur cluster and all the way to the iron centre. So, this extra electrons are coming from outside, but as you have seen this oxygen is forming superoxo by taking the electron from the iron centre ok. Now this superoxide species this is iron III superoxide species can then also be further reduced by another electron; once again from the NADPH or that electron transfer conduit overall channel of the electron transfer.

If this electron transfer gives rise to the iron III peroxo intermediate; this iron III peroxo intermediate, if you look at the oxygen with respect to this oxygen this is doubly reduced its a peroxo species. Now, this peroxo spieces is ready to accept another proton or one proton. So, this proton gives rise to the intermediate compound 0 which is nothing, but porphyrin iron III hydro peroxo species which is I think; it is quite exciting intermediate. Because once this species is form; it is kind of getting ready to react with the substrates throughout this cycle you see the RH is sitting pretty and nice over there, as you have seen the crystal structure that this is sitting very nicely in front of the active site.

Now, we see that this RH is getting ready and of course, RH is ready, but the species was not ready. Now this active site is getting ready another proton is again required to cleave the oxygen-oxygen bond between the iron III hydro peroxo intermediate. This oxygen-oxygen bond cleavage would give rise to the iron IV oxo intermediate iron IV oxo intermediate by removal of water from this equivalent right. So, water goes out and so proton comes in; water goes out iron IV oxo species is formed and a radical cation is also generated during the process.

So, overall it is a two electron process; one electron comes from this porphyrin, another electron from iron; so iron IV. So, that is why the double bond is formed; so this double bond is formed at by taking one electron from the porphyrinand taking one electron from the iron III. So, this double bond is formed and this hydroxo goes out as hydroxide HO minus.

So, HO minus is getting protonated to give the water molecule and this iron IV highvalent oxo intermediate with radical cation on the porphyrin ring is getting generated. Now at this situation this is a really reactive intermediate, this is a super reactive intermediates abstract hydrogen atom from the RH to give you the iron IV hydroxo species; iron IV hydroxo species and, during this process you can see that RH can be homolytically cleaved R dot gets generated that H dot electron can be quenching this porphyrin radical. And during this process we see that see that iron IV hydroxo is getting generated.

Now, at this point R dot sitting with iron IV hydroxo; this hydroxo can be transferred to the R dot and then this two electron from this bond; one electron goes to the O H if you are thinking homolytically and another electron comes to the iron. So, iron becomes iron III and this becomes hydroxy radical, this hydroxy radical binds or combines with R dot to give ROH molecule right that is fantastic. Now this water molecule displaces the ROH to slowly, but steadily form this iron III aqua complex which is nothing, but the resting state of the enzyme.

Note that this is where the radical is getting generated and that is the origin of the radical mechanism over all that we see over here. Now, in case of the peroxides and when mechanism where iron III is getting generated, you do not need all the way traveling by reduction and oxygen activation and one can directly react with this iron III to form the iron III hydro peroxo intermediate. In other word this hydrogen peroxide reacts with iron III to gives rise to the iron III hydro peroxo intermediate; let us discuss that intermediate quickly.

So, we will discuss how these species are forming in presence of in presence of this iron; iron III species.

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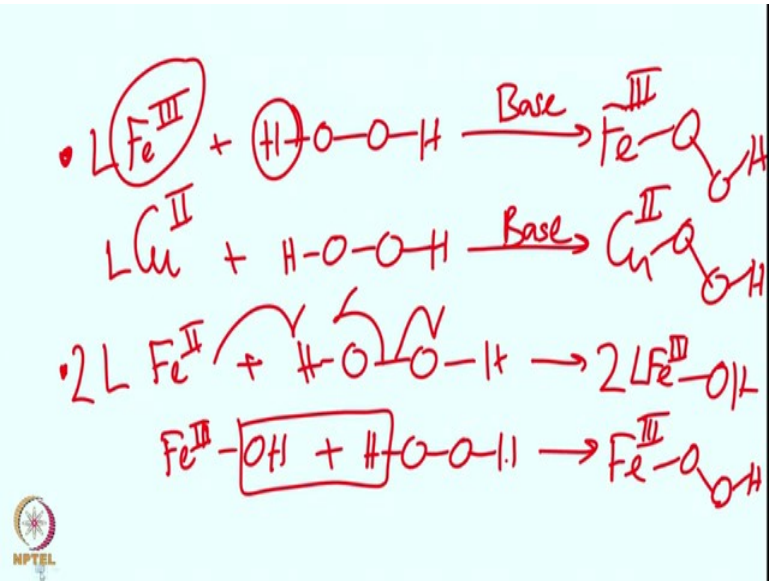

So, if you have iron III and if you are reacting with hydrogen peroxide, this is your hydrogen peroxide right. So, if you are bringing a base in the medium; so in presence of base the protonation will be possible; now this is just like a acid base reaction. So, it can form the iron III hydro peroxo species; if you have a base available hydrogen peroxide this sort of reaction is quite done in terms of iron also and also in terms of copper.

In case of copper, it would be copper II + hydrogen peroxide to gives rise to the in presence of base; it can if this sort of reaction can be done even in synthetic setup. So, that can form the copper II O O H; in these cases you do not need to reduce the iron III to iron II and react with oxygen, it has to have the hydrogen peroxide present. So, and essentially the same intermediate which we are seeing earlier same intermediate can be generated by these processes.

And therefore, this processes are quite effective in their nature overall you form the iron III hydro peroxo alternatively; you are seeing that electron is coming getting reduced to iron II; it is getting or it is reducing iron III to iron II and then oxygen activation was is happening. So, that happens when oxygen molecule is present in absence of oxygen or in absence of the electron which is reducing the iron III.

If hydrogen peroxide is available right over there into the mix it will go on to form the same iron III hydro peroxo species. And once iron III hydro peroxo species is generated one can then go onto the normal catalytic reactivity of cytochrome P450 right.

So, that is a peroxo mechanism or the alternate mechanism; in synthetic setup also one can start with a ligand iron II and iron III and ligand copper II complexes and can react with hydrogen peroxide to form such species. Alternatively, it is also possible to react with ligand iron II and hydrogen peroxide to form a quite interesting intermediate. So, you can have a homolytic cleavage over there; so 2 equivalent of this compound can gives rise to the 2 equivalent of ligand iron II and the hydroxy moiety right.

So, OH radical here OH radical there iron II gives rise to another electrons to form the iron II hydroxy bond. Now once this iron II hydroxo species is formed that can act as a as a base; the base we were talking over here. Similar type of base it can gives rise to the water molecule or H dot iron II dot; overall it can then give you this is hydroxide H plus.

Then overall you can again get the iron hydro peroxo species formation that is quite amazing I would say. And this is where I think this is this is going to be of course, this is going to be iron III hydroxyl; this is going to be iron III; so iron III OH this; this gets oxidized in the process iron III OH + hydrogen peroxide overall gives rise to the iron III hydro peroxo species.

Similarly if one is reacting let me take another page here of course, that is not really relevant over here just for discussion I would like to quickly mention here; that if you are starting if you are starting with copper, it is also possible to do such reaction right.

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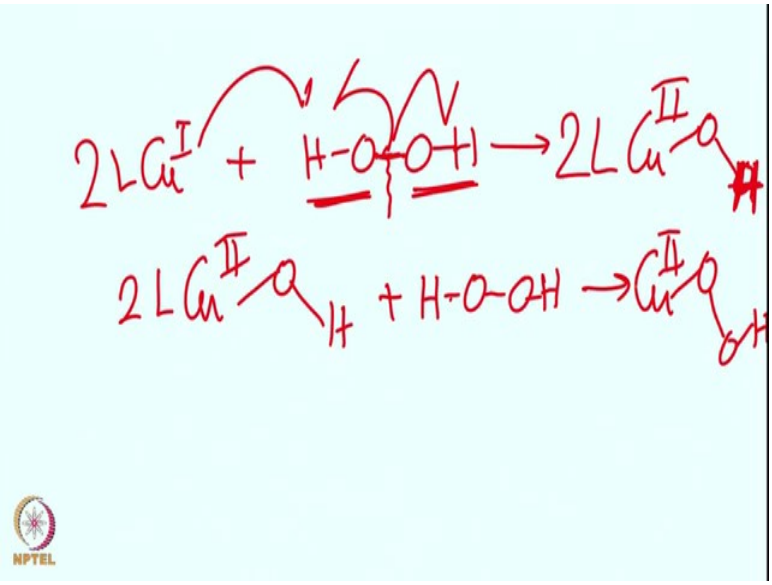

So, where you can take copper I complex just like iron II complex and react it with HO OH this reaction need not be very clean sometime many side reaction also occur in synthetic chemistry. Of course, you have ligand copper I there, no metal ions are completely free; if no ligand such as bidentate, tridentate, tetradentate ligands are there. It could be a monodentate ligand if nothing is available water is there water can; water can form complex with the metal ions. So, it is always coordinated with something where no metal ions are free completely free in the biological condition right. So, this ligand copper I complexes can react once again similar to the iron species in an equivalent manner.

So, this copper I gives one electron hydroxo homolytic cleavage; homolytic cleavage gives you hydroxy radical and hydroxyl radical 2 of them can give rise to 2 L copper II hydroxo species right. Sorry, this is hydroxo, this is hydroxo, this hydroxo, this hydroxo, this hydroxo goes over there. Now once you take 2 equivalent this copper II hydroxo species and then reacting with hydrogen peroxide ok.

Then this water molecule goes out and it just a ligand exchange you can say that hydro peroxo copper II hydro peroxo is getting generated copper II OOH is getting generated. This is similar to what we have seen in the iron case also; of course these are the mechanism by which these iron III hydro peroxy or copper II hydro peroxo species can be generated. Now, that can only happen when this sort of hydrogen peroxide reaction mechanism can only happen when we have the problem and that problem is either the electron is not available or oxygen is not available.

So, there in absence of these if hydrogen peroxide is existing that can gives rise to the peroxides; (Refer Time: 15:34) mechanism nonetheless; this compound 0 is going to be a very reactive under that condition. Once it is formed it can go on to form compound I which is nothing, but iron V oxo, iron IV oxo radical cation. And then compound 2 which is the iron IV hydroxy compounds which are again very great compound to have.

We will see the same catalytic cycle one more time in little bit different context from another review. So, this is by Nam and coworkers where we see that the same reaction mechanism essentially.

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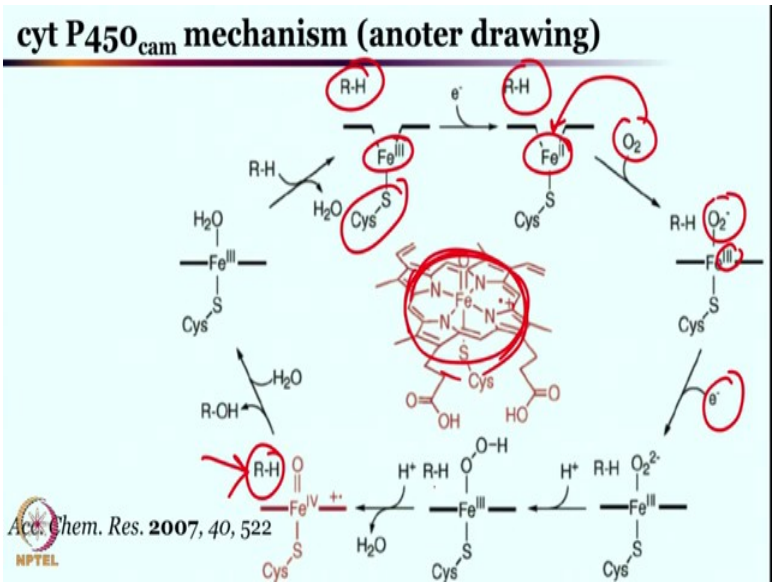

But this is depicting the iron centre outside this is depicting the iron centre outside the box right; outside the porphyrin moiety which is the case. Actually this really nicely depicts how the iron centre is; so this is a porphyrin sides it is abbreviated as a flat line over here iron III plus; it is really outside the outside the cavity of the porphyrin, it is bound with S 16 moiety and the RH at the at the distal site right over there sitting and sitting idle.

This is the resting state of the enzyme, this is the resting state of the enzyme another electron comes in and you get it reduced, but still iron is outside the porphyrin ring RH is sitting pretty right over there. And then we have the oxygen coming in; first of all of course, oxygen first will bind with this centre. Binding will not cause too much of a difference, but the electron transfer once the electron transfer is occurring; so the sixth ligand has come because this is a fourth ligand system.

The sixth ligand system has come this is the fifth one; 4 from here fifth and 6 here and that oxygen is now reduced by iron to give the iron III superoxide. This is a same mechanism what we had seen in the last slide as well right. So, from there on another electron transfer gives rise to the iron III peroxo as we have discussed in the last slide.

And, the protonation gives rise to the iron III hydro peroxo species with the 16 bound. And further protonation double protonation you see electron transfer; electron transfer protonation, protonation all these things are happening in between only oxygen

activation has happened. So, it gives rise to the water molecule and these iron IV oxo radical cation.

Now this is quite interesting over here as you can see the radical cation will be we will be right over here into this porphyrin moiety right. It is delocalized into this porphyrin moiety and this porphyrin moiety is quite interesting that it does not really allow a iron 5 oxo formation rid away. So, it helps this overall process because iron V oxo formation could be little bit more energy demanding and that is why the porphyrin is participating.

You see that is how; how beautiful the natures strategy is when the reactive intermediate is needed to be really high valent oxidize intermediate, then nature decides to oxidize if the porphyrin ring which is visible and it ends up doing it. Instead of forming iron V oxo, it decided to keep it still at iron IV while another centers like another porphyrin centre can be oxidised to a radical cation. I think that is amazing to be able to control these things at a level where enzyme once perfectly is quite phenomenal. And we will see such beautiful chemistry once again or again and again in different enzymatic setup.

Subsequently, this iron IV hydroxo species can then pick up the hydrogen atom from the; some from the RH substrate and at this is when we get the RH formation. So, all these steps where RO; RH is required it is just acting as a substrate without much of the action; this is where it gets into action and gives rise to the product formation ok.

So, I hope the mechanism of cytochrome P450 is quite clear right. And we have seen that how beautifully these reactions can be propagated and can be summarized very very nicely right well right. Let us get out of the reaction mechanism; let us see some of the certain things in here ok.

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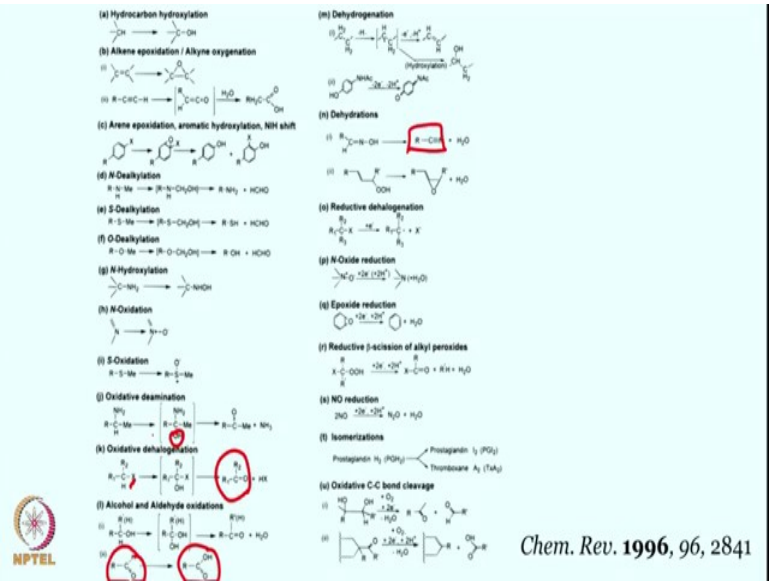

We have seen these reactions earlier in few slides back of course, this that is not an exhaustive list; neither is this one. But this slide gives an overview of various other reaction that you may not have seen earlier can do or can be can be transformed or can be catalyzed by cytochrome 4; P450 enzyme this is once again from this review Chem Review 1996.

You can look it up and you can see that there are various different type of reaction that is possible. So, far we have discussed the first few reactions ok. So, we have not discussed about other reaction it is actually get similar. So, oxidative deamination reaction is also possible when you have in I mean the next alpha carbon centre is getting hydroxylated. And subsequent rearrangement at this centre can give you the ketone formation and the ammonia generation which is fantastic.

If you have similarly an halogenated compound alpha position alpha to the carbon centre alpha to the halogen; halogen can be also hydroxylated and subsequent rearrangement can give you the ketone product as well. You can take an alcohol aldehyde, you can hydrioxilate the alpha position of the alcohol and can give the dihydroxy compound; alpha alpha dihydroxy compound which can which can which can then dehydrogenate; to give you the ketone compound.

See all these cases; all these cases you end up getting the ketone compound. If you have alpha carbon centre alpha 2 amine; alpha carbon centre alpha 2 halogen, alpha carbon

centre alpha 2 hydroxy; all these cases will end up hydroxylated the enzyme perfectly. And that is what the beauty of these reactions; over all these can gives rise to the ketone product formation in all of those cases, but fantastically all this reaction can be done.

Again this over reactive cytochrome P450 as if it is really energized; so, that can sometime be a disguise, sometime can be a sometime can be a very useful, but more often its it can also act as a nuisance anywhere. So, you can also can take the aldehyde as a substrate aldehyde substrate these alpha C H bond can be also hydroxylated to give the acid compound.

That is once again quite interesting; if you have even an aliphatic substrate in addition to the hydroxylation reaction it is also feasible to of course, after hydrogen atom that anyway happen during the hydroxylation. And then with a suitable substrate it is possible to give the give the unsaturation; that means, a double bond is forming; an alkane can give rise to the alkane by utilizing this method. If you have the para hydroxy and it show it a acetanilide type of substrate with which 2 electron oxidation and 2 proton transfer.

You can also get this is semiquinone type of or quinone type of intermediate; aminoquinoline type of intermediate. It is also possible to undergo dehydration such as; as you see over here dehydration reaction on this one will in hydroxy amine will give rise to the to the sino compound or acetonitrile type of compound.

If you have the alpha position hydroperoxylated the next to the olefin, you can you can also react it with to form a nice epoxide alpha to the olefin centre which is quite exciting. You can have a series of other reaction, as you can see all those reactions; we will not be discussing here today, but that gives rise to a number of reaction formation which is which is fascinating I would say.

So, this horizon the; the spectra by which this reactions are happening, I thinks are; I think this is this is quite remarkable none; no other enzyme can be this effective as the cytochrome P450 is. So, no other enzyme can do the better synthetic chemistry perhaps then the cytochrome P450. But the challenges remain the over reactivity getting this reaction very selectively and of course, efficiently without forming other side reaction is always a challenging and it remains a challenge.

And by looking at these a synthetic chemist has tried to develop synthetic methodology; that really can mimic this reaction. These are by far the most difficult and most encouraging chemistry that one can happen; one can find from the enzymatic setup. And again this is really by utilizing oxygen molecule as a reactive species or part of the reactive species ok.

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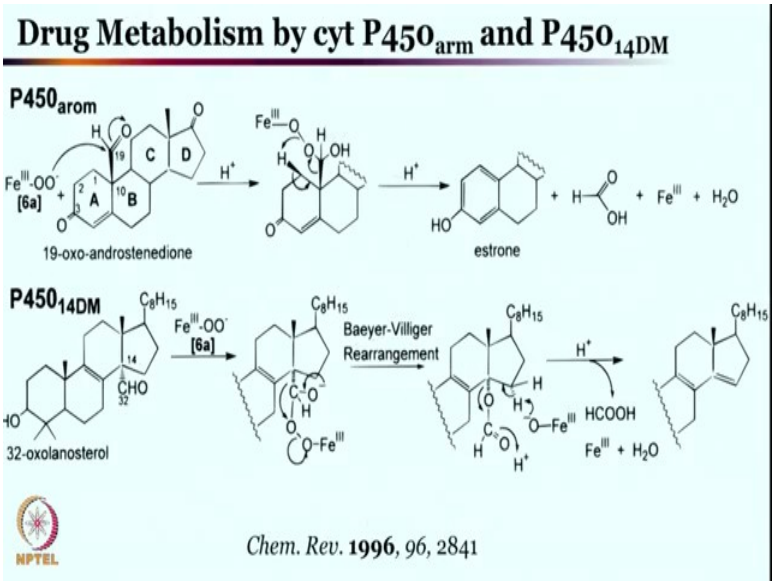

So, we will see few more few more cytochrome P450; there are many different variants of it, depending on the substrate, depending on the certain variation one can think of utilizing different substrate.

For example, over here you see this is an aldehyde a substrate aliphatic aldehyde you can define at now this iron III species iron; iron III species can react with it to gives rise to a pro. So, this is the iron III peroxo species; once again this is from this review, you can look at original paper from there, the references sited there in where you can see that this peroxo species can react with aldehyde to give the iron III hydro; iron III alkyl peroxo intermediate.

This is a peroxo iron III peroxo forming an iron III alkyl peroxo intermediate which can then rebound to give your; then can that can react to give you the formic acid. This is you know overall you can see that the deformylation reaction is happening; this is once again a very very fascinating enzyme, which were or variation of cytochrome P450 which gives the even the deformylation reaction. If you have an aldehyde molecule or aldehyde

moiety and be completely removed from the organic molecule: to give rise to the simple organic molecule or deformylated organic molecule.

Here there is another example right over here in the previous example as you can see the estrone can be generated by such process of starting with 19 oxo androstenedione and by the effect of cytochrome P450 arm aromatic zone or; and then you can have another organic substrate which is similar. But it is little different this is 32 oxolanoterol, you can you can get really beautiful reaction similar to the deformylation reaction above you have seen.

Here once again the iron III peroxo attacks on the aldehyde moiety to give gives rise to the deformylation reaction once again forming the formic acid. We will we will this keep on discussing on cytochrome P450 in the next class. I hope you are able to see that these reactions of cytochrome P450 are very very simple, yet very effective.

They are forming a nice high valent oxo intermediate which is capable of doing any kind of oxidation chemistry that you can think of right. These chemistry are so powerful that that once again pharmaceutical industry has to be worried extremely about this reaction. Because any drug molecule any organic molecule they want to put in our body to solve our problem, it could be metabolized, it could be degraded it could be you know taken to the task by the cytochrome P450.

Cytochrome P450 can be seen as a terror at some point to the; to the pharmaceutical industry. These are so good enzyme I mean; so versatile at such a great synthetic chemistry component can be there in cytochrome P450; that it is unbelievably clean, unbelievably great chemistry and we will keep on seeing this in the next class.

Thank you very much, see you soon.