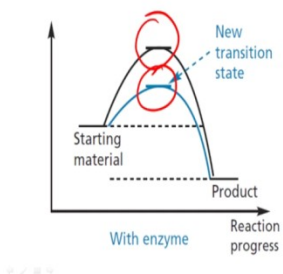


Medicinal Chemistry
Professor Dr Harinath Chakrapani
Department of Chemistry, IISER PUNE
Module 02 Lecture 7
Enzyme Catalysis Part-1

(Refer Slide time: 00:16)

Transition State

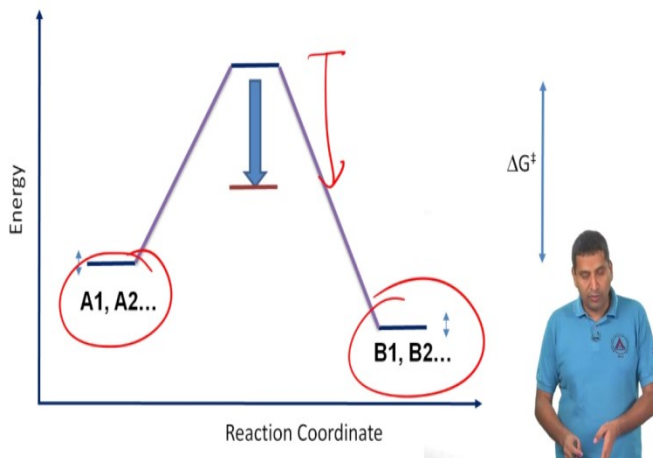
- The transition state involved in the enzyme-catalysed reaction. Indeed, the binding interactions involved are stronger than those binding the substrate, which means that the transition state is stabilized relatively more than the substrate. This results in a lower activation energy compared with the non-catalysed reaction.



In today's lecture we will look at how enzymes catalyze reactions. What are the various mechanisms by which this catalysis occurs? Ok as we looked at last time major part of the enzyme catalysis is in binding, once the enzymes binds the substrate then they must be a reaction that occurs in the active site and once the reaction occurs there is a dissociation to give you the products. And here somewhere along this line there has to be a transition state that is involved in un-catalyzed reaction and now in this transition state since there is an acceleration of the reaction the newer transition state that is being generated must be of lower energy.

So with this general picture, let's look at the various mechanisms by which enzymes can catalyze reactions.

(Refer Slide time: 01:07)



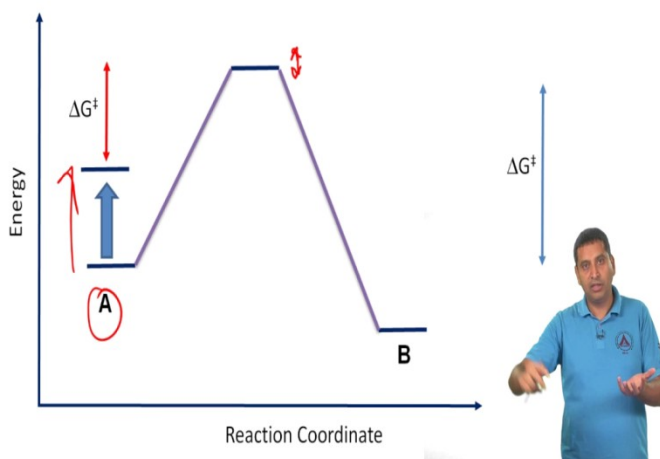
So before that so let's look about general picture of transition states. So imagine a reaction A goes to B ok, typical energy profile will look in the following manner. You have a starting material, you have a product and you have there is a barrier that the starting material has to cross to get across to the product and this barrier is represented by activation energy or by delta G double dagger ok.

Although these two terminologies are not necessarily interchangeable but we will for the purpose of this course we will use them in our interchangeable manner. So intuitively what we can determine from or experimentally what we can determine from a reaction is actually the rate of a reaction. So from the rate we can deduce what is the activation energy ok we will not go into those details at this point however suffice to say that when the transition state is stabilized it would result in acceleration of the reaction.

So what it means is that if this, if the barrier required is actually significantly lower when compared to the parent reaction then the reaction rate would go up. So if you would take let's say a series of reactions of substrates which are related structurally the assumption one could make is that the ground state energy of these reactants do not change a whole lot, neither does the ground state of the products.

(Refer Slide time: 02:45)

Catalysis: Ground State



However, when the transition state is stabilized you will see a an acceleration in the rate ok. So the rate of the reaction is dictated to some extent by the stability that can be imparted to a transition state.

On the contrary, we could also have a situation where the transition state is not perturbed a whole lot so this energy difference is not a whole lot but if the ground state energy goes up ok then we would still have the same result of acceleration in reaction. In reality in enzyme catalysis it is a combination of these two factors that is the ground state energy can also go up and the transition state energy can also go down, if the combination of these two factors results is catalysis.

(Refer Slide time: 03:31)

Mechanisms of enzyme catalysis



So with this background let's look at some mechanisms of enzymes catalysis.

(Refer Slide time: 03:34)

- Approximation,
- Covalent catalysis,
- General acid–base catalysis,
- Electrostatic catalysis,
- Desolvation, and
- Strain or distortion

All of these act by stabilizing the transition state energy or destabilizing the ground state (which is generally not as important as transition state stabilization)



So there are several broad ways in which one understands enzymes catalysis. So we will look at some of these cases in detail over the period of this lecture and the next lecture. So the first one is approximation. So here what we will understand is that when we bring the reactants close to each other then it can result in a dramatic increase in the rate of a reaction. The second concept is covalent catalysis, here (the) you change the outcome of the or you accelerate the reaction by changing the intermediate that is involved during the reaction course and then we will also look at general acid-base catalysis and how transfer of a proton or hydronium or hydroxide ion in appropriate manner can result in catalysis and will also look at electrostatic catalysis, desolvation which results in increased rate and also strain and distortion.

Together these effects results in as I mentioned earlier, result in the stabilization of the transition state or de-stabilization of the ground state and result in acceleration of the rate of the reaction.

(Refer Slide time: 04:47)

Approximation

- Approximation is the rate enhancement by proximity, that is, the enzyme serves as a template to bind a substrate so that it is close to reactive groups of the enzyme (or to bind multiple substrates so that they are close to each other) in the reaction center.
- This results in a loss of **rotational and translational entropies** of the substrate upon binding to the enzyme; however, this **entropic loss** is offset by a favorable binding energy of the substrate, which provides the driving force for catalysis...

Topics, entropy



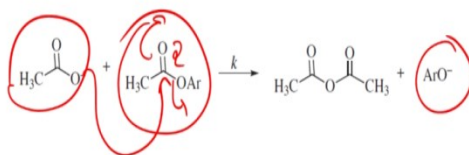
Ok now let's look at approximation. approximation is as I mentioned earlier is a rate enhancement by proximity ok, so for a reaction to occur let's say A and B they need to collide and this collision occurs when they are in closed proximity and they give out the products let's say C and D ok.

But in reality or in enzymes, the enzyme can bind the substrates or in a particular case a single substrate in a manner such that it can bring one of the reactants and the other reactant closed to each other and because they are brought to close to each other the degrees of freedom that are required to be overcome to reach a favorable transition state is lower ok. So off course there is a loss of rotational translation entropy in this process and but this entropy loss is offset by favorable binding energy ok.

So here the binding of the substrate which is the first step in enzyme catalysis provides the driving force for catalysis ok.

(Refer Slide time: 05:58)

- In order to study this effect, the reaction of acetate with an aryl acetate is considered



	k_{obs} (30 °C)
	$3.36 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$

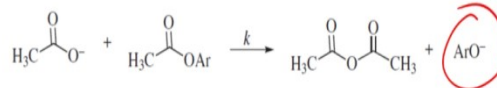
- Second order rate constant for this reaction is $3.36 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$



Now in order to understand this lets take an example of reaction of acetate with acetyl ester or aryl acetate. So now here in this case the acetate attacks the aryl ester and kicks out aryl oxide ion which then which kicks out aryl oxide ion ok. So this reaction as you can imagine would be a second order reaction and if you measure the rate constant at 30 degrees you get a value of 3.36 times 10 power minus 7 per molar per second ok.

(Refer Slide time: 06:44)

	$7.39 \times 10^{-5} \text{ s}^{-1}$
	$1.71 \times 10^{-2} \text{ s}^{-1}$
	$7.61 \times 10^{-1} \text{ s}^{-1}$
	3.93 s^{-1}



And then the effect of decreasing rotational and translational entropy is determined by measuring the corresponding first-order rate constants

However, these are first order rate constants for intramolecular reactions



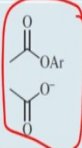
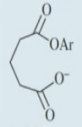
So in order to understand how approximation helps let's study this reaction within intramolecular manner ok. So what we are doing here is we are conducting the same experiment but with an reaction with an intramolecular reaction. So here you have the starting materials are located in

the same molecule this type of mechanism can operate and give out aryl oxide ok, if we measure the rate of this reaction and derive a rate constant, we would get a first order rate constant because this is dependent only on the starting material in this case and the value of this is 7.39 times 10 power minus 5 and similarly when we carry out this reaction over a multiple substrates we find that the rates of the reactions are in this these values ok.

But in order for us to understand approximation, we cannot compare a first order rate constant and a second order rate constant.

(Refer Slide time: 07:42)

Although first- and second-order rate constants cannot be compared directly, the efficiency of an intramolecular reaction can be defined in terms of its **effective molarity (EM)**, the concentration of the reactant (or catalytic group) required to cause the intermolecular reaction to proceed at the observed rate of the intramolecular reaction.

	k_{obs} (30°C)	Relative Rate, k	EM
	$3.36 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$	$1.0 \text{ M}^{-1} \text{ s}^{-1}$	
	$7.39 \times 10^{-5} \text{ s}^{-1}$	220 s^{-1}	220M

- If the rate constant k for this reaction is set equal to $1.0 \text{ M}^{-1} \text{ s}^{-1}$



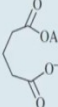
$$EM = k_{\text{First order}} / k_{\text{Second order}}$$



So we need to figure out some way in which we can actually compare these two values and so in order to do that what we do is we define a term known as effective molarity ok. Effective molarity is nothing but the concentration of a reactant required to cause the intermolecular reaction to proceed at the same rate as the intramolecular reaction ok. So if you set the second order rate constant to be 1 per molar per second that is for this intermolecular reaction if you set the second order rate constant to be 1 per molar per second and then what we do is we divide the first order reaction that we are interested in which is the intramolecular reaction and the rate constant of that is divided by the rate constant of the second order reaction.

So that is what we define as Effective molarity ok so if I do this division then I get a value of 220 molar ok.

(Refer Slide time: 08:41)

	k_{obs} (30 °C)	Relative Rate, k	EM
 	$3.36 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$	$1.0 \text{ M}^{-1} \text{ s}^{-1}$	
	$7.39 \times 10^{-5} \text{ s}^{-1}$	220 s^{-1}	220M

$$EM = k_{\text{First order}} / k_{\text{Second order}}$$

What this indicates is that the acetate ion would have to be at a concentration of, for example, 220 M ($220 \text{ s}^{-1} / 1 \text{ M}^{-1} \text{ s}^{-1}$) for the intermolecular reaction of acetate and aryl acetate to proceed at a rate comparable to that of the glutarate monoester reaction



Now, what is the meaning of this 220 molar? What it means is that this is the concentration of acetate that is required in an intermolecular reaction where the rate of the reaction can match the intramolecular reaction ok. So it means that we need a very extremely high concentration of acetate for this reaction to proceed at the same rate as the intramolecular reaction ok. To put this in perspective if you take water, pure water the concentration of water in pure water is 55 molar.

So it is impossible to reach this high concentration of 220 molar of acetate ok. So therefore what is effective molarity is a very useful term for us to understand the effect of bringing together the two reactants in the same molecule. So it appears that holding these groups proximal to one another particularly in the case of the when orbitals are in a favorable overlap condition that these the enzymes substrate complex aligns it in such a manner that the reaction goes in a very rapid manner and it becomes important contributor to catalysis.

I will repeat holding these groups in proximal to one another, especially in an enzyme's substrate complex the reacting groups are aligned in such a manner that favorable interactions occur and it results in a very great acceleration in the rate of the reaction ok and this type of intramolecular reactions can go up to 10 power 16 molar effective molarity can be 10 power 16 molar. So that is a tremendous increase in the rate of the reaction when compared to an intermolecular reaction ok.

So one of the ways in which enzymes catalyze or accelerate reactions is by bringing together two starting materials and enabling the appropriate conformation to be reached so that the reaction goes faster.

(Refer Slide time: 11:01)

	k_{obs} (30°C)	Relative Rate, k	EM
	$3.36 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$	$1.0 \text{ M}^{-1} \text{ s}^{-1}$	
	$7.39 \times 10^{-5} \text{ s}^{-1}$	220 s^{-1}	220M

$$\text{EM} = k_{\text{First order}} / k_{\text{Second order}}$$

- Of course, 220 M acetate ion is an imaginary number (pure water is only 55 M), so the effect of decreasing the entropy is quite significant.
- Efficiency of intramolecular catalysis varies with structure and can be as high as 10^{16} M

Therefore, holding groups proximal to each other, particularly when the orbitals of the reacting moieties in an enzyme-substrate complex are aligned correctly for reaction, can be an important contributor to catalysis.

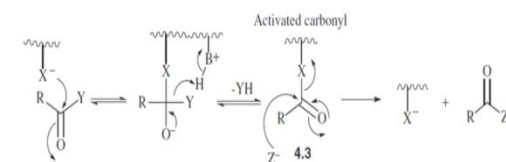


Now let's move on to the second topic which is covalent catalysis.

(Refer Slide time: 11:07)

Covalent Catalysis

- Some enzymes use nucleophilic amino acid side chains or cofactors in the active site to form covalent bonds to the substrate; in some cases, a second substrate then can react with this enzyme-substrate intermediate to generate the product.
- This is known as nucleophilic catalysis, a subclass of covalent catalysis that involves covalent bond formation as a result of attack by an enzyme nucleophile at an electrophilic site on the substrate.

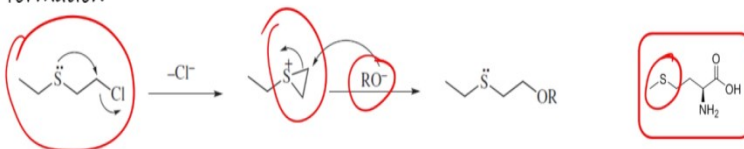


So as we have looked at there are number of naturally occurring amino acids and there are few amino acids which have nucleophilic groups such as a thiol or alcohol ok. So these nucleophilic

groups can participate in nucleophilic substitution reactions or nucleophilic addition reactions and these can enable acceleration in rate of a reaction. Ok so this concept is known as nucleophilic catalysis and it is a subset of covalent catalysis which involves the formation of a covalent bond ok.

(Refer Slide time: 11:47)

Anchimeric assistance is the process by which a neighboring functional group assists in the expulsion of a leaving group by intermediate covalent bond formation



*If the sulfur atom were part of an active-site nucleophile, such as a **methionine**, and the C-Cl bond were part of a substrate, and RO- were HO- generated by enzyme-catalyzed deprotonation of water, this would represent covalent catalysis in an enzyme-catalyzed reaction, where the covalent adduct is represented by the **episulfonium** intermediate.*



In order to understand this better let's look at an example of the reaction of this thioether. So in organic chemistry we are all familiar with the concept of neighboring group participation also known as Anchimeric assistance. So what happens in neighboring group participation is that a neighboring group carries out or a neighboring group facilitates a reaction by changing the path way which is taken for a reaction to occur and a product to be formed ok.

So here you can imagine that reaction of an alkoxide with this alkyl chloride which is normally an extremely slow reaction is dramatically accelerated when you have a neighboring group participation. So here this lone pair on the thioether can move in here kick out a chloride and produce a highly reactive episulfonium ion intermediate which then is opened up by alkoxide to give you the product. So when this type of catalysis occurs what in essence happens is that the path of the reaction is actually changed ok and these results in acceleration. So one of the amino acids that can be involved in this type of term a covalent catalysis is methionine.

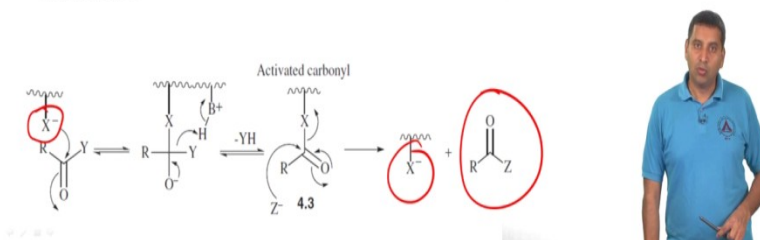
As you can see methionine has a thioether functionality and once it attacks in this case it would be it reacts with a substrate it produces an intermediate which is highly reactive and that covalent

adduct up can now be opened up by let's say an alkoxide or water to give you the final product ok.

(Refer Slide time: 13:33)

Covalent Catalysis

- Some enzymes use nucleophilic amino acid side chains or cofactors in the active site to form covalent bonds to the substrate; in some cases, a second substrate then can react with this enzyme-substrate intermediate to generate the product.
- This is known as nucleophilic catalysis, a subclass of covalent catalysis that involves covalent bond formation as a result of attack by an enzyme nucleophile at an electrophilic site on the substrate.



So in this case so we can look at the formation of an amide bond here you have X minus which can be a cysteine or serine reacting with an amide to form this is generic reaction of carboxylate group so here the X minus attacks the C double bond O produces a tetrahedral intermediate and now this tetrahedral intermediate is actually activated and then subsequently attack by a nucleophile can give you back the reactant and give you a different product.

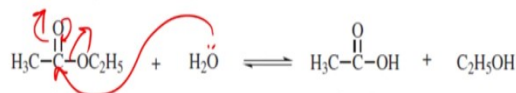
So this can for example be a transesterification reaction or even amide formation or amide hydrolysis type of reaction can occur through this type of mechanism ok. So covalent catalysis is very important mechanism by which enzymes can accelerate a reaction.

(Refer Slide time: 14:27)

Specific Acid-Base Catalysis

If catalysis occurs by a hydronium (H_3O^+) or hydroxide (HO^-) ion and is determined only by the pH, not the buffer concentration, it is referred to as specific acid or specific base catalysis, respectively.

Consider the hydrolysis of ethyl acetate



This is an exceedingly slow reaction at neutral pH because both the nucleophile (H_2O) and the electrophile (the carbonyl of ethyl acetate) are unreactive. The reaction rate could be accelerated, however, if the reactivity of either the nucleophile or the electrophile could be enhanced.



The third major way in which catalysis can occur is through acid-base catalysis. As we all know, amino acids have both acids in the form carboxylic acid as well as a base in the form of amines ok and in the broad terminology we have lewis acid as well as bronsted acids but suffice to say anything that can donate a proton is considered an acid and anything that can donate a lone pair is a considered a base.

So with that definition, we can look out acid-base catalysis and there are (two) generally speaking there are two forms of acid-base catalysis, one is called a general acid-base catalysis and the other one is called a specific acid-base catalysis ok. So let's look at the hydrolysis of ethyl acetate as an example so ethyl acetate if you put it in water, water is being a nucleophile can attack and produce acetic acid and ethanol ok.

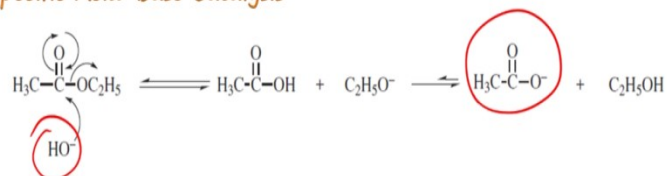
Now in the case of first example that we look at is specific acid-base catalysis here if the catalysis occurs by hydronium ion or by hydroxide then the rate of a reaction is determined only by the pH and not by the buffer concentration and therefore any form of catalysis by hydronium or hydroxide but not the buffer concentration is referred to as specific acid or specific base catalysis respectively.

So let's look at the example of hydrolysis of ethyl acetate, so this is extremely slow reaction and what happens is that water attacks here and generates tetrahedral intermediate and then the

tetrahedral intermediate collapses and kicks out ethoxide ion which can in turn pick up a proton from acetic acid and give you ethanol. So this reaction is very slow right, but one could think of accelerating this reaction by either by one can think of increasing the rate of a reaction by increasing the reactivity of either the nucleophile or the electrophile.

(Refer Slide time: 16:43)

Specific Acid-Base Catalysis



An increase in the pH increases the concentration of hydroxide ion, which is a much better nucleophile than is water, and, in fact, the rate of hydrolysis at higher pH increases

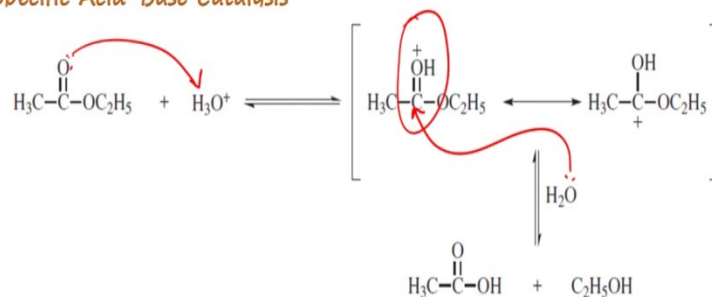


So this can be achieved by converting water to hydroxide. So hydroxide is a better nucleophile when compared to water and therefore just increasing the pH of the reaction can increase the rate of the reaction. So what we have done here is converted water the previous nucleophile to hydroxide and now hydroxide can come and attack form the same tetrahedral intermediate and kick out ethoxide and ethoxide and then subsequently it equilibrates to form acetate and ethanol ok.

So indeed when we carry out hydrolysis of ethyl acetate in basic pH we do find very high rates of reactions.

(Refer Slide time: 17:28)

Specific Acid-Base Catalysis



Likewise, a decrease in the pH increases the concentration of the hydronium ion, which can protonate the ester carbonyl, thereby increasing its electrophilicity, and this also increases the hydrolysis rate



Similarly we you lower the pH that is increased the concentration of hydronium ion you also find an increase in the rate ok and the way we understand this is that when you have hydronium ion the lone pair and the carbonyl can react with H_3O^+ plus and form a protonated carbonyl intermediate which offcourse exists in this resonance form but now because we are generating this very highly reactive protonated carbonyl intermediate the attack of the nucleophile at which case it is water happens much faster and now the reaction rate actually ends up going up ok.

So therefore acid-base catalysis, here is a concept where we would increase the reactivity of nucleophile or increase the reactivity of electrophile by simply protonation or de-protonation reaction ok.

(Refer Slide time: 18:27)

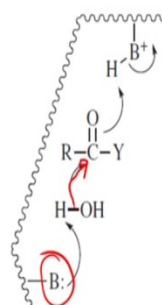
- That being the case, then the hydrolysis rate should be doubly increased if base and acid are added together, right?



Now so therefore in principle if I add both acid and base into a reaction medium, I should be able to increase the rate correct. However in reality that does not occur because what happens is that acid-base reactions are very fast and therefore you will have neutralization and you will lose all any potential for catalysis.

(Refer Slide time: 18:49)

- Unlike reactions in solution, however, an enzyme can utilize acid and base catalysis simultaneously
- The protonated base is either an acidic amino acid side chain or a basic side chain in the protonated form, and the free base is either a basic residue or an acidic residue in the deprotonated form.



It is important to appreciate the fact that the pKa values of amino acid side chain groups within the active site of enzymes are not necessarily the same as those measured in solution.



Now in the context of an enzyme the situation is very different ok, so in the enzymes since we looked at how the proteins fold up and there are certain residues which are present in specially

in a particular area those can facilitate protonation or de-protonation which results in acceleration of rate of reaction. So here is an example of a relay type of mechanism, where this base here can donate a proton to water in which it will generate hydroxide ion which can then attack carbonyl and which in turn pick up a proton from another base from another acid and produce the base ok.

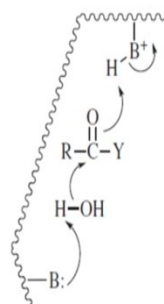
So here is an example of or a hypocritical example of a relay mechanism and since enzymes have very hydrophobic cavities and there are residues there which can be in both protonated as well as de-protonated forms this type of mechanism is possible ok. We need to understand here we are all familiar with the concept of pKa which is a measure of how strong a particular acid is, but we need to appreciate that pKa measurements are typically or always carried out in water ok and once we change the medium the pKa of a particular group can change.

So the way we understand is pKa is that the pKa would be lower that means it will be a better acid if the conjugate base can be stabilize better ok. However in an acid in an enzyme maybe a hydrophobic cavity the conjugate base can be stabilized in a different way in a more perhaps sometimes which can result in a better acid or it can be de-stabilized making the better acid actually a worst acid. So this are a little difficult to understand conceptually however it has been established the pKa values of amino acids very quite a bit in proteins. So this is something that we need to be sensitive about ok.

(Refer Slide time: 21:02)

It is important to appreciate the fact that the pKa values of amino acid side chain groups within the active site of enzymes are not necessarily the same as those measured in solution.

pKa values can change drastically in hydrophobic environments. Therefore, removal of seemingly higher pKa protons from substrates by active-site bases may not be as unreasonable as would appear if only solution chemistry were taken into consideration.



In within the active site therefore it is possible that you can have a weaker base picking out a proton from a stronger base or vice versa ok, so therefore, it is not unreasonable to expect that certain reactions will occur which would never happen in solution chemistry. So that is one of the interesting and important things about enzymes.

(Refer Slide time: 21:32)

General Acid-Base Catalysis

General acid catalysis occurs when acids other than hydronium ion accelerate the reaction rate. Similarly, general base catalysis occurs when bases other than hydroxide ion accelerate the rate.

In solution, general acid-base catalysis can be demonstrated when the reaction rate increases with increasing buffer concentration at a constant pH and ionic strength, and shows a larger increase with a buffer that contains a more concentrated acid or base component.

In enzyme-mediated catalysis, general acid-base catalysis occurs when an acidic or basic residue at the active site is used to facilitate proton transfers in the reaction.



The second form of catalysis is general acid-base catalysis. So here we define general acid catalysis occurs when acids other than hydronium ion accelerate the reaction rate ok and similarly general base catalysis occurs when base is other than hydroxide accelerate the rate ok. So inside an enzyme for example the amount of free water that is present is generally very low and therefore we would have other sources of acid and base which can do the protonation and de-protonation.

Now how does one understand or how does one distinguish general acid-base catalysis from specific acid-base catalysis. So we looked at previously specific acid-base catalysis if I increase the pH the rate of the reaction goes up and if I decrease the pH the rate of the reaction goes up. So therefore in specific acid-base catalysis the reaction rate is dictated exclusively by the pH of the media. In general acid-base catalysis even if the pH is the same but if you increase the buffer concentration and if you observe that the rate of the reaction goes up then we would conclude that general acid-base catalysis is operational ok.

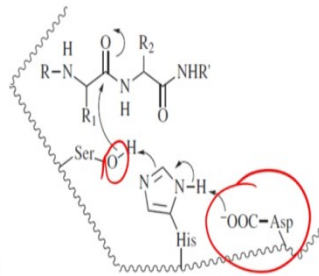
So at constant pH as well as ionic strength if you have an acceleration in the rate right, by just by increasing the buffer concentration you have acid-base catalysis. Enzymes mediated catalysis are general acid-base catalysis reactions ok and here as mentioned earlier acidic or basic residue at the active side is used to facilitate the proton transfer. If you recall we were looking at the relay mechanism where a base can transfer a proton to the other end of the active side by just a relay mechanism ok.

(Refer Slide time: 23:45)

Chymotrypsin

The generally accepted mechanism of action of this enzyme, which features nucleophilic attack by a serine hydroxyl group onto the carbonyl group of a peptide bond

Although the serine hydroxyl group is not normally regarded as a potent nucleophile, aspartic acid and histidine residues nearby have been implicated in the conversion of the serine to an alkoxide by a mechanism called the charge relay system



So let's look at example of Chymotrypsin, so chymotrypsin is a enzyme that cleaves peptide residues ok, so here this aspartic over here which is in a de-protonated state can pick up a proton from histidine and histidine is one of the functional groups which can act as a acid as well as a base depending on the context ok. So when here it is you can imagine that the carboxylate picks up a proton from histidine and this proton in turn and this histidine in turn can pick up a proton from serine and now the serine residue is actually the alcohol in a serine is actually made alkoxide which is an extremely good nucleophile and that attacks an amide and hydrolysis it.

So this relay charge relay mechanism has been really well worked out and you have what is known as a catalytic triad which does this then takes care of this catalysis ok.

(Refer Slide time: 24:46)

Acid-Base Catalysis

- This catalytic triad involves the aspartate carboxylate (pKa of the acid is 3.9 in solution) removing a proton from the histidine imidazole (pKa 6.1 in solution) which, in turn, removes a proton from the serine hydroxyl group (pKa 14 in solution).
- One explanation could be that the pKa values of some of these acids and bases at the active site are different from those in solution.



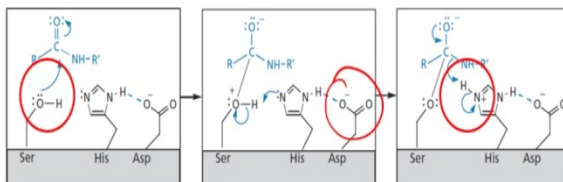
So the catalytic triad is nothing but aspartate, carboxylate, histidine which has an imidazole group and serine ok. But if you look here the aspartate carboxylate acid the pKa is 3.9 in solution which is going to remove a proton from histidine imidazole whose pKa is 6.1 which in turn removes a proton from hydroxyl group of serine whose pKa is 14.

So any well respected any good organic chemist who would never agree with this kind of a reaction this kind of a mechanism that is operational in solution, however it can occur in an enzyme ok. One of the explanations is what we alluded to earlier was that inside an enzyme or inside a protein the pKa values of this acids and bases can be vastly different.

(Refer Slide time: 25:42)

Acid-Base Catalysis

- A full deprotonation is likely not occurring; rather, because these groups are held close together at the active site, as the proton is beginning to be removed from the serine hydroxyl group, the charge density proceeds to the next step

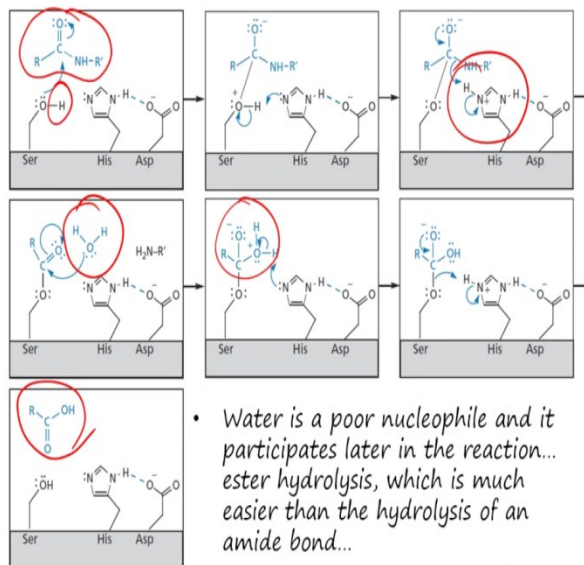


The approximation of the groups and the fluidity of the active-site residues working in concert permit reactions to occur that would be nearly impossible in solution.



The second explanation is that it is likely that the full protonation or de-protonation does not occur. So you can imagine that this lone pair of electrons from serine is attacking here and this serine residue is not completely de-protonated whereas it is partially de-protonated, Whereas the aspartate over here is pulling the electrons from histidine and there as well it is not completely de-protonated or protonated in that case and so in this realm one can imagine that there is a differential distribution of charged density which helps or facilitates the reaction. So here a combination of approximation of the groups which is present which is basically bringing this groups in proximity to one another which is impossible in solution occurs which can change the which has a tremendous impact on the rates of reactions ok.

(Refer Slide time: 26:43)



To complete this reaction mechanism, serine attacks the amide and this proton of serine is then picked up by histidine and since histidine is a good acid-base catalyst histidine is a very good imidazole is a very good acid-base catalyst it can in turn donate a proton to aspartate or it can form a weak interaction with aspartate and now water attacks this now which is now an ester water attacks this ester forming the tetrahedral intermediate which then subsequently undergoes decomposition to produce a free carboxylic acid.

So that the mechanism the reaction basically is the conversion of an amide to a carboxylic acid and it is catalyzed by these three residues and now as we can see this three residues are recharged to do to carry out this reaction once again ok.

So hydrolysis on an amide bond is extremely difficult to carry out in solution and you need a number of reagents to do that but in presence of this enzymes such as chymotrypsin one can carry this out with relative ease.