Metals in Biology
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Lecture – 31
Concerted vs radical pathway for CH 4 to CH 4 OH conversion

Welcome back. Today, we will continue discussing on the reaction mechanism of formation of methanol right. So, from methane we are talking about, methane is such a great starting material to convert or to be converted into biomass right. So, methane to methanol is hugely important and very difficult transformation.

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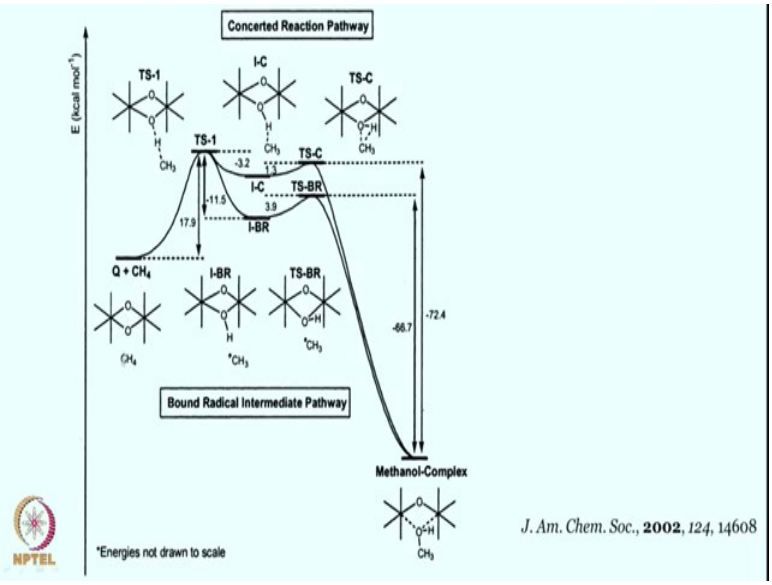

And, we had seen in the last class that these di-iron bismuoxo intermediate where iron is in +4 oxidation; state iron is in +4 oxidation state and oxide oxide is reacting with methane to give an intermediate or in this case the first the transition state TS-1, CH3 HO where both bond making and bond breaking is happening simultaneously.

From there the we have seen the intermediate where CH3 and OH are interacting still which each other and subsequently a concerted pathway where CH3 and OH is intimately involved in the CH3OH molecule formation. This pathway so called concerted pathway is little bit energy demanding compared to the one the bound radical intermediate pathway.

So, in the bound radical intermediate pathway we see the exactly same transition state where bond breaking and making is happening simultaneously, from there a distinct radical intermediate involving CH3 dot radical an oxo hydroxy iron IV, iron III intermediate is forming; now, that I would say quite interesting. So, what has happened over here this one of the iron center is iron IV plus, another iron center is iron III plus. Now, the oxo is no longer oxo it is hydroxide HO minus 1 minus, this is oxide O2 minus right.

So, iron Iv iron to III mixed valent intermediate is formed along with the formation of the CH3 radical. This is what is I-BR intermediate of this which is really preferred over that you see for IC intermediate for concerted pathway, intermediate for the radical pathway is this one. This radical then can react with this OH of course, the OH has to orient properly and of course, we will see in a moment that this rearrangement or the electronic reassignment has to be done, some electron spin has to exchange, overall will see that that the CH3 and OH. Once the OH is properly oriented and ready to react with the radical HO is HO minus; that means, two electrons are there.

So, two electrons over here, one electron over there it is not going to be a radical mechanism. So, this double electron has to be a single electron by transferring one of them to the iron center and this is where this iron intermediate we will come into the picture and this OH radical and this CH3 radical will combine with each other right, to give the CH3OH.

Let us look at this pathway which is the preferred one in little bit more detail as you see the energy levels are not too high, it is achievable and these reactions are achievable at 20 degree C at to nearly room temperature or less than room temperature at pH 7. So, to be able to convert methane to methanol at room temperature at pH 7, I think is phenomenal right. This is quite exciting such a transformation remained very difficult to do even for the synthetic setup. Enzyme can do it perfectly because everything all the active site is perfectly.

We will look at these step, how methane is approaching this intermediate Q, how this transition state is forming, how these intermediates are and how the next transition is forming and subsequently the overall product formation is happening by the DFT studies in a little closed manner ok.

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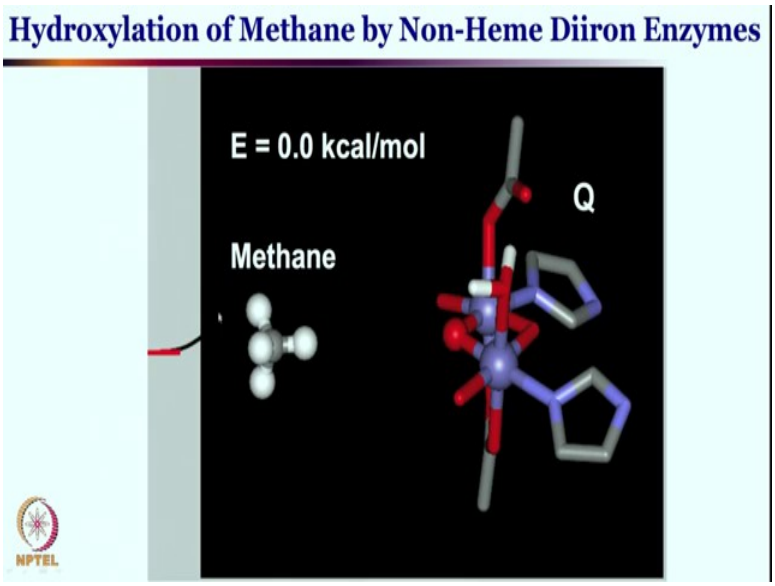

So, this is your methane is approaching the intermediate Q. So, you see these two intermediate these two iron centers are iron IV + 4 plus oxide oxide and rest of the cycle or rest of the ligands are as it is as in the active site. So, you have iron sites oxo, oxo and the methane this approach or this is set at 0 because this is when it is going to come and start reacting. So, this is set at 0 kcal and we are going to look at this overall hydroxylation of methane to methanol by this non-heme di-iron enzyme ok.

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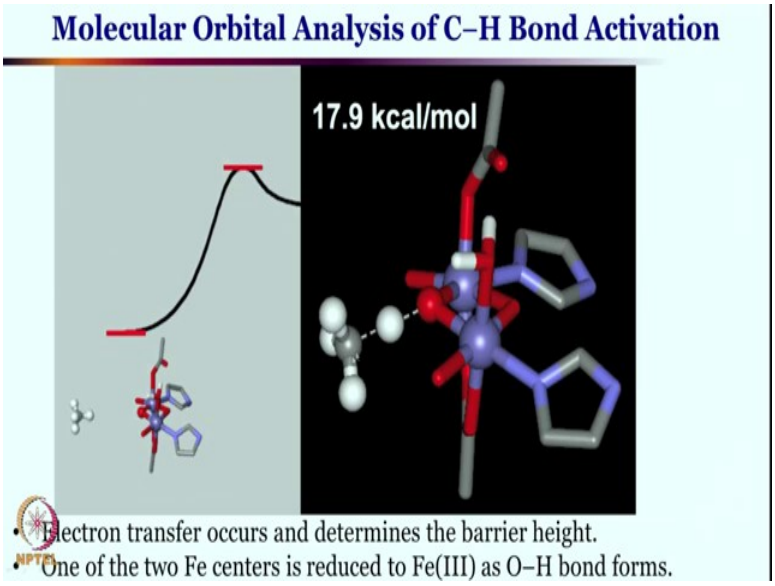

Subsequently this is the transition state that we that we discussed where C-H bond breaking and OH bond making is happening ok. The electron transfer in this state, electron transfer from this C-H bond, C-H bond electron transfer occurs and determines the barrier height here it is 17.9 kcal per mole which is right which is achievable at 20 degree C. So, this is the highest energy demanding step and this is the step why we do see kinetic isotope effect when we displace CH4 with CD4.

If these are deuterium this C-H bond breaking becomes further slow because this is the one which is the most difficult step to carry out, C-H bond breaking and OH bond formation. This is the corresponding transition state; C-H 4 versus CD4 will give a kinetic isotope effect value of nearly 22 as you have seen earlier ok. During this process one of the two iron centers will be reduced to iron III as this OH bondage formed. This is the transition state by the nor the intermediate. So, this is the structure looks like in this transition state.

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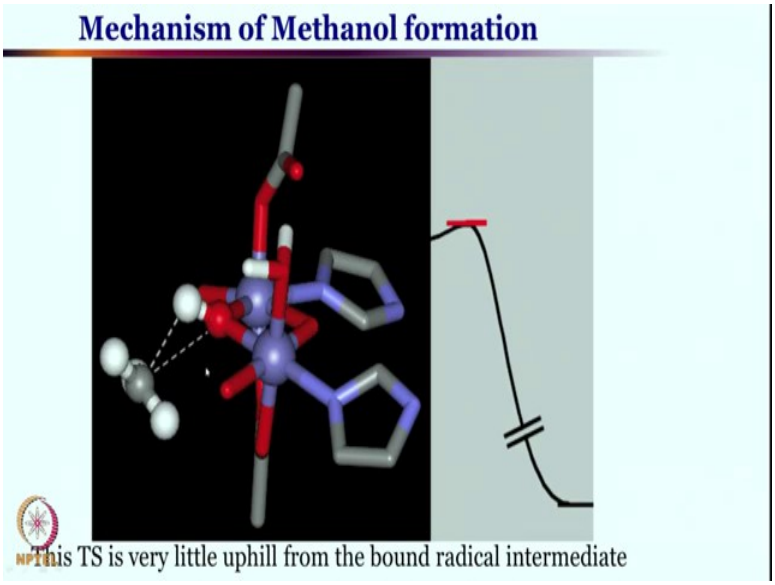

If you move on from there on what will happen? Once the OH bond formation is happening we are looking at the second transition state. This OH is now formed and it has of course, have undergone the electronic rearrangement. Overall now this OH is ready to bind with CH3 and the corresponding transition state will be looking like this. This transition state is very little are filled from the radical intermediate that we have discussed two slides back.

So, these are the two transition state. Once again this is the first CH breaking OH bond formation step transition state which is the most demanding step and this transition state is not that very demanding, it is rather easy to form the rather less energy demanding where OH rebounding with the CH3 radical. So, this is of course, oxygen rebound or OH rebound step with the CH3 radical, but before that the rearrangement of electron has to happen.

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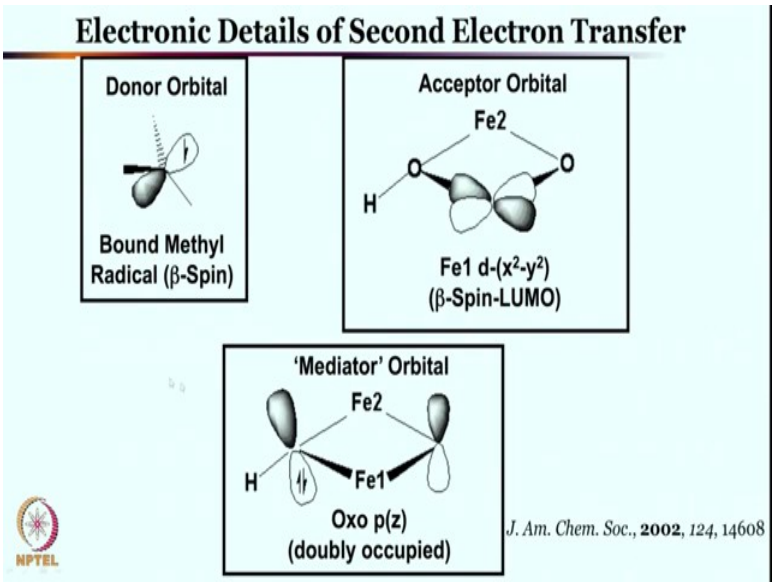

Let us look at those rearrangement of electrons. So, this is once again from this book, sorry from this reference, from this research paper where this is the radical bound or bound methyl radical. So, CH3 dot H H H here is carbon. This is the p-orbital of carbon; this is one electron over there CH3 dot donor orbital is there. Here is the acceptor orbital. This is one way of to looking at it one of the orbital we are looking at dx2 y2 of the iron center which is having beta-spin-LUMO. So, we will see that in a moment.

If you are looking at hydroxide HO minus; HO minus have two electrons and so, the two paired electron one up another down this doubly occupied intermediate or doubly occupied state, then we will transfer one of the electron from here to the iron center right. Let us look at that. So, this is not really this OH minus and this double electron is not going to react with the CH3 radical that very easily. This OH also has to form the radical; that means, only one electron should be over here another electron should go somewhere most likely in this iron center we will see that in a moment.

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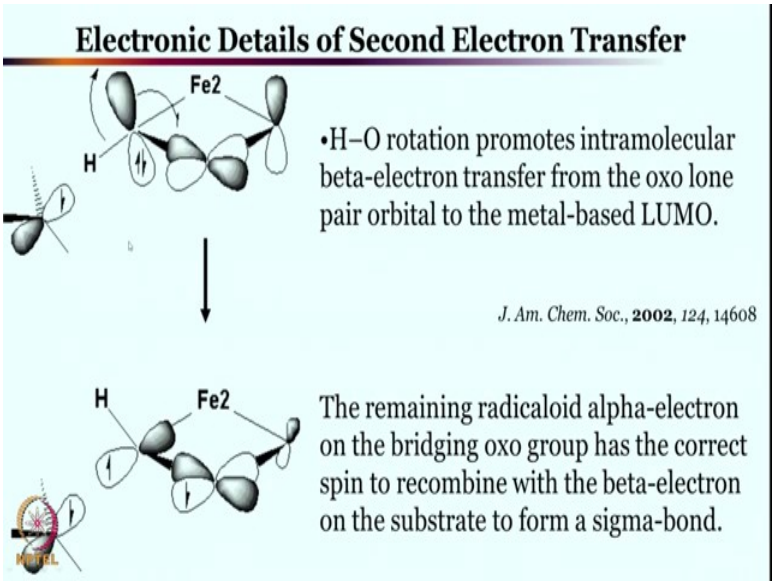

So, what you need to do you need to overlap. So, the rotate this p-orbital or rotate over all over here to do the bonding we between these d-orbital of the iron center, let us say iron one center and subsequently one of the electron from here will be transferred to the LUMO metal based LUMO and then you left up with one down spin beta spin, one alpha spin O on the OH and the beta spin on the LUMO of the iron center.

So, what has happened the alpha spin over here is now reacting with the beta spin of the radical to form the CH3 OH bond. So, these will form the new bond and then there is the one electron reduction center for the iron center making it from iron IV to iron III. So, this HO rotation, this rotation promotes intramolecular beta-electron transfer. So, intramolecular beta-electron transfer from the oxo lone pair orbital to the metal based LUMO. So, this is the metal based LUMO. So, oxo lone pair to the metal based LUMO this transfer is happening. The remaining radical it that is over here after one electron transfer, the remaining alkaloid alpha-electron on the bridging oxo group has the correct spin now to recombine with the beta-electron of the sigma of the CH3 radical and these two now will form the sigma bond.

So, what is essentially happening? From here if you look this is the CH3 radical, this is the di iron center, this is the beta-spin-LUMO or that means, dx2 y2 orbital for the iron 1 center we are not looking at the iron 2 center. If you are looking at OH 2 electrons over there it will have to rotate. In any case this is the two electron, so, this is single electron,

this is two electron. It will have to rotate one of the electron has to be the beta electron has to be transferred to the iron center and this is what you see over here.

The alpha spin sorry the beta spin and the alpha spin upon rotation and transfer of one electron from this OH to the iron center gives rise to a situation where OH radical is there and the CH3 radical is there. Now, this two radical will combine to form the sigma bond. So, we have seen how CH4 to CH3 radical and CH3OH bond is forming.

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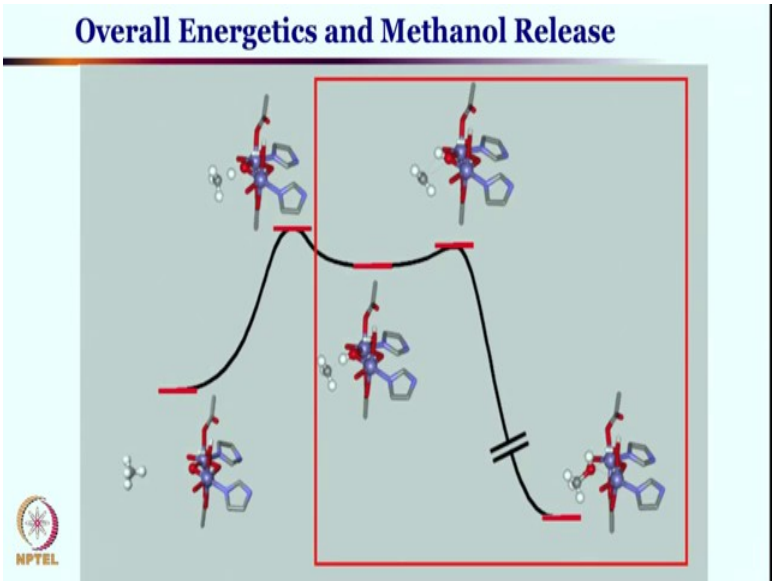

So, overall this is the diagram where you started as we have seen with the bismuoxo species so called the Q intermediate and this is the Q star intermediate. Q intermediate the acting or approaching methane is approaching towards is that it say to 0 and this C-H bond breaking and OH bond formation is happening. So, C-H bond breaking and OH bond formation is happening. You are having a transition state right over here.

This is the most demanding transition state 17.9 kcal per mole, where if you have the CD4 you will also get the same thing, but it would be significantly slower and then therefore, you will see that the CH3 radical and OH is now form O. This hydroxide still has to form the OH radical and that is what is happening over here. OH radical is form and CH3 radical is over there. So, they will combine. This is not that very energy demanding step and this is when they will combine with each other and will give you the CH3OH bound intermediate that we have seen earlier.

So, to summarize simply in this case what we have seen that a diiron-mu-oxo intermediate is approached by CH4, radical obstruction or you see that CH3 radical formation or OH formation is going on between the iron 2 centers. From there on electronic rearrangement has to happen to give you the hydroxy radical and then you also have the CH3 radical ready. They combine to give you the product that is all, nothing else is there ok.

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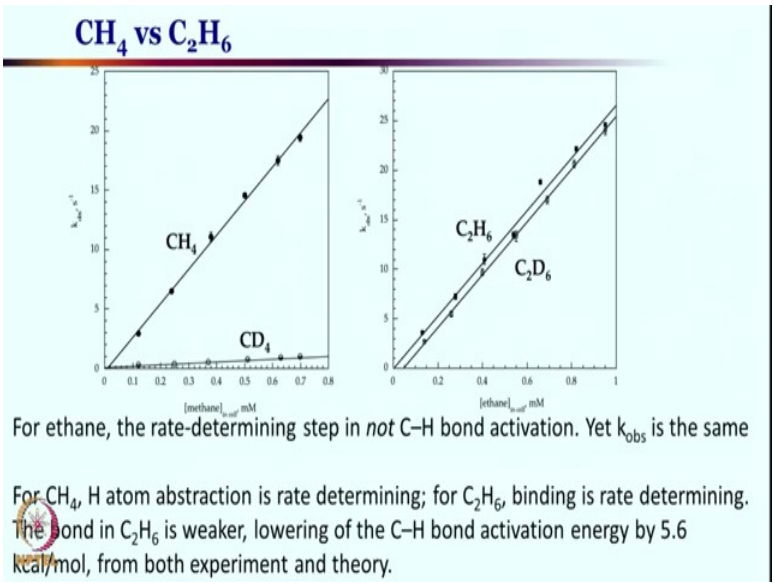

So, if you are looking at CH4 by versus CD4 that we have seen that this is having a high kinetic isotope effect value nearly 22. Quite surprisingly if you are changing the methane substrate from methane to ethane, just change of substrate methane to ethane and if you are studying this enzyme you see that there is no kinetic isotope effect value between C2H6 and C2D6. Here CH4 versus CD4 you have a huge difference in the reaction rate right.

Over here C2H6 versus C2D6 which is ethane versus deuterated ethane there is no difference in the kinetics data or k abs data. So, this is quite amazing right. For ethane the rate determining step therefore, is not the C-H bond activation step. So, what we are trying to tell here is the scientist has now figured out quite phenomenal observation and that is methane and ethane reacts completely differently or at least the intricacies of the reaction mechanism are different.

In case of methane C-H bond activation is the rate determining step. Can you imagine that and just you change from methane to ethane C-H bond formation is no longer the rate determining step. You do not have a kinetic isotope effect value at all, it is kind of KIE is 1. Although the k obs is same which is fascinating I would say.

So, for methane hydrogen atom abstraction is rate determining; for C2H6, the binding, the approach the C2H6 is approaching the Q, the diffusion control is happening con. So, the binding of the ethane with respect to the Q; intermediate Q is important. See this is how nature has evolved in such a way the enzyme is designed in such a way overall enzyme pocket the approach road is such that it just fits methane perfectly ok.

If you want to fit in ethane that is also not possible and this is why one should realize that although nature is really great, but methane is only designed for one substrate and this is also true why we have such difficulty in synthetic set up in mimicking these chemistry and more importantly having a broad substrate scope for this sort of this sort of active site if we are able to mimic in synthetic setup. Because nature can do it for one substrate, but synthetic chemist one every substrate over there that is not even nature has done it right that is going to be even more challenging.

That is why synthetic methodology development or catalyst development for a wide variety of substrate is always going to be challenging, one thing perhaps would be better for synthetic chemist to do is to try identify one great reaction and try to do these transformation effectively just what perhaps nature is doing, but you know synthetic chemists demands are little different. In any case, C2H6 as we are discussing the binding is the rate determining step.

The bond in C2H6 is weaker. Of course, this is weaker significantly I would say by nearly 5 to 6 kcal compared to CH4. CH4 was 104 kcal per mole and C2H4 or ethane C-H bond breaking would be something like you know 104 minus 6, let us say 98 or 98, 99 kcal per mole. So, this is also saying that C-H bond dissociation is not the rate determining step; that means, C-H bond dissociation is relatively easy, indirectly saying.

This is also can be proved by experiment and theory in any case the C-H bond dissociation is not the rate determining step, but the approach of ethane or diffusion of ethane to the intermediate Q is the rate determining step. But, for methane diffusion was

not of a problem, but C-H bond breaking was the problem right, for ethane you can see that the approach of ethane towards the Q is problematic or the most demanding ok.

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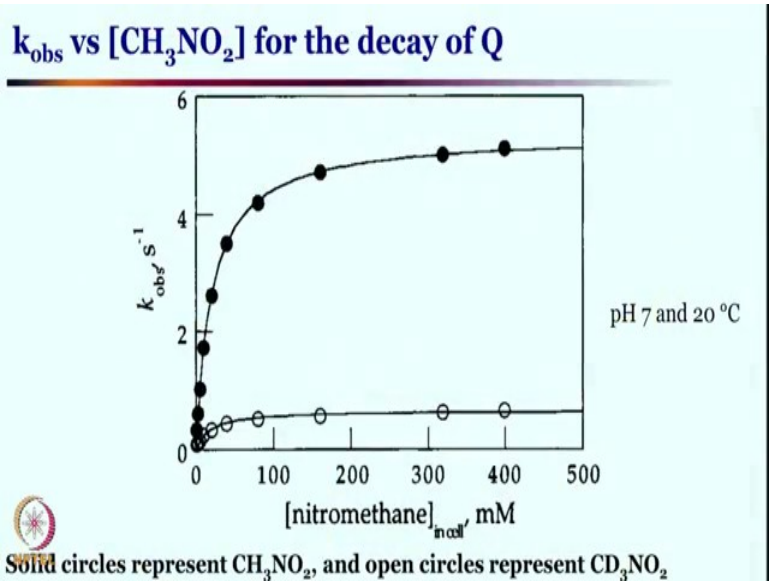

So, in number of substrate one can study. One such substrate that has been studied, again this is a different paper. So, that is the nitro methane and in this case actually react IR can be used to monitor these by IR-spectroscopy. You know kind of the dissociation or the CH bond breaking or the new product formation can be followed by the react IR.

So, CH3NO2 versus CD3NO2 can be studied by reactor of course, one can still think of doing the UV visible spectroscopy, but this is a fantastic way of studying the reaction mechanism once again. The CH3NO2 and CD3NO2 will give this short of plot k obs versus nitro methane at pH 7 and 20 degree C. This also gives rise to a very high kinetic isotope effect value. As you see between CH3NO2 which is first are CD3NO2 is significantly slower ok.

So, this is not by UV visible spectra study, but by IR study you can follow the kinetics of this reaction with respect to the concentration, different concentration of CH3NO2. The study is done similarly as we were seeing for intermediate Q and then change the CH3NO2 to CD3NO2 and vary the concentration of CD3NO2 you will get the plot as it is over here.

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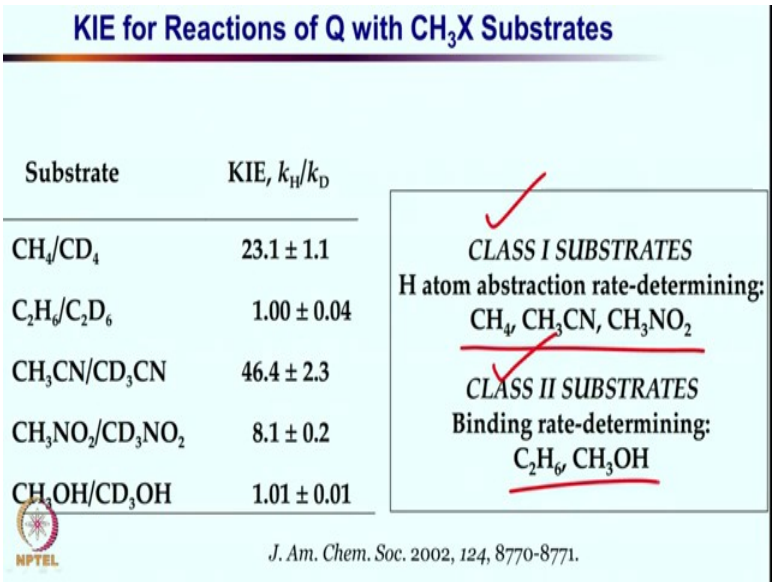

Overall many different substrates are studied and this remains quite interesting for methane as you see nearly 23, 20 to 23, 23.1 plus minus 1.1. This is the kinetic isotope effect value. As you see that is for CH4 and CD4, but the C-H bond breaking is the rate limiting and therefore, a huge kinetic isotope effect.

For CH3NO2 and CD3NO2 once again there is a large kinetic isotope effect; not as large as CH4 CD4, but CH3NO2 and CD3NO2 is still high kinetic isotope effect. This is saying that once again both for methane and nitromethane C-H bond breaking is the rate limiting step. One can even study the night nitrile, let us say acetonitrile with for it is hydroxylation chemistry, acetonitrile versus CD3CN is giving perhaps the highest kinetic isotope effect value in this series which is once again saying that the C-H bond dissociation is problematic step rather than the diffusion which is happening in the other case. This is the diffusion control process.

Now, all for methane, acetonitrile and nitromethane it is a significant kinetic isotope effect value indicating that these are the substrate wherein C-H bond dissociation will be the rate limiting. I think it is also quite fascinating as we have discussed that ethane, methane to ethane you change the dimension completely. Methane C-H bond was the difficult steps; ethane, of course, C-H bond is little weaker nearly 98-99 kcal per mole bond dissociation energy, but this is no longer, C-H bond dissociation is no longer the rate determining step.

The rate determining step is going to be the one wherein it is defusing or approaching the intermediate Q and that is the difficult step rather than the C-H bond breaking. And, such a similar case will be happening in case of methanol; methanol versus deuterated methanol is also having kinetic isotope effect value of 1. So, methane to sorry ethane and deuterated ethane, this kinetic isotope effect value of 1 indicates that that the C-H bond dissociation energy is not the rate determining step, but the same thing is also true for methanol versus deuterated methanol where once again the C-H bond breaking is not the rate determining step.

So, based on these studies one can think of having two different classes of substrate. Class I substrate where hydrogen atom abstraction is rate determining. As you see in these three substrate, let us say those are studies methane, acetonitrile and nitromethane. For class II substrate binding rate is the rate determining step, substrate binding with respect to the active site or active species that is the intermediate Q that diiron, diiron IV plus with the bismuoxo intermediate that is the rate determining step right. That is quite fascinating I would say and this is true for C2H6 and methanol ok.

One can perhaps think of studying many other substrates as well, but therefore, overall the whole spectrum can be split into two; one is substrate I category I and the category II. But, I think you have seen the power of these reactions or power of this active site and that is this bismuoxo species are capable of forming the great methane to methanol ok.

I think you have seen that there is similarity between the iron chemistry and copper chemistry I will take that reaction mechanism in a moment where you will see that iron chemistry and copper chemistry are similar.

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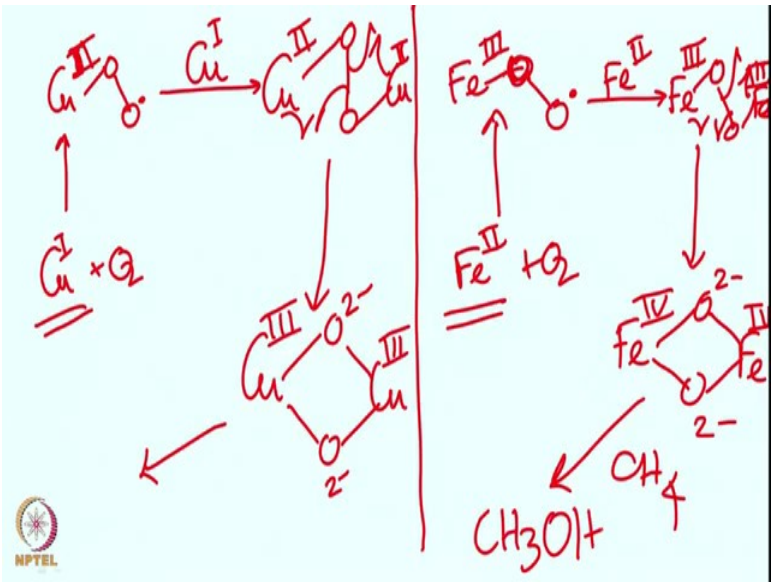

So, let me let me divide. So, let me try to draw the copper chemistry what you have seen in case of copper chemistry, you have seen that copper oxygen or superoxo species is forming from copper I right. So, this is I am drawing copper II ok. So, you have taken copper I reacted it with oxygen right. So, one electron transfer happens to give you that and from there on you have seen another copper can come in copper I can come in to give you copper II O-O, let us say copper II.

I am deliberately drawing that such an end-on that will be easier to follow and from there on you see the one electron breaking from there and one electron from there another electron from there will give rise to the copper III intermediate right copper III bismuoxo intermediate. So, this is oxygen 2 minus this is oxygen 2 minus right.

So, similar thing you have seen in case of iron iron II plus, let us say let me draw the similar intermediate; iron III would be there similar looking intermediate; if we are trying to draw iron II is reacting with oxygen right. So, if we start from copper I, we start from iron II. It is a iron III super oxo and from there on another iron II can come in; although this could be a side on geometry, just I am drawing as an end on just to keep it clarity iron III can form.

Now, this iron III intermediate can then once again give one electron break homolytically and this can give one electron this one electron and these can give one electron overall you can form iron IV these new oxo species. Now, you see the

similarity. So, this is a copper III copper III bismuoxo intermediate over here you have a iron IV iron IV bismuoxo intermediate, only difference is one electron ok. We start with copper I we start with iron II. So, we go to copper III, we go to iron IV right that is very simple.

This is why you can see that these species are very reactive and can effectively do a very great chemistry and these are of course, can do many chemistry as you have seen over here. Here you can react methane to get methanol there are copper enzyme also which is responsible for this methane monooxygenase. These are the chemistry and these are the studies which are ongoing. The detailed understanding are still not 100 percent clear ok.

I will not divert too much right now. We will come back later with the summary between the copper and the oxygen similarities and summaries between them in some classes later. Let me get back to where we were discussing and that is these are the class I substrate and the class II substrate we have seen and these are the two different types of substrate that we have seen over there right, that is fantastic.

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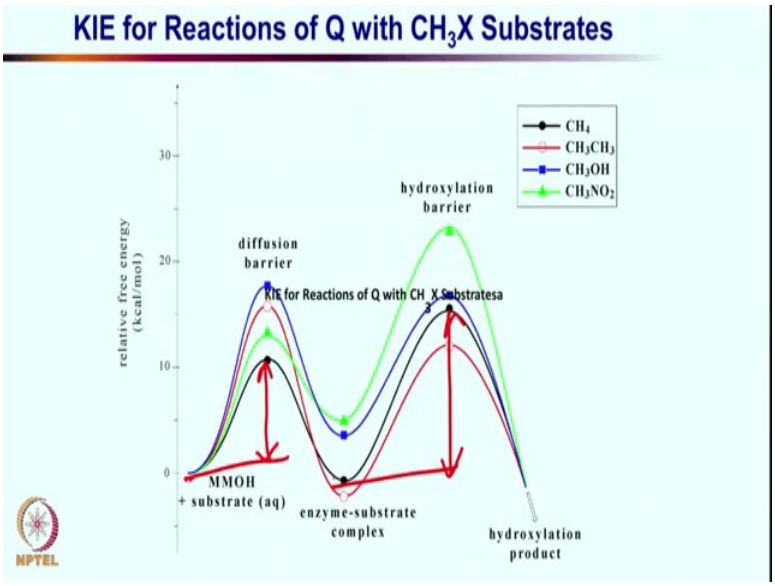

Now, let us look at the overall program then. So, this is a once again from professor Lippard's note where we see that the first case if you are looking at methane that in the black here. So, relative free energy if you are looking at the black one, where it is showing that this is not really the high energy. This is diffusion is not the rate limiting of course, this is energy demanding, but not to energy demanding, but if you follow the

black line, black line is having the C-H bond dissociation is as the rate limiting space and then the hydroxylation product is forming.

In case of ethane, that is in red you follow the red line here, red line is very up. So, this transition state where the diffusion barrier is quite high on the other hand C-H bond dissociation energy of course, is energy demanding, but not too very high. So, this energy is much higher than this one. So, that is why this diffusion is the rate limiting the CH bond activation is not the rate limiting.

In case of methanol, the blue line over here once again you see the in case of methanol which is the class II substrate. If you have forgotten methanol is the class II substrate. Here you see that diffusion is far more energy demanding it is very high energy. Of course, C-H bond activation is also demanding, but compared to the diffusion, it is less energy demanding. So, diffusion is the controlling factor over there. In case of nitromethane as you have seen C-H this nitromethane diffusion is not too much of higher energy, but the C-H bond dissociation is high energy.

So, as you can see over here show this relative energy plot. So, let us say just for methane if you are looking at that is the black line over there. So, this is the diffusion energy compared to methane the C-H bond activation energy is quite high as you see over here. That is indicating that this is not going to be too much troublesome, but doing this C-H bond activation the black line if you are following is going to be the most determining factor or the difficult factor.

So, to conclude for this methane monooxygenase, I think you know we are very lucky to have such a great enzyme which where you have non heme diiron center in the very high oxidation state iron IV iron IV in the bismuoxo bound intermediate which has similarities between the copper III, dicopper III bismuoxo intermediate, but more importantly it can convert methane even methane to methanol. All other substrates are I would say relatively easier not all, but most of them are relatively easier.

There are two class of substrate that you can study with respect to these are methane monooxygenase. One is class I or substrate class I, another is substrate class II. Substrate class I is the one where we see that kinetic isotope effect value is more than 7 or you know significant kinetic isotope effect value is there. If kinetic isotope effect value with

equal to 1 then that is the substrates class II. Those are the one let us say for example, ethane that is what we have seen and in case of also methanol we see the similar cases.

So, the class II substrates are also can be utilized for its hydroxylation chemistry, but the diffusion is controlling. You have seen stepwise how these bismuoxo species is abstracting hydrogen atom from methane and then how little rearrangement is happening making the hydroxyl in-situ form ready for the rebound and then subsequent chemistry is following that and fascinating.

We will come back with a much more chemistry in the subsequent classes. I hope you are studying these in detail and also we will summarize them to make it a little bit easy and compare contrast among the different enzyme so that you can follow well in the exam.

Thank you very much. See you in the next class. Bye bye.