Introduction to Complex Biological Systems
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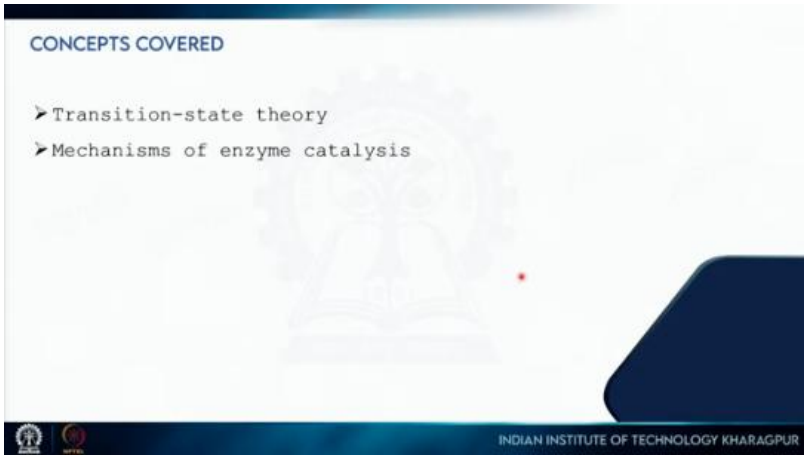
Lecture 17
Theory of enzyme catalysis

Welcome back. So, in today's lecture I am going to discuss the mechanisms by which enzymes catalyze reactions and they achieve this fantastic rate of catalysis. So, I am going to talk about a very important theory which is Transition-state theoryand then I will discuss several mechanisms by which enzymes catalyze reactions. So before I get into that, this is something that is important to discuss.



So enzymes, depending on the type of reactions they catalyze, they are classified into seven groups. So a few years back, there were six classifications of enzyme and the seventh one is a new entry. So enzymes based on the reactions can be classified into these seven groups and they are called enzyme classification. So, the first one is oxidoreductases, which means that some sort of oxidation reduction reaction is happening. So, either hydrogen atoms are removed or transfer of electrons takes place.

Second one is called transferases. So in this case, certain functional groups are transferred between donors and acceptors. For example, kinases, so these are enzymes, they transfer phosphate group from ATP to other molecules and this other molecule can be other proteins also. Hydrolases, so these are enzymes which will break a bond by the addition of water molecule. For example, we will see the example of chymotrypsin.

So, a peptide bond will be broken by addition of a water molecule. So, that COH group and an NH_2 group will be formed. So, that is a hydrolysis. Liases, they add water,

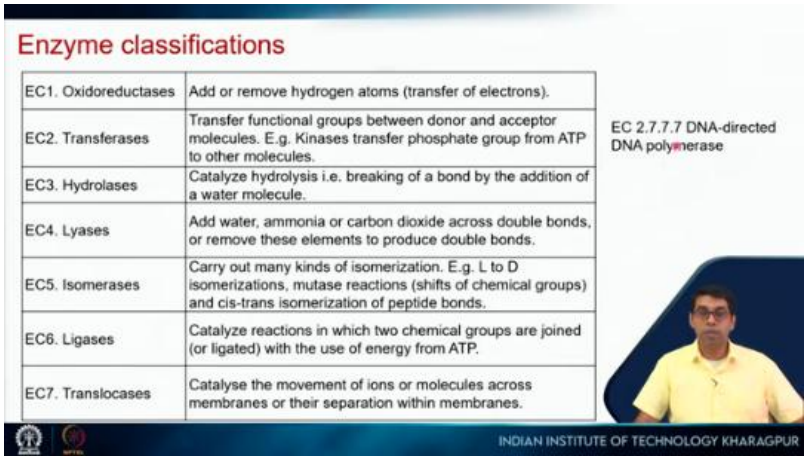
ammonia or carbon dioxide molecules like that across a double bond or remove these elements to produce a double bond. So, both reversible reactions will come under the same category.

So, these are liases. Isomerases, so they carry out isomerization of different isomeric groups for example, *L* to *D* transformation or if we think about a peptide bond then the peptide bond can have *cis* or *trans* conformation. So, there are enzymes called isomerases which will catalyze this *cis trans* isomerization of peptide bonds right. Then there are ligases.

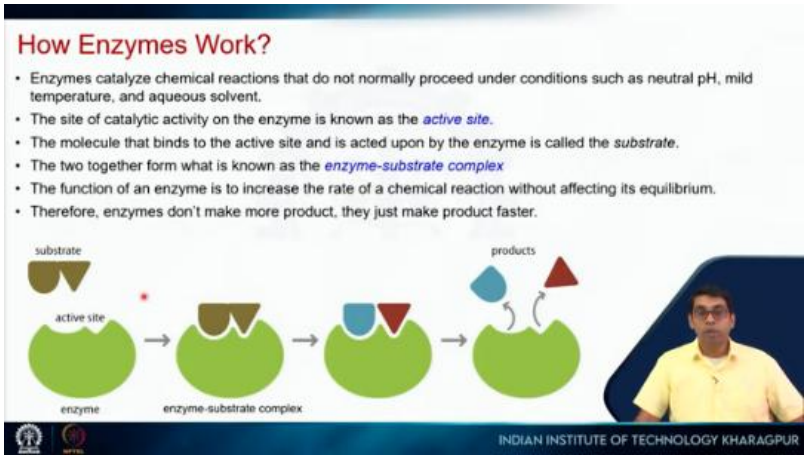
So, they catalyze the reaction in which two chemical groups are joined together. For example, in so, there are many ligases we have already seen ligases. And then this fourth one is translocases. So, in this case movement of ions or molecules across membranes or their separation within membranes is catalyzed.

Now, every enzyme is given a unique enzyme classification number. For example, this enzyme classification number 2.7.7.7 is actually for DNA polymerase. So, DNA polymerase or not just any DNA polymerase it is DNA dependent or DNA directed DNA polymerase. So, what it does it transfers this. So, it takes ATP or GTP or any trinucleotide and then transfers that molecule to the growing chain of the DNA.

So, it will come under the classification of class of transferases that is why this first number 2 and then there are sub classifications for which this subsequent numbers come in. So, every enzyme will have its own enzyme classification number and from that number you can actually tell what is what this enzyme does. You can actually tell a lot about that particular enzyme. So, how do enzymes work we have already seen that that enzymes they have an active site where the substrate comes and binds and we discussed again two mechanisms lock and key and induced fit. So, the shape of this active site will determine the binding of the substrate and this is important because enzymes want to their specific substrates.



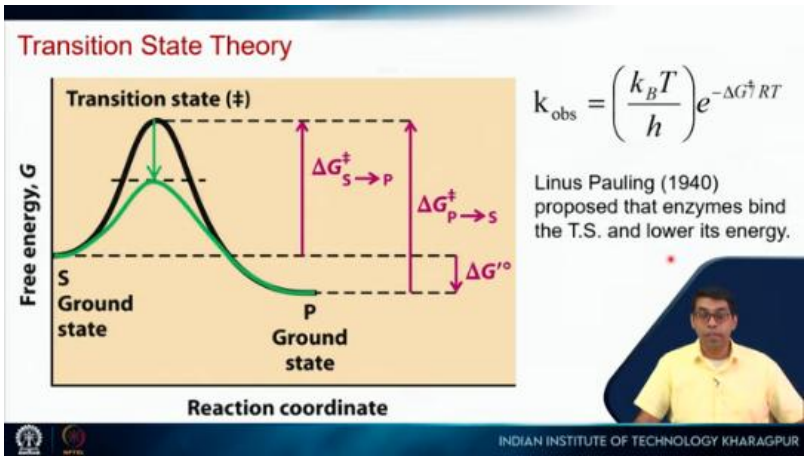
But we will have to modify this thought a little bit in the subsequent slides and I will come to that. So, we have seen that substrates they bind to the active site, they have all these complementary interactions where you have hydrophobic interactions or electrostatic interactions or complementary hydrogen bond formation. So, they together give the specificity of the enzyme towards its substrate. Now how do enzymes catalyze reactions so fast? So to understand that let us look at how reactions happen. So we have a substrate and that substrate gets converted to the product now depending on the reaction mechanism. There will be some state where it will go through a particular transition state.



So, let us say some OH group is like if we think about hydrolysis of peptide, let us say OH group is attacking the carbonyl carbon and then this O minus intermediate is formed, but before that intermediate is formed. It will go through a very high energy state and that state is referred to as the transition state and that has the highest energy. Now, higher that energy slower will be the rate of the reaction. So, the observed rate of the reaction depends on this energy barrier by this relation.

So, k_B is the Boltzmann constant, T is the temperature in Kelvin that is the absolute temperature, h is Frank's constant, ΔG is this energy barrier and then R is the gas constant and T is against the absolute temperature. So, higher the barrier lower will be the observed rate. It is hypothesized that enzymes lower this energy barrier. So, if I can lower this energy barrier like this, then of course, my reaction will become faster. This was proposed by Linus Pauling in 1940, He proposed that enzymes bind the Transition-state and lower its energy.

That is how they can achieve such fantastic enhancement in reaction rate. So, let us look at it in more details. So, we have already seen this enzyme plus substrate the substrate binds to the enzyme. So, we have enzyme substrate complex while it is bound the reaction happens. So, the substrate gets converted to the product.



So, we have enzyme product complex and then this product will readily diffuse out. So, the enzyme is regenerated and we have product formation and then this enzyme can again participate in the reaction by binding another substrate. So if I draw the free energy diagram for all these different conformations of the enzyme and the product. So this is our reaction coordinate. So this is the free enzyme plus substrate.

This is the enzyme substrate complex. So the formation of this complex will go through some energy barrier. Then this gets converted to the enzyme product right so the reaction happens. So this will go through another barrier and then the product will be released. So this will also go through another barrier. Now it turns out that this barrier between the substrate and the product enzyme substrate and the enzyme product complexes where the actual reaction happens. This will be the highest barrier and it will determine the slowest reaction step. So this will be the rate determining step of our complete reaction going

from this to this. Now let us say this is the original barrier of the actual reaction when there is no enzyme.

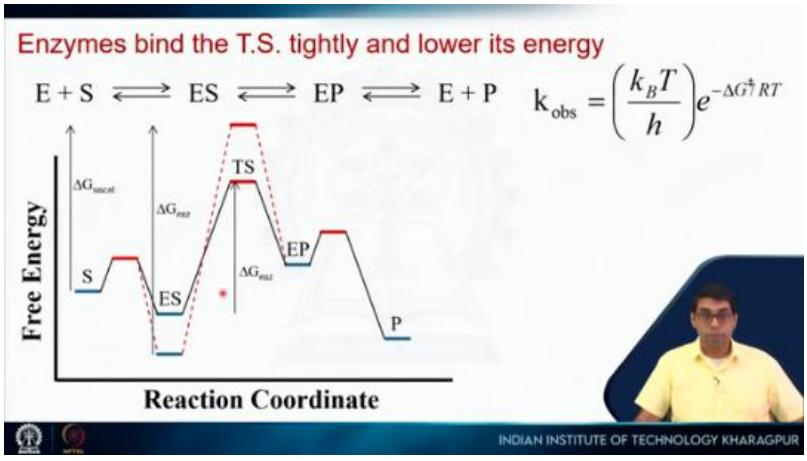
So the substrate goes to this transition-state and then it goes to this product. So, if we go to the first slide where I say that enzymes bind to the substrate, they are complementary in structure. So, if the enzyme is complementary to the structure, if it is optimized for binding the substrate, then it will bind the substrate very tightly. It will not do anything to the transition state. So, now what happens?

You see that originally this was the substrate and this was the TS. So, the gap was this. Now what we have done is we have lowered the enzyme substrate complex here and the gap is this. So we have increased the ΔG. So what will happen to the reaction? If my ΔG is increased, the reaction will become slower.

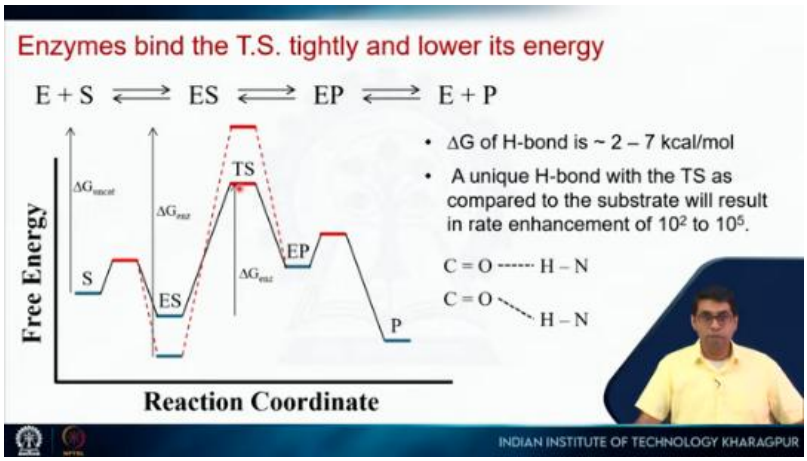
So if the enzyme binds the substrate tightly, not the transition-state, then we will have actually a slower reaction. So instead what the enzyme does, of course it will bind the substrate because you need specificity. So it will lower it. But, then it will bind the transition-state even more tightly. So, this lowering is much more than this lowering.

So, instead of lowering it up to this the ES goes here. So, the energy difference is this and in this case the energy difference is this. So, uncatalyzed reaction the ΔG will be this. If it binds to the substrate more tightly, then it will be this. So you can see that this is much bigger than this.

So the reaction will actually become slower. But instead what it does is it binds the substrate, but it binds the TS much more tightly. So this ΔG in the presence of enzyme or we can call it ΔG of our reaction rate. So, how can we do that? This substrate and the TS will not be very different right.



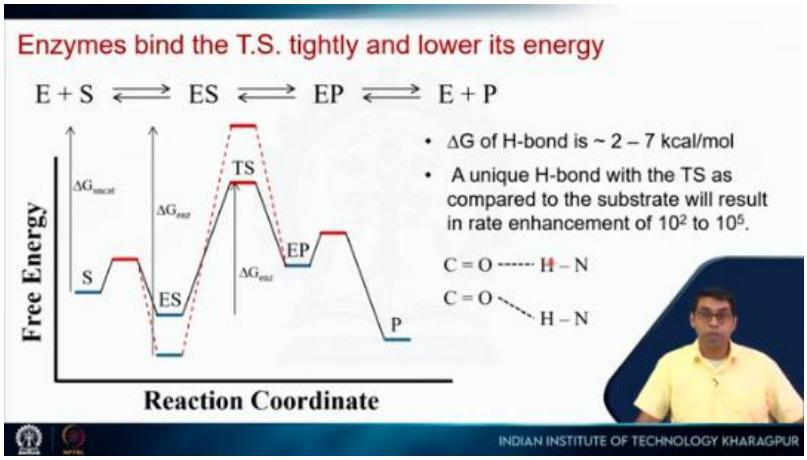
So, they will be somewhat similar. So, similar interactions will be there, but since the TS is different, the enzyme can actually introduce some extra interactions or interactions which are more specific for the transition state compared to the substrate. Let's for example that all the interactions for the substrate and the TS are exactly the same except that the enzyme forms one extra hydrogen bond with the TS. Now one the ΔG of hydrogen bond is around 2 to 7 kcals per mole right. So, if we have an extra hydrogen bond here then this is stabilized by 2 to 7 kcals per mole more with respect to this right.



So that extra stability, if we put it in that equation, it will result in 100 to 100,000 times faster reaction. So, now why do we have this range of hydrogen bond? This range of hydrogen bond is there because hydrogen bond depends on lot of things. Let us say primarily it depends on the geometry. So, it is a dipole-dipole interaction.

So, if my carbonyl carb, so there is an hydrogen bond between C double bond O and this NH, if all four are collinear, this will be a very strong hydrogen bond. But if they are something like this, then this hydrogen bond will be relatively weaker to this. And we have already seen this in the example of beta strands, parallel versus anti-parallel beta strand. So, the energy of the hydrogen bond will depend on this and also other factors. So, there is a range and and it turns out that enzymes are very good.

So, they the interactions are all optimized. So, you can expect that it will form a very optimal hydrogen bond and just one hydrogen bond extra can give you a rate enhancement of 100000 fold. So how enzymes work? This is our uncatalyzed reaction substrate goes through this high tension state into this product. In the presence of the enzyme, it forms this enzyme substrate complex, goes through a Transition-state, gets the enzyme product complex and then the product is released.

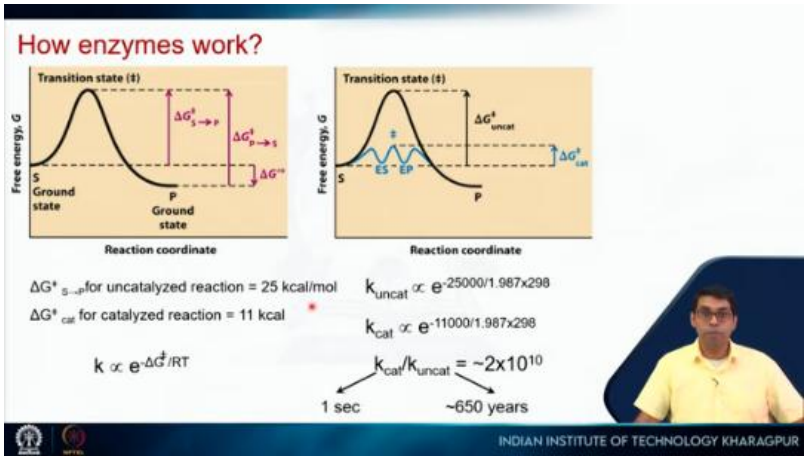


So, again you see that this ΔG between the substrate and the product is not changed. So, your final equilibrium will not change, only the rate will enhance, which means that you will reach equilibrium much faster. So, this is my ΔG uncatalyzed and this is my ΔG catalyzed in the presence of the enzyme. And now we know, so let us say my ΔG uncatalyzed is 25 kcals per mole and ΔG catalyzed is 11 kcals per mole. So, there is a reduction of 14 kcals per mole right.

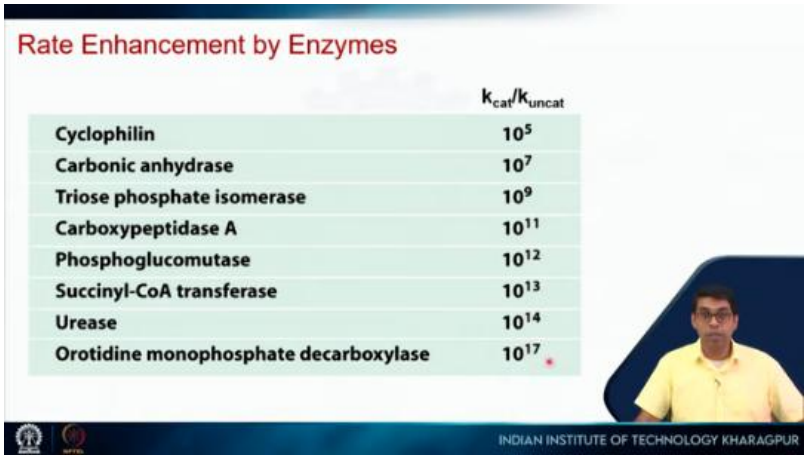
So, it means maybe couple of hydrogen bonds or some one hydrogen bond and some more hydrophobic interactions. So, all of these things have some electrostatic interactions. So, all of these things are stabilizing the TS. So, that it results in a 14 kcal per mole. Now, we know that the rate is proportional to ΔG like this. So, I have removed all these constants because we are going to take a ratio.

So, we do not need that. So, my K uncatalyzed is proportional to this. I am just plugging in the numbers K catalyzed is this. It turns out that K catalyzed over uncatalyzed will be in the order of 10^{10}. So, how big this number is? If my catalyzed reaction happens within a second, it means my uncatalyzed reaction would actually take 650 years to reach equilibrium.

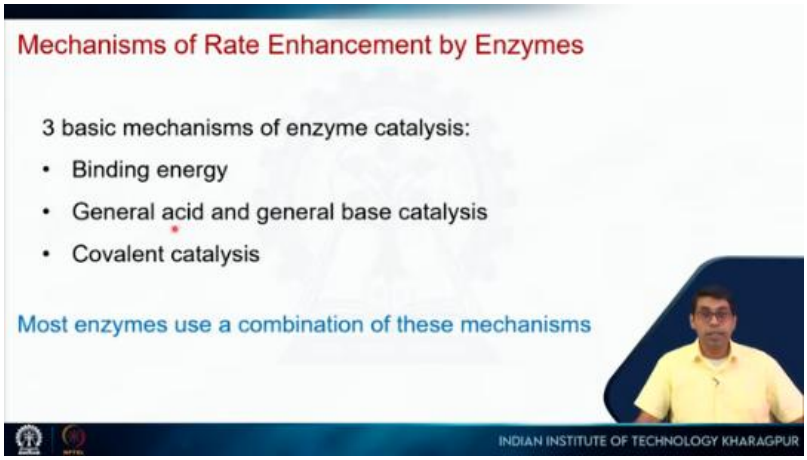
So, something that would take 650 years will be done within a second if the rate enhancement is this or in other words, if my transient state is stabilized by 14 kcals per mole. So, there should be kcals per mole here. So, this rate enhancement is not something that is unheard of. So, there are many enzymes, you can see all these enzymes, they have much higher rate enhancement compared to what I have shown.



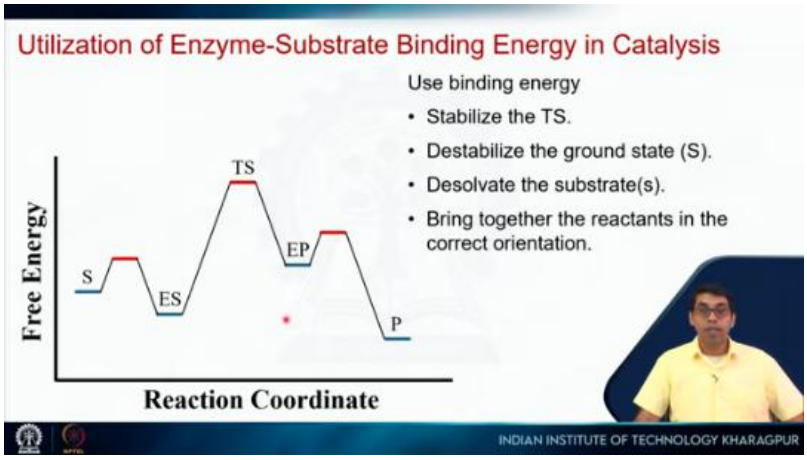
So, this is 10^{17}. So how do enzymes achieve this? They achieve these fantastic rate enhancements by using multiple mechanisms. The first one is binding energy. So I have already mentioned binding energy where it is using this binding energy to bind the Transition-state more tightly than the substrate.



Then there is general acid base catalysis. I will discuss this and also covalent catalysis and it turns out that most enzymes will not use only one of these three, they will actually use a combination of these three at least two to get this rate enhancement. So the ones which you see have more than 10^{10} and will most probably use all three so utilization of the enzyme substrate binding energy in catalysis. So it binds the substrate specifically. So it uses all these interactions and then it will bind the TS even better right so that is why we get a stabilization of the TS. So it uses this binding energy to stabilize the tension state it can also utilize this binding energy to destabilize the ground state. It can actually take it up, it can use this to dissolve with the substrate. So, typically the substrates they will have water molecules around them and if they are polar they will have this hydration layer. So, you have to strip off those and that is where this binding energy is utilized



and then it will also use this to bring the reactants together in the correct orientation. So, if it is a bimolecular reaction, A plus B then A and B have to be in the right orientation so that the reaction can happen. Enzymes provide a scaffold where these two reactants are put together in the correct orientation so that the reaction can happen. So, we will see examples of all of these when I discuss chymotrypsin in the next lecture. So what is acid base catalysis?

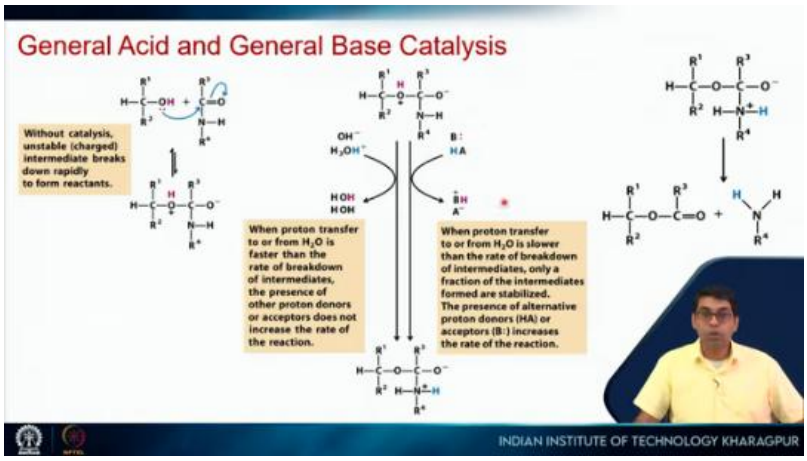


So let us look at this reaction. So this OH group attacks this. So it will form this intermediate and then this bond has to break. So then it will proceed. So this is the intermediate.

This can actually go back to the reactants if this bond is broken but we do not want that but what we want is this O minus should come here and then this bond should break. So, to do that this proton has to be transferred from here to this nitrogen and then only we can make this bond weaker compared to this CO bond. So, this will act as a better leaving group and this bond will be broken. This transfer of proton can be catalyzed by general

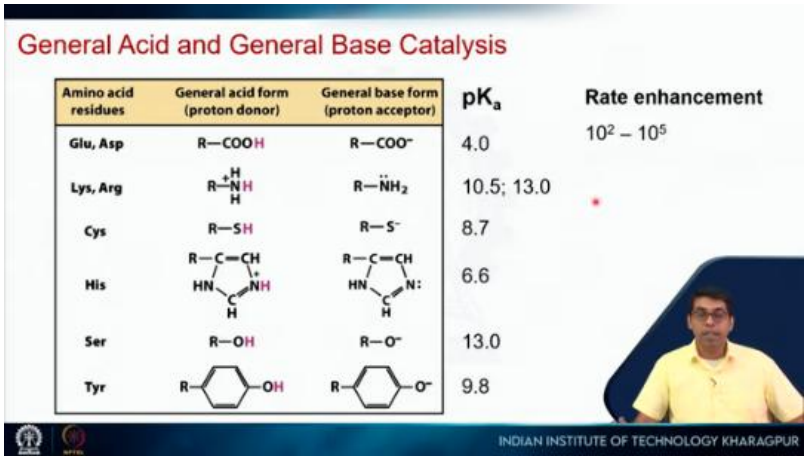
acid or general base. So if they are present then this general acid can abstract the proton from here and this base can donate the proton here. So the acid can donate the proton here the base can abstract the proton here.

So, now the intermediate is changed and then it will undergo this reaction. So, you see that this bond is more stable, this is more vulnerable. So, this negative charge comes here and this bond breaks and that completes the reaction right. So, the presence of acid or base can catalyze this reaction and it turns out that enzymes will use various side chains like serine to act as this proton donor or histidine as a proton acceptor and it will catalyze reactions like this. So we will again see the example of chymotrypsin where it will be clearer.

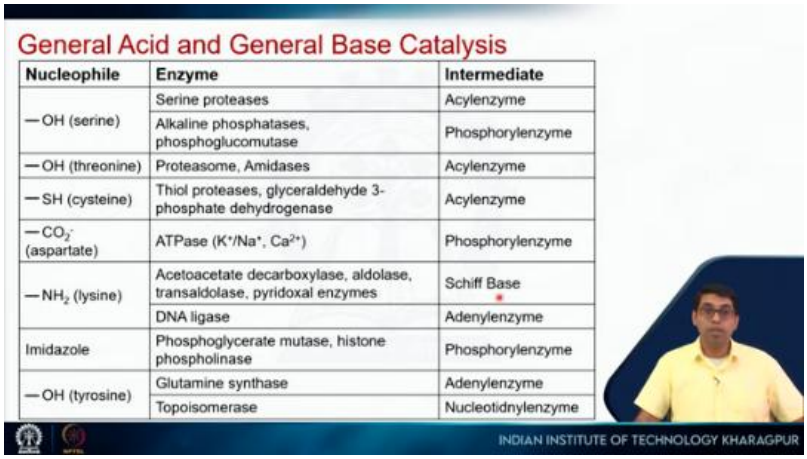


So, we have all these different side chains which can act as a general acid or a general base so proton donor or proton acceptor. So, we have glutamic acid, aspartic acid, their pKa is 4, lysine arginine, pKa of much higher pKa 10.5 and 13, cysteine, histidine, serine and tyrosine. So, these amino acid side chains in the active site can act as general acid or general base and catalyze the subsequent reaction.

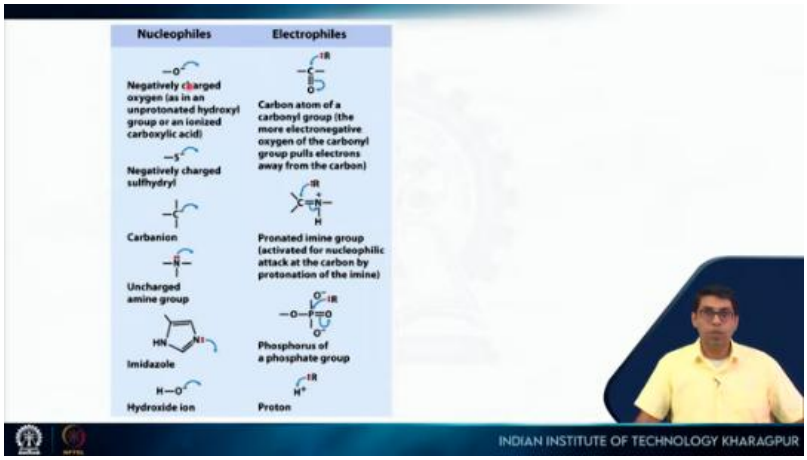
And this type of catalysis that is general acid- base catalysis can result in another 100 to 100,000 rate enhancement. So, let us say we have binding energy stabilization of the Transition-state. So, together they provide us 10^7 and then this acid base catalysis provides us another 10^5. So, together they can give us 10^{12} rate enhancement. These rate enhancements to these different mechanisms is not always straightforward, but over the years scientists have done lots of experiments to show that from this type of mechanism, we get rate enhancements in the range of 100 to 100,000.



So these are some examples. These are the side chains, serine, threonine, cysteine, aspartic acid, lysine, imidazole coming from histidine, OH from tyrosine. These are the enzymes and these are the intermediates that are formed. So acyl enzyme, phosphoryl enzyme. So these are shifts based. So these are all the intermediates which are formed by these side chains.



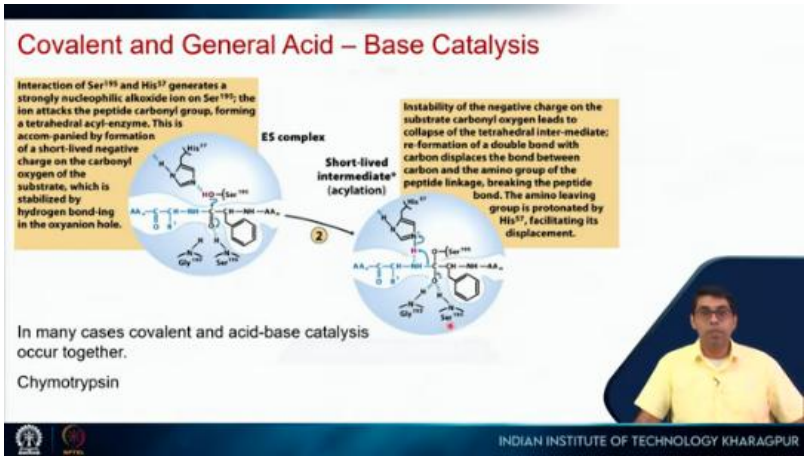
So, some of those intermediate and structures are shown here. So, these are the list of nucleophiles and these are the list of electrophiles which are present in this different reaction. So, enzymes can also form covalent bond and I am just showing you as one reaction step and we are going to see this in more detail in the next lecture. So, this is chymotrypsin, it catalyzes the hydrolysis of a peptide bond.



So you see this peptide bond $NHCO$, it will hydrolyze this peptide bond and it will separate this. So this is my polypeptide chain. Now chymotrypsin, it is specific to those peptide sequences where the C-terminal amino acid has a bulky hydrophobic group. So it can be a phenylalanine like this or a tyrosine or an isoleucine or a methionine. So long hydrophobic side chains.

In this case we will see a combination of two things, one formation of a covalent bond. So covalent catalysis and two general acid base catalysis. So histidine in case of chymotrypsin, there is this catalytic triad serine histidine and there is aspartic acid, histidine will abstract this proton this becomes O minus it attacks this this C double bond O or this O minus that will form here the acyl intermediate is stabilized by hydrogen bond formation by this, is stabilized by the hydrogen bond formation by these two residues. So, you can see that in this step, it actually utilizes all the mechanisms. There is covalent bond formation; there is acid-base catalysis. So, you see now this is forming a bond with this and it will act as a proton donor here so that this bond is broken, not this bond.

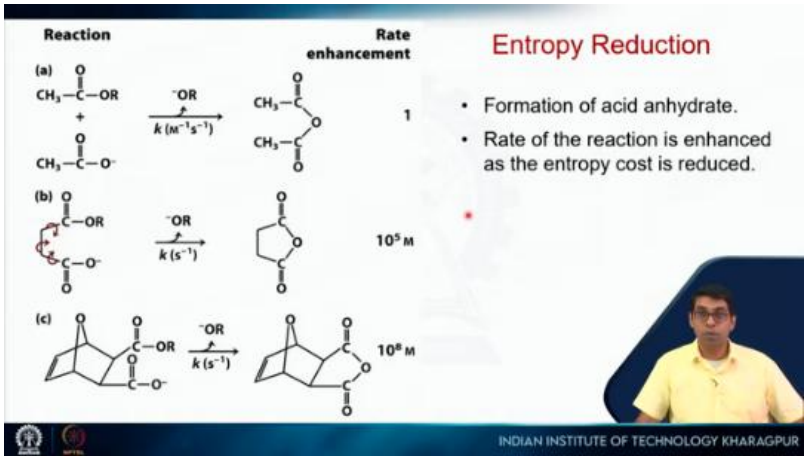
So, there is acid-base catalysis and also there is stabilization of the Transition-state by all these different interactions. So, we will see all of this in more details when I discuss chymotropism. Another mechanism that enzymes use is entropy reduction and this is something that is that becomes useful for reactions where you have two different components so A plus B. So, if you look at this reaction.



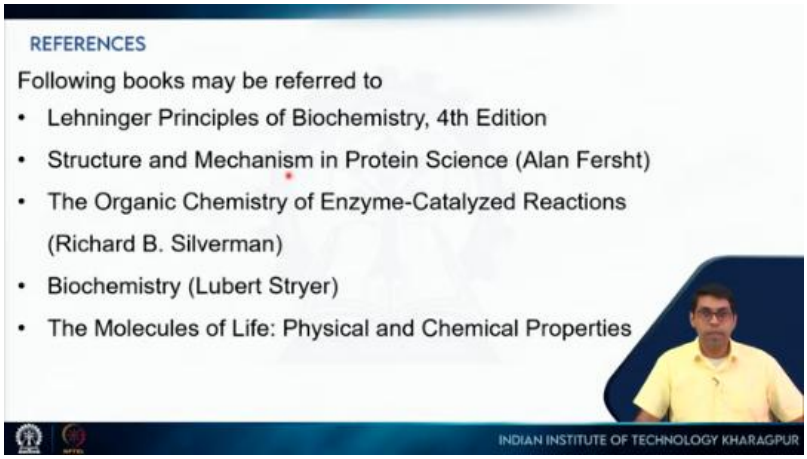
So, this is the formation of an acid anhydride two molecules will attack here this OR group will leave. So, you will get the formation of this acid anhydride. So, if the rate enhancement rate is 1.

Instead of two different molecules if we put them together. So if we join these two carbons by a bond then it will look something like this. Now there is one difference though this was a bimolecular reaction, this becomes a unimolecular reaction. So the rates become different this is per molar per second this is per second but, if we take the ratio of these two we get something in the range of 10^5 molar. It means that we will get the same rate of reaction in this condition if both are present at this concentration 10^5 molar. So, you need a very high concentration to get a similar rate of reaction.

But still this is not that great because we still have entropy, we can have rotation about this bond, about this bond and about this bond. So if we make this even more rigid like this, so that even the entropy cost is reduced here, then the rate enhancement is even more. So we get another 1000 fold rate enhancement. So this is what enzymes do. They will take these two molecules. Both will bind to the enzyme in its active site so that they are in the right orientation and the entropy is cost is reduced and the reaction is proceeds and then the reaction will proceed. So, entropy reduction results in faster rate of reaction and that entropy cost is actually paid by the binding energy. So, this is where the binding energy will come.



So, whatever I have discussed you can again go through these books for the Transition-state theory and for these different mechanisms of enzyme catalysis.



Thank you.