Mechanisms in Organic Chemistry
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Lecture-37
Enzyme Catalysis

So in the last class we had wrapped up acid base catalysis. So we had looked at both specific acid catalysis as well as general acid catalysis.

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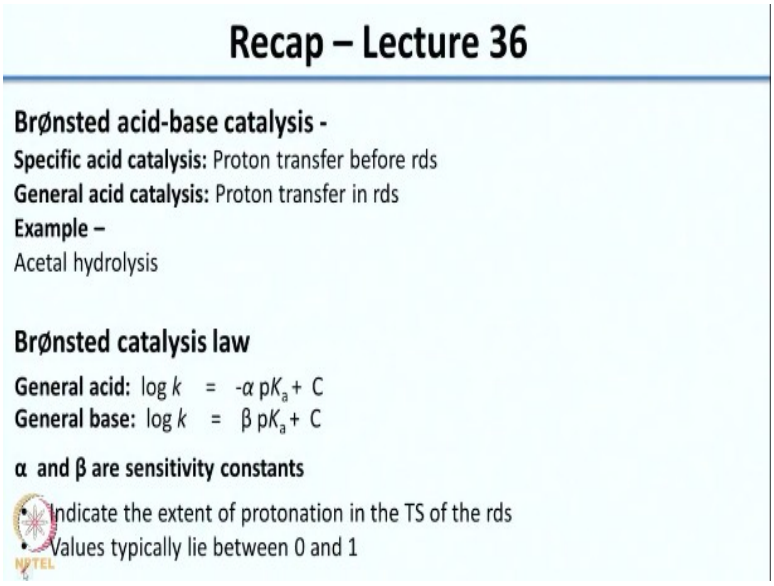

So specific acid catalysis is where proton transfer occurs before the rate determining step whereas general acid catalysis is where proton transfer occurs in the rate determining step. Now remember specific acid means the acid is the protonated solvent. So it is water it is H_3O^+ . So that is why it is called specific acid and specific base whereas general acid can be any acid that you add in the reaction medium that is why it is general. And we had looked at the example of acetyl hydrolysis and we had seen that in which cases you see specific acid catalysis and we had looked that an example where you see general acid catalysis.

Then we had looked at how you can correlate the rates of these reactions following general catalysis with the pKa. So this is called as a Bronsted catalysis law so for general acid the log k is given by - α pK a + C where is for general base the log k is given by β pK a + C. So this is

similar to a linear free energy relationship. So on the left you have the rate constant for the reaction which is being protonated by the general acid and on the right you are looking at the ability of the acid to protonate water because that is what your pKa is.

So you are correlating this thermodynamic parameter with the rate constant k. Now the negative value of α is because the rate increases for a general acid as the pK a decreases because the general acid becomes a better acid. So we had also looked at what α and β signify. α and β are called sensitivity constant and they indicate the extent of protonation in the transition state of the rate determining step.

So typically, the values lie between 0 and 1 and we had seen that in some cases you see a negative value. The negative value indicates that your rate constants for protonation does not follow the same strength as the pKa so the acid which is strongest would actually undergo a slower reaction. So that indicates a negative α value and we had seen an example where this is observed. So typically but the values lie between 0 and 1.

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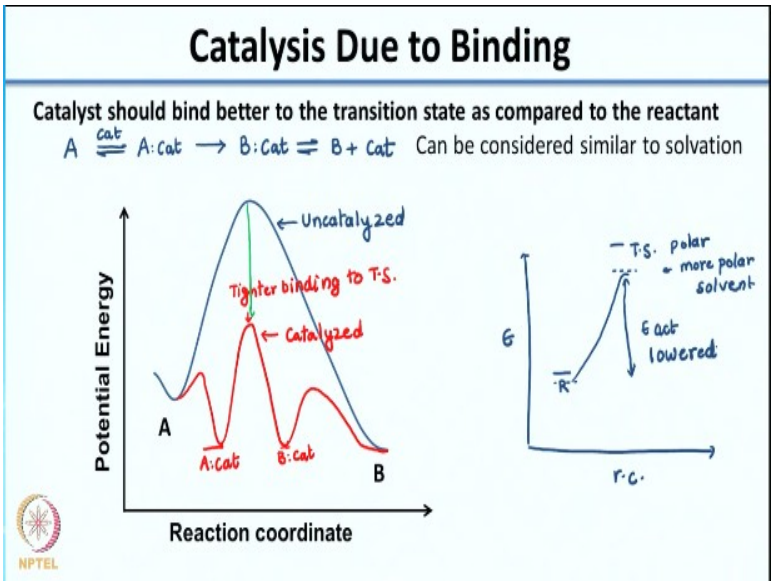

So now will move on to other types of catalysis. The first will do in today's class is catalysis due to binding. Now catalysis due to binding we had looked at reaction coordinates before and you can express catalysis due to binding using this generic equation. So you can have your reactant,

in this case your reactant is A and let us say you have the reactant binding to the catalyst. So you will have the complex of reactant with catalyst and then you will have this being converted to the product B which is still bound to catalyst and then ultimately you will get B + catalyst.

So this is how you can write this entire process. So this here is the uncatalyzed reaction and we had done this reaction co-ordinate before. So this is just like revision for you. So can you then go ahead and draw the reaction co-ordinate diagram for the catalyzed reaction? You can press the pause button and draw it in your notebook. So let us see if you were able to draw this properly. So the first thing you have is the complex between the reactant and the catalyst and as I said since its binding to the catalyst it indicates a lower energy than A.

So you have this. Then you have conversion of A:cat to B:cat. So that if it is actually catalyzing the reaction what you would see is you would see a decrease in activation energy and then finally you will have formation of your product. So here what is important if you remember we had told you, so I told you that for the reaction to be catalyzed efficiently by the catalyst what you need to have is you need to have a tighter binding with the transition state compared to the reactant.

So you have to have and we had looked at different scenarios if you remember as to how the reaction coordinates will look if it does not bind tightly to the transition state. Now in this case what happens is because it binds tightly to the transition state, you have a lowering in energy as compared to the uncatalyzed reaction. So it is very important that your catalyst binds tighter to the transition state as compared to the reactant. Now if you were to think about it similar to a concept that you had studied earlier remember initially.

when we were looking at various factors affecting reactions I had told you it is always the relative difference. Remember we had spoken about climbing a mountain and if you have to reach so if you need to reach the peak faster you would need to either lower the height of the peak or increase your starting point. So either increase your starting point so that you have a shorter distance to cover or you lower the height of the peak so that you have a shorter distance to cover. So you can think of this similar to solvation which you had studied earlier.

So when you had studied solvation earlier, if you remember I had told you what is important is the relative stabilization. So say I have a reaction so this is my reactant and this is the transition state and suppose the transition state is quite polar and now I do the reaction in a more polar solvent and let us assume the reactant is not as polar as transition state so what would you see is that for your reactant the lowering in energy is less whereas for the transition state it is more. So this is a more polar solvent.

So now what you see is you have a lowering in activation energy. So always remember it is these relative differences which are very very important when you study kinetics. It cannot be absolute. Just a very tight binding to reactant will not help your reaction. There needs to be a tighter binding to the transition state. So now with this understanding let us look at specific examples and one of the classic examples is seen in biology when you look at enzyme catalysis.

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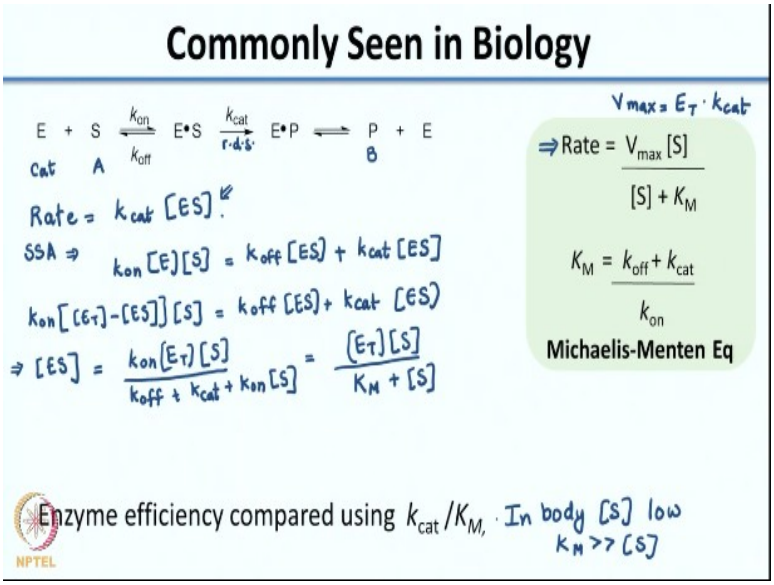

So in enzyme catalysis, so here we are representing the reactant as S which is a substrate. This is the common terminology used which is why I am using that. E is your catalyst, which is the enzyme, ES is your enzyme-substrate complex. So your reactant-catalyst complex, like before. EP is your enzyme product complex. So this is similar to the catalyst B complex you would seen before and finally you have P + E.

So if you are getting confused I will just write below this so that you are not confused what each of these corresponds to. So enzyme is your catalyst, substrate is your reactant, P is your product. Alright? So than the rest of it will make sense to you. So the rate law for this is given by the Michaelis-Menten equation shown on the right. Now you might think that you need to memorize this Michaelis-Menten equation. But then you do not need to do that. Remember earlier we had studied how to derive the rate laws?

So you can do that very systematically using whatever you had studied earlier for chemical reactions. So what I want you to do now is given that this is the rate determining step and applying the steady state approximation for ES what I want you to do is derive the rate law for this enzyme catalysis. So the transformation given on your screen. So you can press the pause button and work it out. So let us see if you were able to work it out. So the first thing that you have is the rate is given by k_{cat} concentration of ES.

Now assuming that the enzyme-substrate complex once it is formed it is consumed to give you the enzyme-product complex you can apply the steady state approximation. So applying steady state approximation what are the things which lead to formation of ES? So you have k_{on} [E] [S] concentration of enzyme into concentration of substrate is equal to k_{off} so these are the things which lead to consumption of ES + k_{cat} [ES].

So now when you think of a reaction, essentially you will have all the enzymes which are unbound then when you add the substrate you will have the substrate binding to the enzyme. So now what this would lead to is that you will have some enzymes which are free and some which are bound to the substrate. So anytime the enzyme concentration would be given by in E_{total} − [ES].

So then you can substitute here. So what you will get is k_{on} into concentration of total enzyme which is (E_{total} − [ES]) × substrate complex would be given by again k_{off} [ES] + kcat [ES]. So from this you can get the concentration of enzyme-substrate. So enzyme-substrate will be given by so I am not going to show you all the steps but it would be given by k_{on} [E_T] [S] / k_{off} + kcat + k_{on} [S]. Alright?

So now if you divide this whole thing by k_{on} which is from the numerator if you bring that down what this would simplify to would be $[E_T]$ $[S]$ / k_{off} + kcat by k_{on} which is given by K_M + substrate. So now if you substitute this value of ES into the rate equation given here you will get an expression similar to what is shown here. The only difference being what you see is here you have V_{max} when you substitute this V_{max} would be given by enzyme total into k_{cat} and this makes sense correct?

Because when will you have V_{max} you will have V_{max} when you have a very very very very high substrate concentration. So essentially what will happen is if you look at this expression for ES what you would see is this expression of ES because the substrate concentration will be very very high, the denominator becomes equal to almost substrate concentration because substrate concentration would be much greater than K_M. So what will happen is you will have both of these getting cancelled so you will have concentration of ES equal to total concentration.

So then if you put that in this equation the rate will become k_{cat} into a total concentration which is the maximum rate or V_{max}. So here what I shown you is you can very very systematically derive the Michaelis-Menten equation using whatever you had studied earlier when we were studying kinetics. So it is not that complicated. Now a lot of times when people compare enzyme activity, so if I were to see that which is a better enzyme

when I am trying to figure out the two catalysts are the two enzyme. Efficiency is compared using this term k_{cat} / K_M. So people look at the value of k_{cat} / K_M and say that ok this enzyme has a greater k_{cat} / K_M that means it is a better catalyst. Now why do you think that is the case? I want you to think about it. Alright. So when you had, remembered I told you the case of V_{max} is when you have a very high substrate concentration you have maximum catalysis then.

In general in our body what you see is the amount of substrates that you have is in the nano molar concentration. So it is very very very very very very dilute. So now let us see what happens to this rate expression when you have a very very very dilute solution. So when you have a dilute solution essentially what you would have is, so in our body concentration of S is low. So what

you will have is you will have K_M becoming now greater than [S]. So now K_M is greater than concentration of S.

So now what you would see is in your expression you would have the rate determination, the rate dependence directly related to kcat over K_M. So you can see that by substituting in the expression so now you have K_M value becoming much higher than substrate so you can ignore the substrate concentration and then what would you see is in your reaction, so your reaction rate is given by k_{cat} [ES]. So that would be given by k_{cat} $[E_T][S]$ divided by K_M.

Now enzyme total is how much enzyme you are taking and that would be similar for two enzymes. The substrate concentration is very low. So what would be important would be the ratio of k_{cat} / K_M. So hopefully this gives you an idea of how binding is seen in biology for catalysis and its very very important in several several biological functions. Now let us look at the other factor which contributes to catalysis, which is proximity. So when you think of a chemical reaction you think of an electrophile you have a nucleophile and you have a solution

So you have all these molecules in solution and you have your electrophile and nucleophile which have to meet for your reaction to take place you. So you need a collision to take place for the reaction to occur and because of the collision the transition state that you get has a greater order. So entropically formation of this transition state is not favored because essentially you have greater disorder when the molecules are alone. And then they join together and now you have greater order in the transition state.

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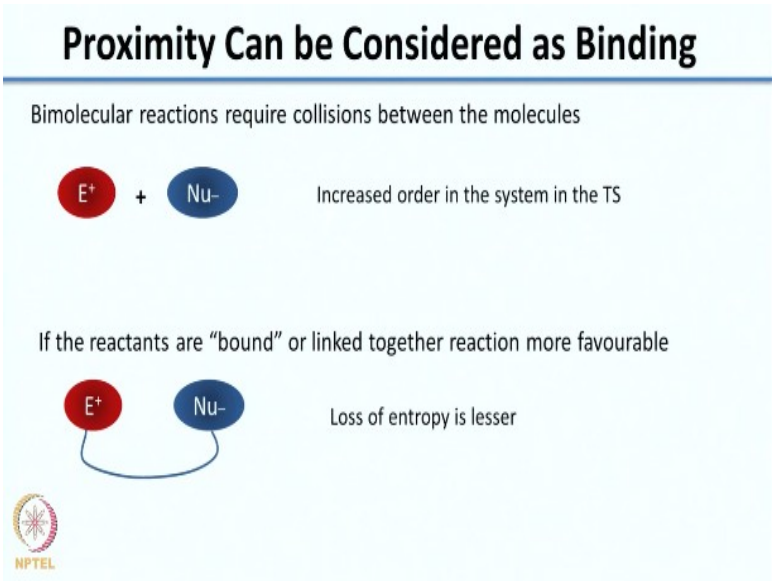

So what is seen is that if you bind the two reaction at the two reactants so you bind the two reactance with some sort of a linker, this loss of entropy is lesser show the reaction is thermodynamically more favorable because what is seen is since now the reactants are bound so you do not have as much disorder as you had with the free reactant. The second thing is having then close to each other improves the probability of collision and that is why the reaction rate and enhances. So both these factors help in catalyzing reaction by keeping the two reactants close to each other.

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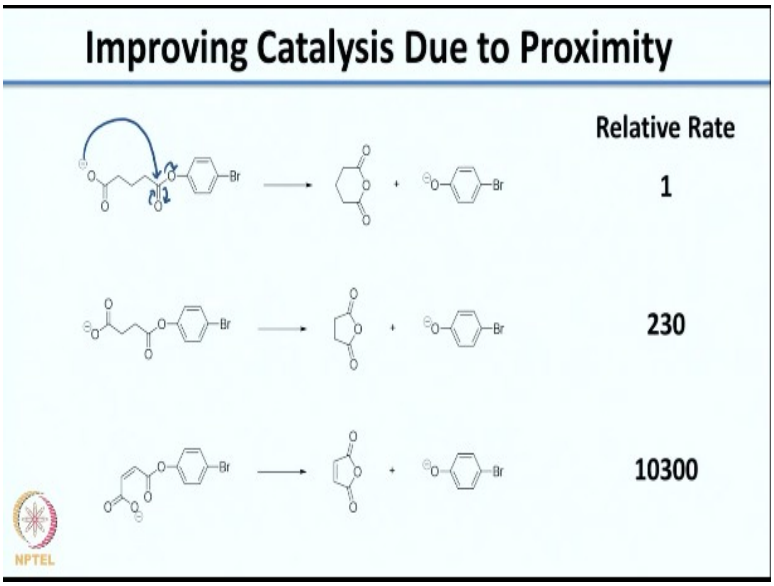

So now let us took examples where you can see this. So here what you see is the reaction that is taking place is formation of the anhydride. So what you have is you have this coming in right,

and then it forms the tetrahedral intermediate which then comes back and pushes out this phenoxide. Alright? So here what you see is both of these are tied together by a linker. Right? So this reaction would be quicker than a reaction where you have two different acids coming together to form an anhydride. So the cyclic anhydride would be easier to form.

Now let us compare three of these cases. In the second case what you see is the length of the linker is slightly shortened. So by slightly means by 1 CH_2 unit. So now you are forming a five membered ring instead of a six membered ring and the third case you have put a double bond. So the number of carbons is the same but you have now put the double bond and the geometry of the double bond is cis. Alright? So these are the three scenarios and I want you to think of how the kinetics will change. So if you put if you were to compare the reaction rates for these which do you think would be faster?

So think about this proximity effect you can press the pause button and spend more time thinking about the answer. So alright I give you the answer and let us see if you can understand why. So the relative rates are given by one for the first example where is performing a six membered ring and then for the two five membered rings. So what you see is when you do not have a double bond the rate is slower as compared to the case where you have a double bond.

So why is this so? So what would you see is when you have a shorter linker you are improving the proximity of this O^- to the electrophilic carbonyl centre which is why you have an enhanced rate of two thirty. Alright? Also the approach is very important when you think of these reactions. So probably you have a better approach in this case. So what you see in the third case is that now you are really restricting the proximity by putting an sp^2 centre. So having sp^2 centre makes these two highly proximal to each other.

So now there is a greater greater enhancement of the reaction rate. So it is almost ten thousand three hundred times the first case. So in this case what you see is even orientation is improved for the reaction to take place. Now here the double bond geometry is cis. What do you think will happen if I take a trans double bond geometry? Obviously the rate will decrease because now I am keeping them far away from each other and unlike the previous case where the molecule had

flexibility with the trans geometry it does not have that flexibility. So it will be more difficult for it to reach this electrophilic carbonyl centre.

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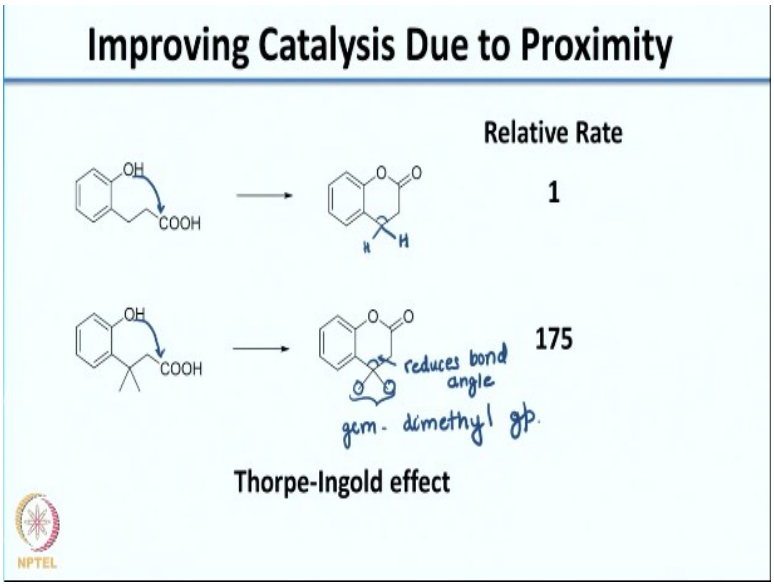

Another example is shown on your screen. So here your forming the lactone and not the anhydride. So here again you will have the O attacking the carbonyl carbon. So here you have an aromatic ring which again, similar to the sp^2 case that we had seen earlier, it helps in keeping the two reactants proximal to each other. Now what is seen is when you add this gem dimethyl group here to form

now the same six membered lactone, you see and enhancement in the rate and this is a very standard observation that is seen quite often. And this effect is called the Thorpe-Ingold effect. So what the Thorpe-Ingold effect does is that when you put these gem dimethyl group, so gem is for germinal; so having this two groups you have the CH_3, CH_3 which are large what it does is it reduces the bond angle. So reduces it relative to the case where you just have two hydrogen.

So compared to the two hydrogen when you have the dimethyl group, because it is larger and needs more space this bond angle becomes reduced. So having this bond angle reduced, improves the proximity of the oxygen to the carbonyl group. So here you see how catalysis due to proximity can also be thought of like a binding effect where you have the two reactants bound to each other. So we will stop here and in the next class we will look at the other types of

catalysis which I had shown you on the first slide of catalysis. So thank you and see you in the next class.