ENZYME SCIENCE AND ENGINEERING

PROF. SUBHASH CHAND

DEPARTMENT OF BIOCHEMICAL ENGINEERING
AND BIOTECHNOLOGY
IIT DELHI

LECTURE – 4

ENZYMATIC CATALYSIS

We will continue today our discussion on enzymatic catalysis and particularly the ways in which the enzyme can lower the free energy of activation of a chemical reaction it catalysis.

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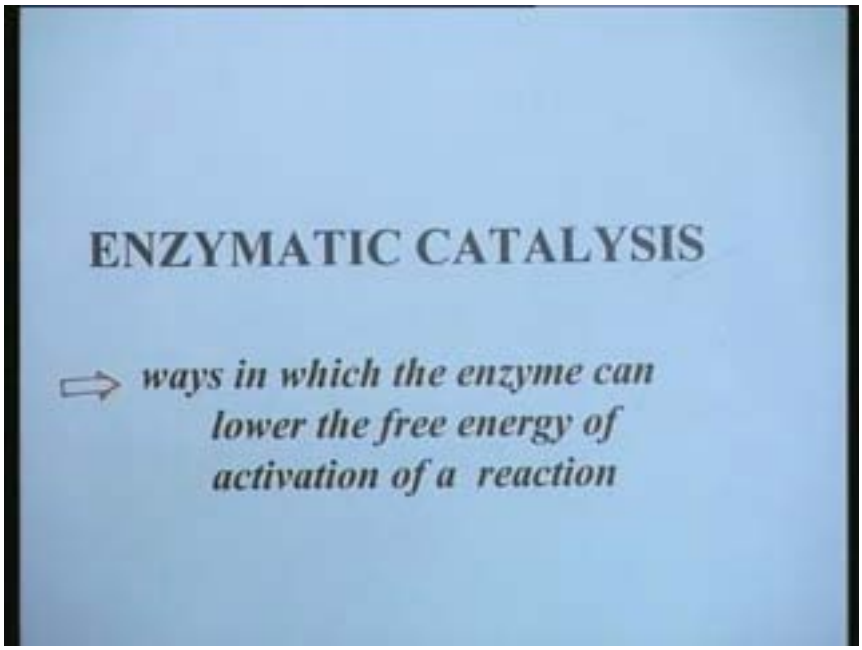

In our earlier lecture we have noted that enzymes can lower the free energy of activation of a reaction very effectively as compared to chemical catalyst. However this catalytic efficiency is attributed to two major molecular strategies that we had discussed earlier and they are binding of the substrates to the enzyme on the active site mainly the major play ground here is active site present on the enzyme molecule.

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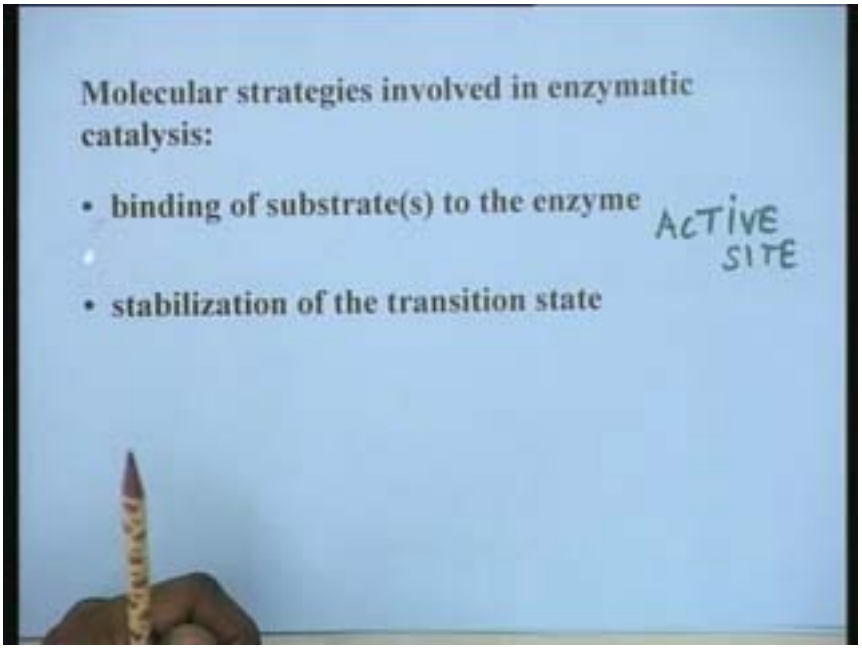

On the other hand the stabilization of the transition state of the chemical reaction which is the desired reaction which you want to catalyze. These are the two molecular strategies which are responsible for the catalytic efficiency of the enzyme. Here I like to draw your attention about active site. You see in both cases the active site on the enzyme molecule play a very key role in the variety of functions or variety of mechanisms that we will discuss today. Before we go in to main mechanism I like to summarize some of the characteristic features of enzyme active sites at least the major one which may be of interest in today's lecture.

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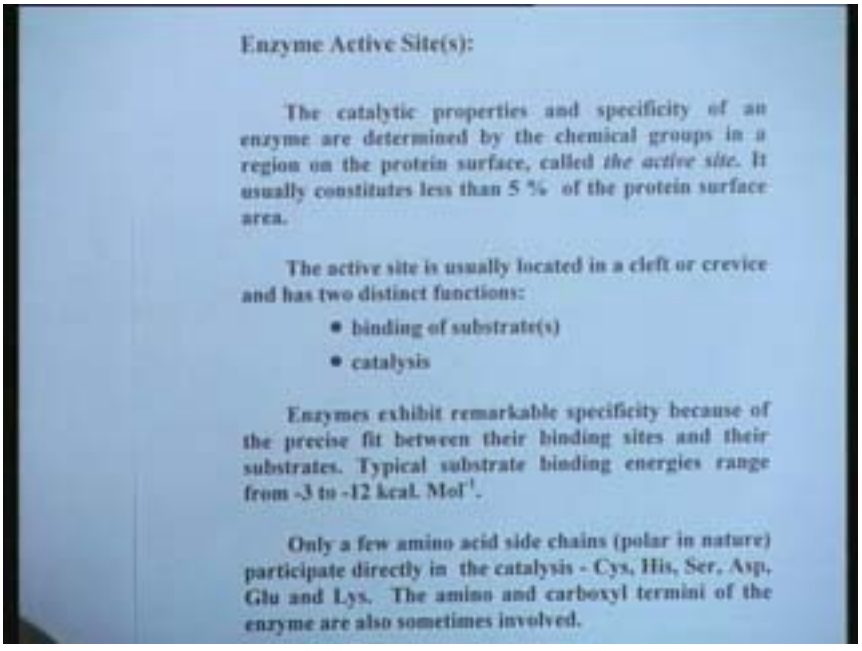

The enzyme active sites determines the catalytic properties and specificity of the enzyme and these properties are determined by the chemical groups attributed by the amino acids present on the particular small region what we understand as active site. This region, the so called active site may constitute less than 5% of the total surface area present on the enzyme protein.

So it's a very small area on the total enzyme surface and rest of the enzyme surface either in its proximity provides some favorable reactions or very often it may be considered as only a sort of building framework structural feature which creates the active site. The creation of active site on the surface of an enzyme molecule is brought about by tertiary folding of the enzyme protein. All the enzymes we talk about are all globular proteins, structural proteins are not involved in catalysis and therefore the rest of the amino acids by virtue of their folding pattern creates certain niches, certain crevices, cleft on the surface of the molecule which acts as active site and the functional groups attributed by the amino acids in that cleft take key role in binding as well as catalytic process. The active site is usually located in a cleft or crevice.

Active site although present on the surface has its own three dimensional entity. It has two distinct functions binding of the substrates and catalysis and both the functions are a result of the amino acid residues present there primarily and then the shape, which is brought about by the conformation.

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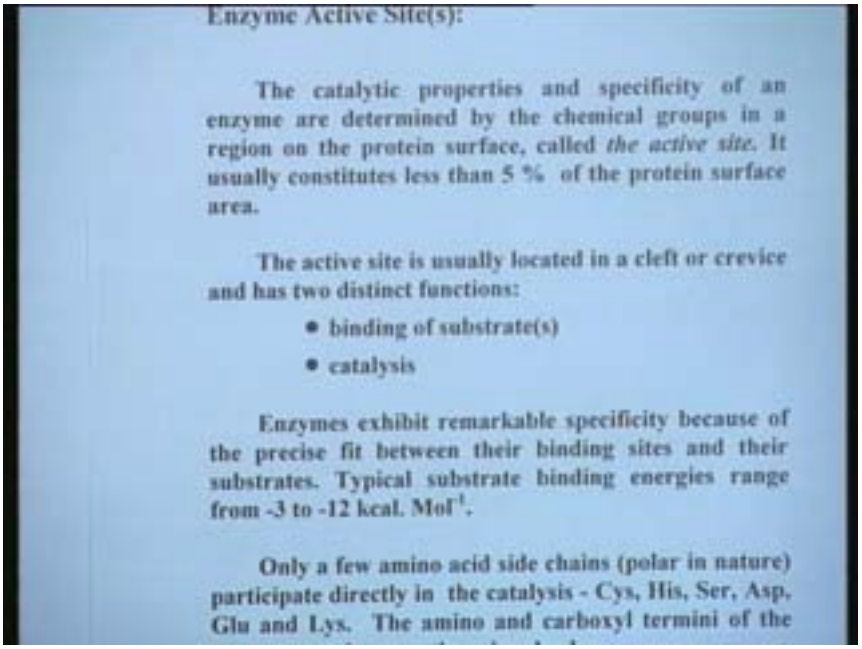

The enzymes exhibit remarkable specificity because of their precise fit between their binding sites and their substrate. Considering the active site it is a combination of two functional entities: one is the binding site, substrate binding site plus the catalytic site. Enzyme protein, as some of you may be aware, has enough number of ligand binding

sites. Substrate binding sites are one of them. The active site consists of the substrate binding site plus catalytic site. It does not catalyze the reaction it will only remain as a ligand binding site.

Typical substrate binding energies range from 3-12 kcal mol^{-1}. That is the order of magnitude which the binding energy goes in the binding of the substrate. Another feature is that not all the twenty amino acids that are present in protein are involved in active sites. Just by observation of the variety of enzymes which have been studied so far, it is noted that only a few amino acids usually polar in nature they participate directly in catalysis - cystein, histidine, serine, aspartic acid, glutamic acid and lysine. You can see that all of them are polar in nature and these are the usual amino acids which constitute the primary functional groups on the active sites.

In some cases the terminal carboxylic and amino groups are also involved whenever by tertiary folding they come in the proximity of the active site. Not that on the terminal side if they are far away from active site they will play a role. Once by folding process if they come and become a part of the active site, the amino and carboxylic groups are also important in the catalytic function but again they are also polar in nature.

By meaning that they participate directly in the catalysis I mean that they are present at the active site. The involvement of certain amino acids at the active site is a result of the whole folding process in which all amino acids are involved. So while we say that these are amino acids that are present on the active site and they participate in the catalysis does not mean that the other amino acids which are playing a role in the folding process have no relevance. If one of the amino acids is transferred or removed the whole folding will change and primarily involved means those which are physically present on the active site. But their presence in the active site may result from the whole composition of the protein as well as the folding process.

Another feature which we need to keep in mind is that certain enzymes require cofactors. The precise reason for that is amino acid side chains cannot catalyze all the chemical reactions. That is one of limitations although the protein design or the enzyme design has been so beautifully evolved that a large variety of reactions can be catalyzed but still there are limitations.

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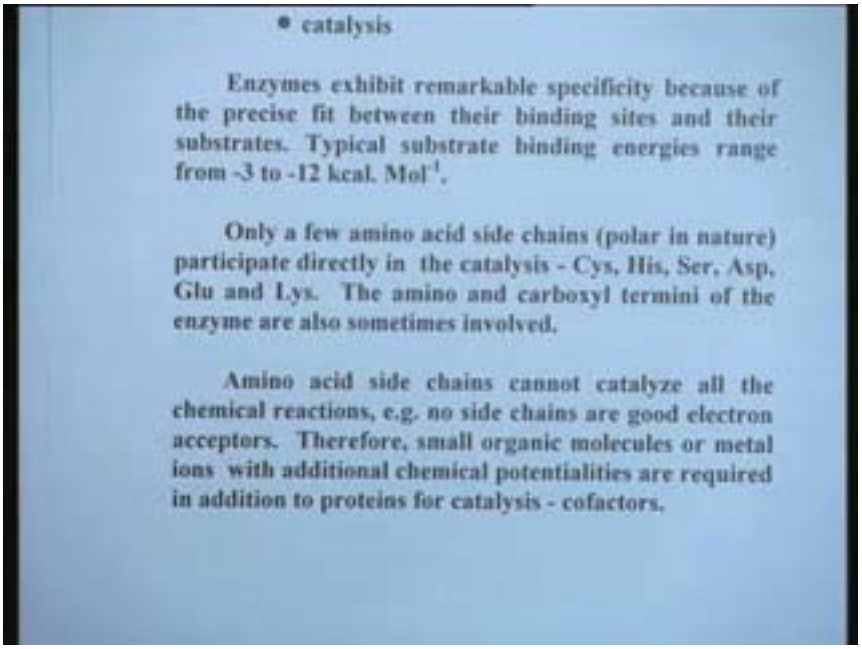

One of that is that the available twenty amino acids are not able to catalyze all the reactions. The limitation is on the electron acceptors. None of the amino acids are good electron acceptors and many reactions which are primarily based on electron acceptors require additional input in the form of cofactors. For example the cofactors like NAD, NADP, they are all ideal electron acceptors and they participate in the catalytic process simply by oxidation, reduction or whatever mechanism is involved. Therefore small organic molecules or even metal ions provide electron acceptor or some other chemical potentialities in addition to proteins for catalytic function. So that is one limitation that while most of the reactions can be catalyzed by the amino acids present on the active site, certain reactions which are not possible to be catalyzed by this can be carried out by cofactors and that provides a key role for catalytic function.

Just to recall our earlier discussion on the previous day we mentioned that the energy of activation E^* can be broken down into two components that is your enthalpy contribution as well as entropic contribution.
We can break down the reaction and the classical Arrhenius equation, the expression for rate constant can be written as

$$k = RT/Nh \exp(\Delta S^*/R) \exp(-\Delta H^*/RT)$$

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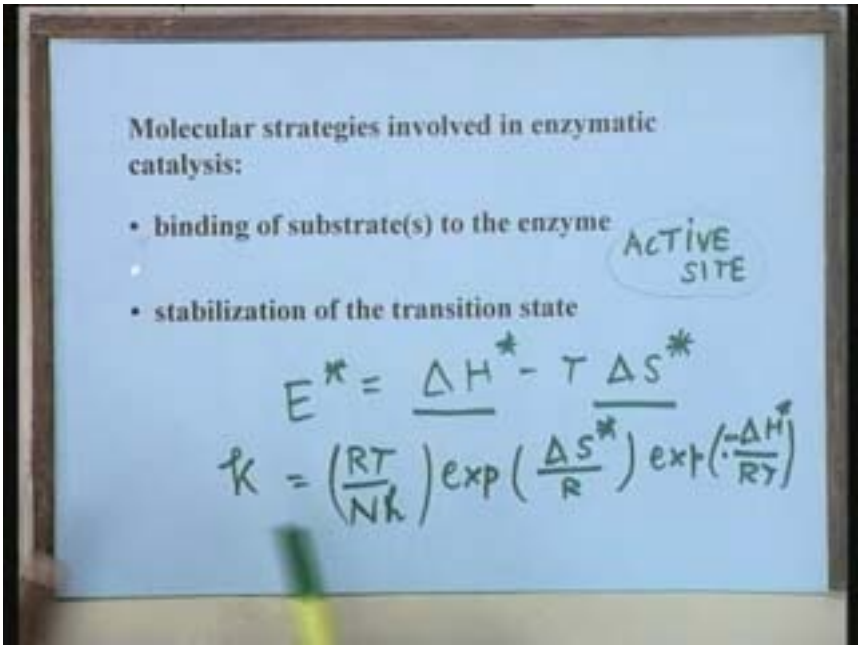

The rate enhancement process consists of two different components or the energy of activation consists of enthalpic as well as entropic contribution. So by any means if the enzyme can either reduce the enthalpic contribution to the free energy of activation or entropic contribution, the net result will be a catalytic function. While considering the various ways we will consider the lowering of energy of activation through each of the contributions and first we will see lowering of energy of activation through entropic contributions.

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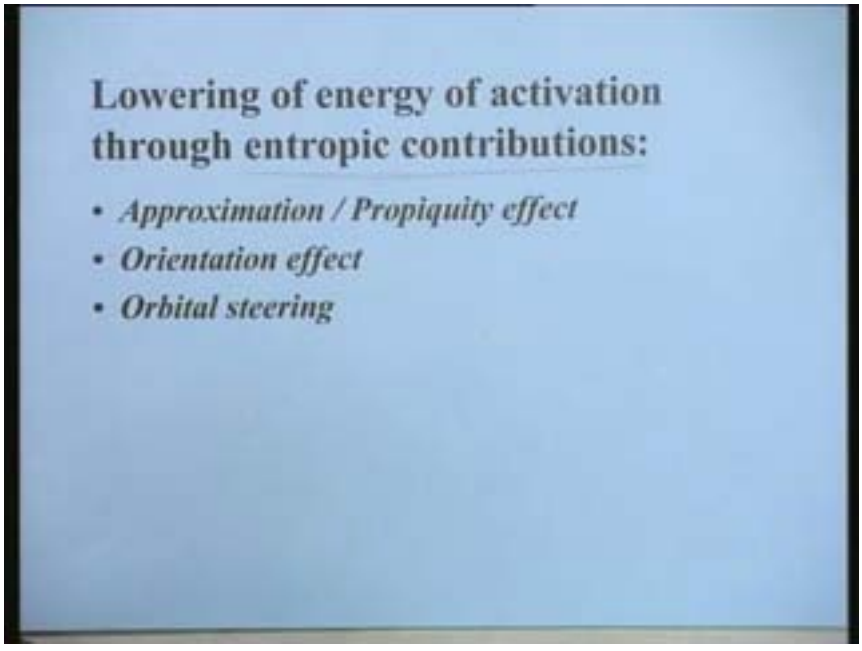

Consider a bimolecular reaction in which A and B participate to from AB^* as a transition state. The entropy of these molecules will consist of translational as well as rotational degrees of freedom. Three degrees of translational freedom and three rotational for each of the molecule A and B. The two molecules will have six degrees of freedom.

Once the reactants A and B bind to an enzyme molecule at the active site there are only half of the their degree of freedom that means they become one entity and they lose six of their degrees of freedom and thereby there is a loss of rotational and translational entropy and the transition state will have only three degrees of rotational and three degrees of the translational freedom each.

You must understand that the net result of binding of the two reactants to the active site involves in reducing the degrees of freedom of the two reactant molecules and therefore there is a loss of entropy. In the case of A and B acting in the absence of any catalyst, in our case we are considering enzyme as catalyst, when the transition state compound is formed there is a loss of entropy. Now this loss of entropy is accounted for in the energy of activation. Whereas in the case of enzymatic catalysis the loss of entropy as a result of combining into one entity is attributed in the binding process which are already taking place in the binding process.

When the enzyme substrate complex forms a transition state there is no loss of entropy affecting and the loss of entropy as a result of enzyme binding compensates for the reduction in the free energy of activation. This binding energy can be of the order of about 10-15 $kcalmol^{-1}$ at 25°c and if you take the two reactants at one molar concentration, the computation will tell that it will be about 10-15 $kcalmol^{-1}$. So simply by entropic contribution of binding the enzyme can reduce the energy of activation by about 10-15 $kcalmol^{-1}$.
Just to repeat, we should consider two different cases. In one case A and B are present in a solution in which there is no catalyst. Ultimately for the reaction to take place they have to pass through a transition state. This A and B both have to colloid and make an unstable compound. It will lead to loss of entropy and this loss of entropy will then be accounted for in the energy of activation. This loss of entropy has to be provided in terms of the energy of activation. But when you talk about enzymatic catalysis this loss of entropy is compensated by binding process. That means whatever loss of entropy is there in the binding process that is not to be provided in the energy of activation. There will be loss of entropy but that will result in the range of energy during binding process. Whereas from the bound enzyme substrate complex to go to the transition state there will be no loss of entropy and that entropy gain will account for advantage in the entropic contribution to the energy of activation and this is one of the major contribution in reduction and activation energy as a result of substrate binding.

One point must be kept in mind that such an advantage due to entropic contributions for the energy of activation will be more pronounced if they are multi substrate reactions. If suppose there is a unimolecular reaction one may say that there is hardly any advantage. There are hardly any unimolecular reactions as far as an enzyme is concerned expecting

certain isomerases because most of the reactions are bi or trimolecular. There are some, but not many in numbers. In the case of unimolecular reactions this entropic contribution will not be very significant. There will be another mechanism which will play a role. The reduction in the activation energy is not through one mechanism. There are various mechanisms and the net gain is sum total of the kind of mechanism that are involved in specific enzymes and in case of unimolecular reactions the entropic contribution may be quite insignificant.

The binding of the substrate to the enzyme molecule is not a process which is within the purview of activation or transition state. The enzyme substrate complex must not be confused with a part in the transition state complex. It is a totally different process which, particularly the enzyme substrate complex is a result of a large number of non covalent interactions between enzyme and substrate, hydrogen bonding or Vander Waal's forces. Whereas from the enzyme substrate complex to transition state is a totally different phenomenon and the energy required to go from an enzyme substrate complex to transition state is the amount of activation energy required for the reaction. The binding energy is a totally different game and therefore that should not be confused. Very often we might make a judgement that the energy which has gone into enzyme substrate binding is not a part of activation energy, is not required for catalytic process, for activation energy to be equalized.

This effect of entropic contribution to the energy of activation is called approximation effect or propinquity effect.

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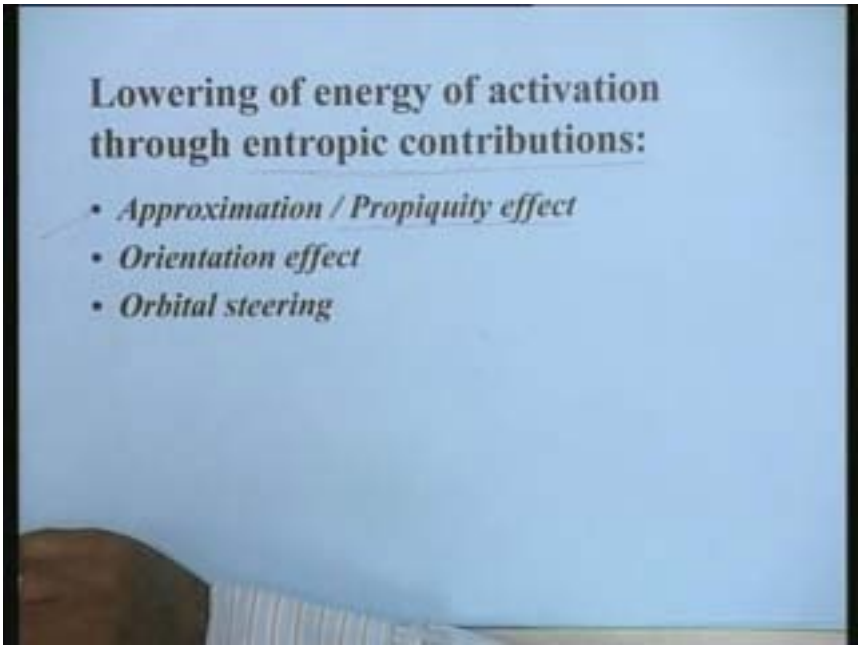

You bring the reactants into to a close box so that they act as one and they function. As a result of this approximation one can also consider another feature. When the substrates

are bound on the active site the effective local concentration of the two reactants in the active site will also be much larger than the concentration in the free solution. The substrate is now localized for the reaction at the active site whatever is the volume of active site in a given enzyme molecule. This concentration of the reactant at the active site will be many fold higher compared to the actual concentration in the bulk phase in the aqueous medium in which the substrates are dissolved. The higher concentration of the substrate at the active site means that the enzyme provides a new micro environment in which the substrate concentrations are much higher than in the bulk and you get the net effect of higher substrate concentration on the reaction rate. Whatever mechanisms you can use to increase the frequency of collision, it could be the increase in reactant concentration, it could be temperature change, they will all result in increased reaction rate.

The net result of substrate binding to the active site which will lead by the approximation or propinquity effect into to a higher substrate concentration will also lead to an increased catalytic rate. The approximation or propinquity effect will lead to benefit in the reaction rate, one by a reduction of energy of activation as a result of entropic contribution and the other is as a result of apparent higher concentration at the active site. Here also the same situation will apply that these effects of approximation will not be very, very significant if you are talking about unimolecular reactions.

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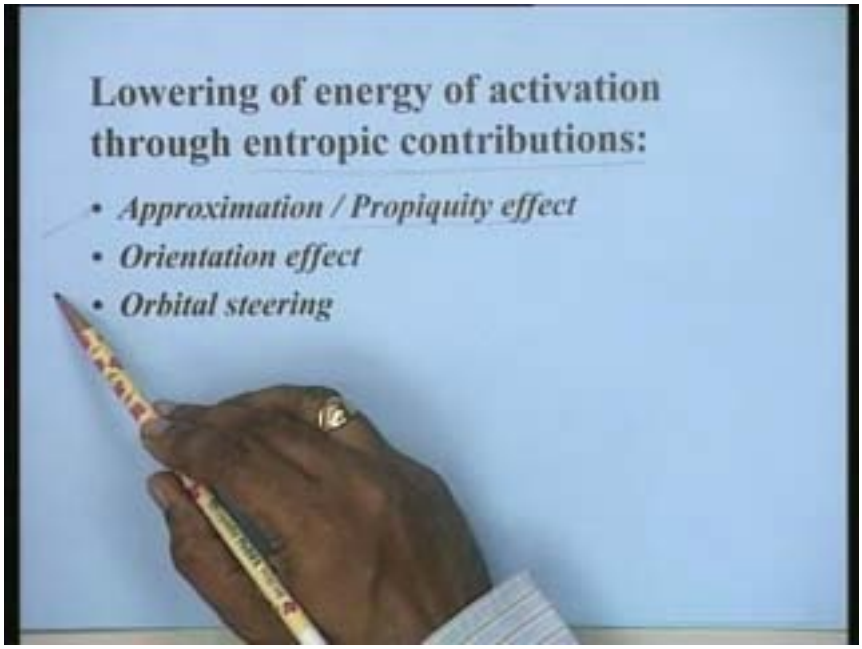

The other effect based on the entropic contributions is orientation effect. While we discussed reaction rate theory we noted that collision of the molecule is a pre requisite for reaction to take place. Not all the collisions result into product. One of the reasons we considered was that the molecules have to posses a certain minimum energy, potential

energy so as to reach to the level of energy of activation before they can break down into product from the unstable state.

Another reason of the non release of product from collisions could be that the molecules during collisions may not be in the right orientation because orientation at the time of collision may also be significant in many cases and enzyme can play a key role. Substrate binding to the enzyme site on the active site may also play an important role in providing the substrate molecule in the right orientation. Orientation effect means the aligning of the substrate molecule on the enzyme active site in the right orientation which on collision will result into the product. In higher frequency large numbers of total molecules that are present participate in the reaction process, a feature which will result in higher reaction rate.

The three dimensional confirmation of the active site takes care of that. That means the evolving of the design of three dimensional structures to which this substrate molecule will fit will be exclusively specific for the orientation which is required for the catalytic reaction. The orientation could be in the form of certain functional group on the substrate molecule, could also be the correct alignment of the electronic orbitals of the colliding molecules and a small departure from the optimum alignment may result in lower reaction rate or when we say lower reaction rate, increased energy of activation. Theoretically this concept of orientation was considered that the enzyme is able to steer the reactants into a correct orbital alignment at the active site and therefore this effect was also called as orbital steering. The two phenomena of orientation effect and orbital steering are related.

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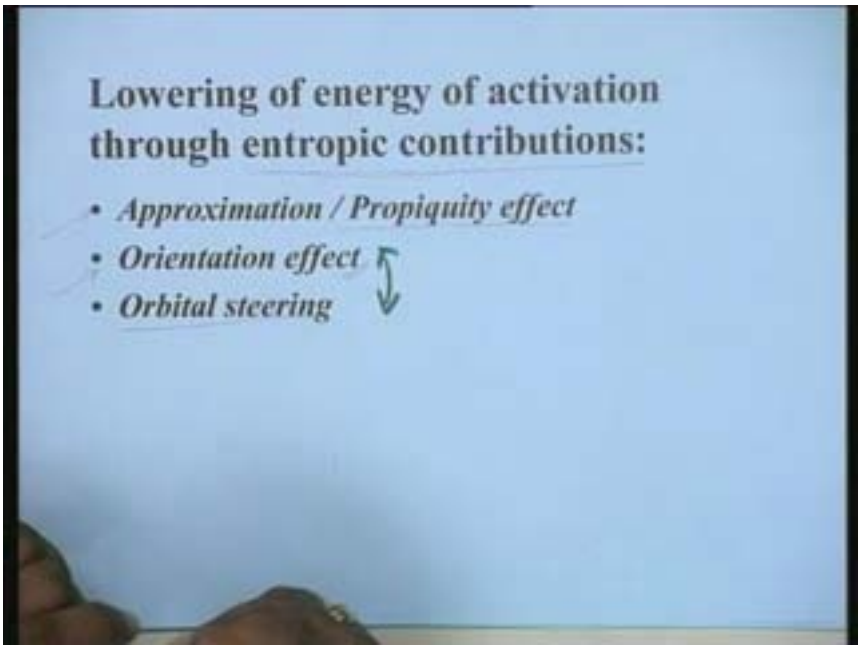

Orientation effect is a much broader term in the sense that it will include orbital steering as well as the aligning of certain functional groups on the substrate molecule but orbital steering is aligning of only electronic orbitals in a particular fashion at the site, which will result in the net catalytic function. Mainly these two behaviors, approximation or orientation effects are the major contributing factors in all catalytic reactions catalyzed by enzyme molecules. Another group of mechanisms that we will discuss are enthalpic factors.

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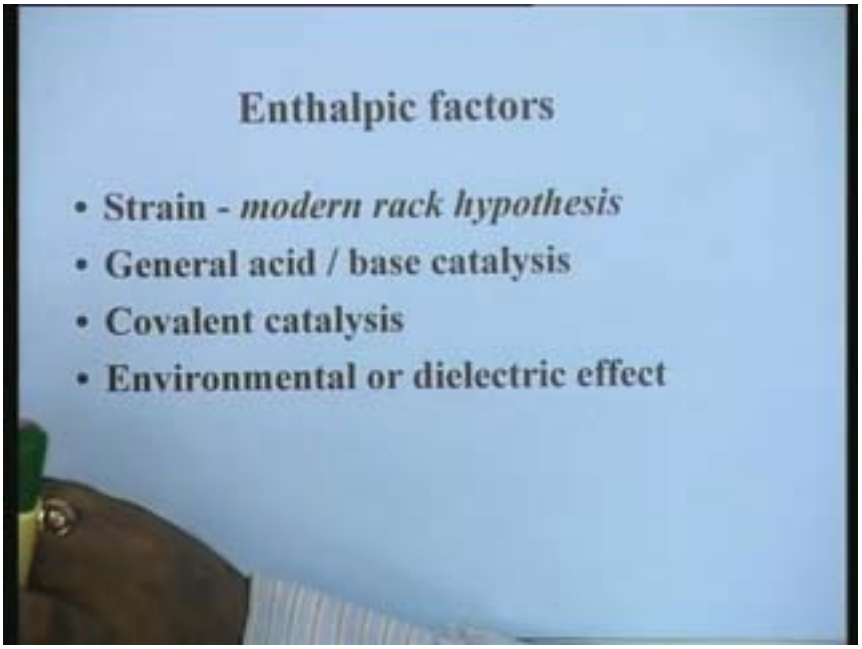

If you consider the whole energy of activation, part of it is contributed by entropic modifications and part by enthalpic contributions. If you just understand the enthalpic factors we must consider that the transition state is like a molecule with some of its chemical bonds in an unusually extended or strained form. They are unstable molecules. The bonds are partial bonds. They are not completely formed. Either they are quite weak bonds and either they are in process of forming or breaking somewhere in between. That is what the transition state is.

Any mechanism by which if you can take the reactant closer to a transition state not by formation of transition state any other mechanism which can do that will facilitate or reduce the energy of activation. If you look into the mechanism of substrate binding to an enzyme active site something similar happens. If during the binding process, the reactant or the substrate has to undergo some change in shape in order to fit very snugly into the active site there might be some distortion or strain on one of the bonds which is required to be broken. All these factors will depend on the design of the active site which the nature has evolved in the evolutionary process.

None of these active sites have been designed so for at least for commercial purposes or for practical purposes. There have been some academic efforts to take advantage of anti bodies to provide a catalytic function, to take advantage of stabilizing effect because antibodies have very high values of affinity to their substrate. The antibodies and enzyme molecules in terms of their dissociation constant for their counter part in the case of enzymes it is substrate in case of anti bodies, they are antigens. Their dissociation constant values are very, very small in the case of antigen antibody interaction. If similar forces are involved they can also act as much better catalyst theoretically. There have been efforts in which this has been successfully proven, of course in very specific cases. Not a very general behavior. Still we depend on the natural design on the enzyme active site for all practical purposes.

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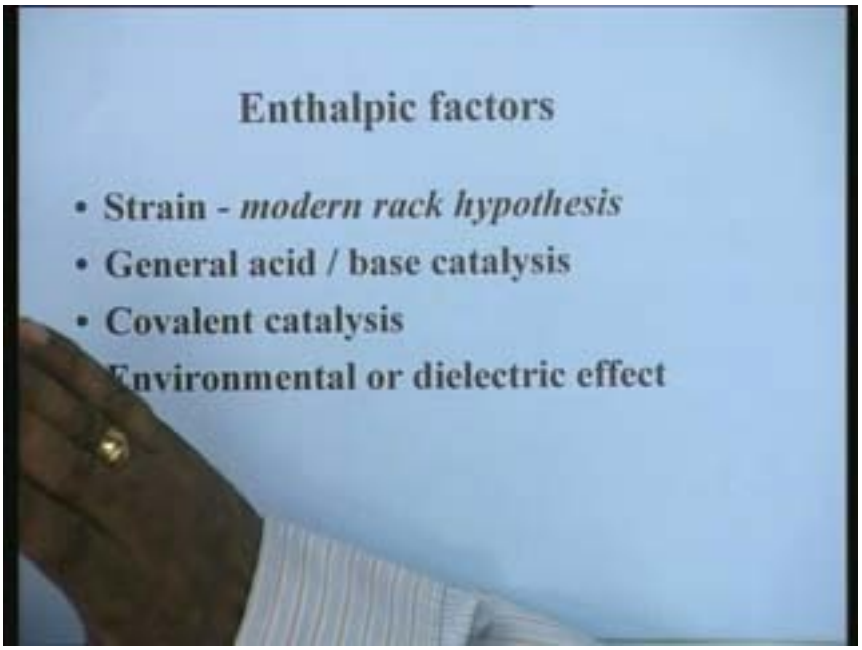

When you consider the strain or the distortion of the substrate molecule before it can go into the active site for binding purposes, if the desired bond which has to be broken during the reaction is partially strained or distorted then part of the job is already done. Energy of activation required for conversion of enzyme substrate complex to the transition state will be smaller.

Consider simply a hypothetical case in which we have enzyme active site shown by this x,z ellipse. A substrate molecule with a central carbon atom and a, b and c functional groups is present on this substrate molecule. These are supposed to bind with x, y and z which are the amino acids residues on the active site. We have considered that two of the functional groups on the substrate b and c are able to bind to y and z functional groups. But one of them, a is not easily accessible to x in the in the native form of the reactant. It has an attractive feature for binding purposes between a and x.

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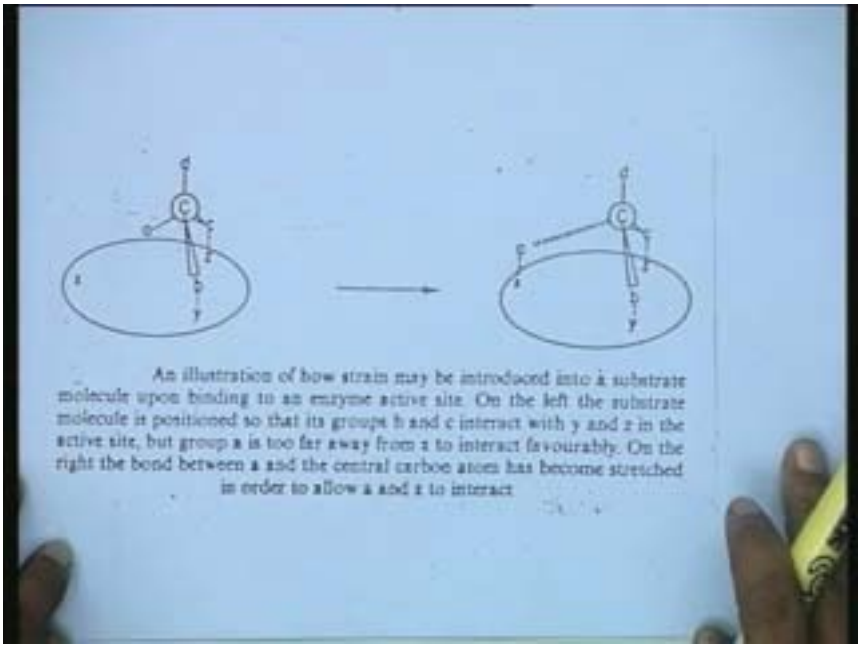

One of the mechanisms by which enzyme function is that the bond between the a and the central carbon atom gets distorted or strained, such that the binding gets facilitated to the x. That means that this bond is already now partially broken and if this molecule then goes to transition state the energy of activation required will be much less. In many cases such a thing happens and such a behavior is often called as modern rack hypothesis

Today it is proven by many experimental designs that partial breakage of the bond can take place on the enzyme site but in earlier days people have thought of the concept that enzyme tears off the whole substrate molecule which has proven today by modern rack hypothesis and which illustrates that the enzyme can accommodate a substrate in which one of the bond which is required to be broken for catalytic reaction to take place can get distorted partially during the binding process thereby reducing the energy of activation. If you consider even from energetic point of view let us say the binding process of these three residues requires 5 $kcalmol^{-1}$ for example and in the distortion process let us say already 2 kcal have been spent hypothetically. So for this binding of a to x only three are required. Instead 15 $kcalmol^{-1}$ involved in binding of the normal molecule for the three bonds, you will require only thirteen.

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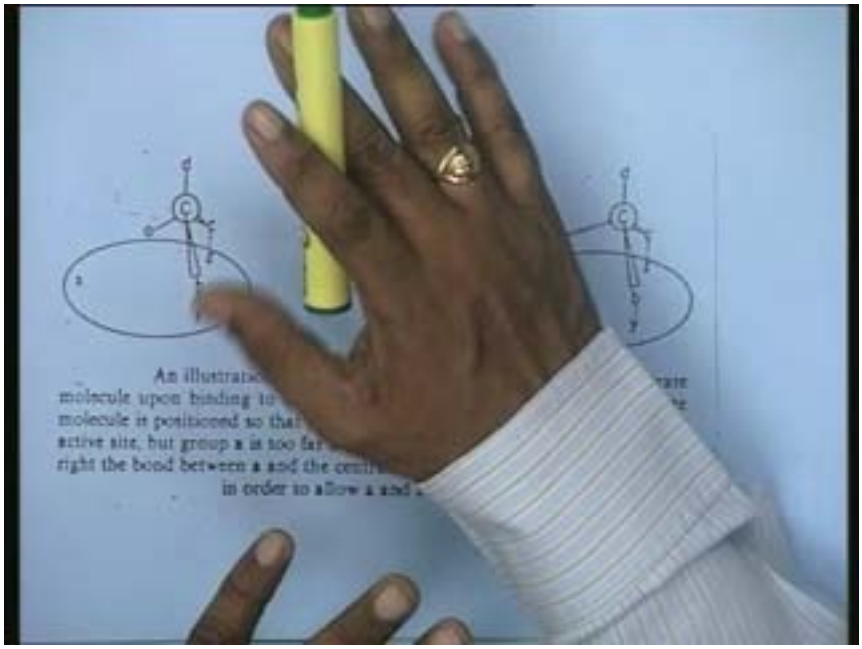

Therefore while going for the activated state, 2 kcal are already saved as a result of partial distortion and that is another mechanism what we call as modern rack hypothesis.

That is the component of the x y and z, are all on the active sites. Here if you consider x y and z are the three residues which are the part of the active site which will bind to a, b and c functional group on the substrate. Two of them are complementary and can easily bind without any problem. One of them is out of the way and requires some distortion. Enthalpic contribution might reduce energy by 2 kcals. The energy required for transferring the enzyme substrate complex to activated state will be reduced by 2 kcals because 2 kcals have already been consumed in the distortion process at the binding site. The binding energy should be distinguished from the energy of activation. Energy of activation is energy required for the catalytic part. Binding part whatever is consumed is different and that doesn't account for because we are talking only one phenomenon, the increase in the catalytic rate and that takes place as a result of decrease in activation energy.

The other mechanism by which the enthalpic factors contribute to reducing energy of activation is general acid base catalysis.

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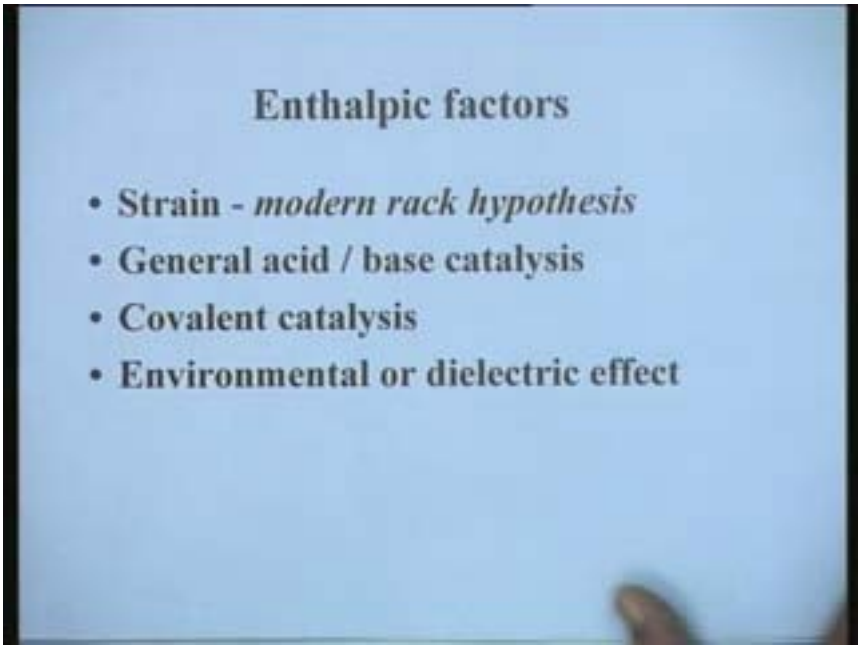

This is the mechanism which is very often employed even in the case of chemical catalyst. Many of the reactions can be catalyzed simply by acids or alkalis and the basic mechanism is that by certain transfer of electrons or protons to the reactants the catalyst can reduce the energy of activation.

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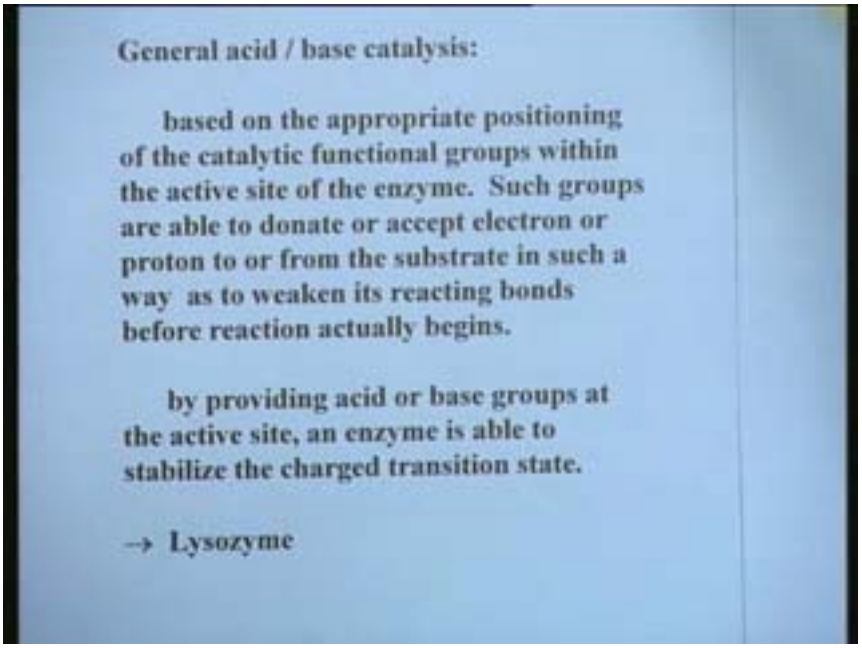

The basic principle of acid base catalysis is more or less identical. Only difference is that the role of acids or alkali in the chemical catalysis is played by the functional groups on the active sites. This is based on the appropriate positioning of the catalytic functional

groups within the active site of the enzyme. Such groups are able to donate or accept electrons or protons to or from the substrate in such a way that it weakens its reacting bonds before the reaction process actually begins. Again a similar theory just like in the case of the modern rack hypothesis we assumed that the binding process induces distortion on the reaction bond.

In the case here by the participation of the functional groups or the acids or basis we are weakening the bonds which are involved in catalysis. Therefore the energy of activation will be reduced by that amount. By providing acid or base groups at the active site an enzyme is able to stabilize the charge transition state.

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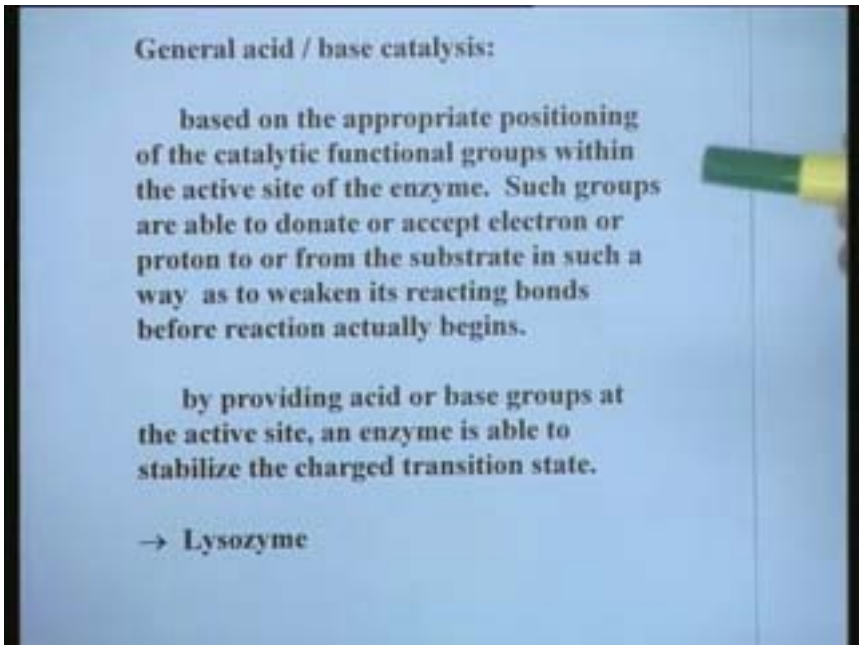

It is quite logical that in such cases it will be mostly the ionic species like glycine or glutamic acid or proteic acid will be the functional group that will be responsible for acid base catalysis. A very typical example in which acid base catalysis is predominantly found is the action of lysozyme. Lysozyme is an enzyme which breaks down bacterial polysaccharide by virtue of acid base catalysis predominantly.

Positioning of the functional residues at appropriate positions so that it weakens certain bonds can also be considered covalent catalysis.

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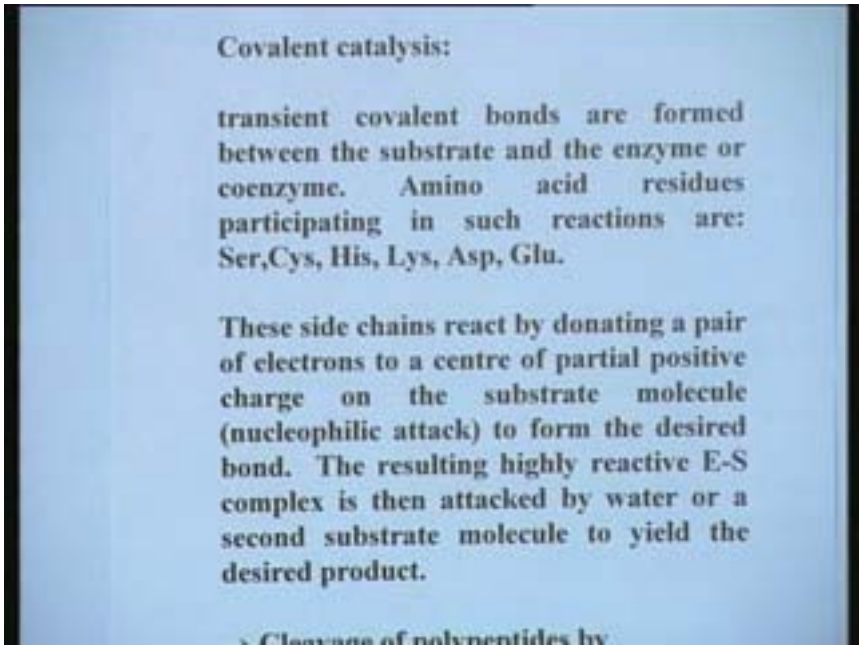

The difference is that in the case of acid base catalysis there is no covalent bond formation. It is only transfer of electrons or protons to or from the substrate. In the case of covalent catalysis transient covalent bonds are formed between the substrate and the enzyme or the coenzyme. Amino acids residues participating in such reactions are serine, cysteine, histidine, lysine, aspartic acid and glutamic acid. They participate by forming transient covalent bond. That means the enzyme substrate complex which results as a result of these covalent bonds are highly reactive. They have a covalent bond. Bond energy is not that high and therefore even under ambient conditions by attack of another molecule they can further be broken down to the resulting products.

These side chains react by donating a pair of electrons to a centre of partial positive charge on the substrate molecule, a nucleophilic attack. These pair of electrons looking for a positively charged nucleus donates a pair of electrons to form the desired bond. The resulting highly reacting enzyme substrate complex is then attacked by water in the normal cases if it is a hydrolytic reaction. Like in the case of lipase or protease, water can be the second substrate or it could be the second substrate itself to yield the desired product.

Again an example of such catalysis is cleavage of poly peptides by carboxy peptides.

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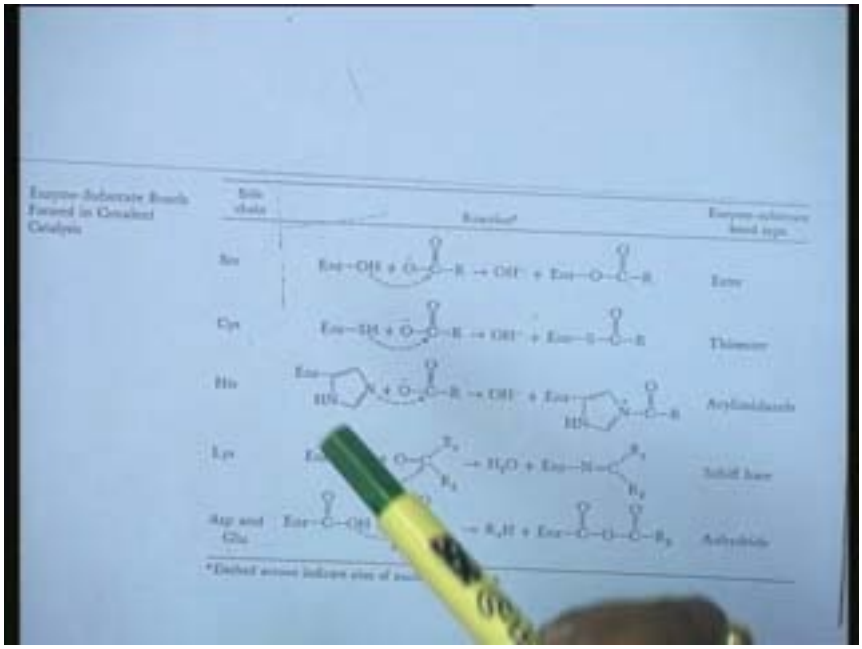

In some of the examples in this table you will notice that on the first column you have side chain serine, cysteine, histidine, lysine, aspartic acid and glutamic acid. The enzyme containing serine residue or hydroxyl group on the active site attacks at the positively charged nucleus, the central carbon atom and there by produces an enzyme substrate complex by nucleophilic attack and the type of bond formed is ester type of bond. This is quite a highly reactive molecule. That means not a very stable molecule as a covalent compound and therefore when it comes in contact with water it breaks down into the respective acid or alchol. But on the carboxylic group this serine functional group of side chain of the active site can attack.

Similarly in the case of cysteine the type of bond formed is thioester. In the case of histidine the bond formed is acylimidazole; lysine forms Schiff's base and aspartic and glutamic acid form anhydride bonds and these anhydride bonds then can be attacked by the second substrate. Second substrate could be even water and thereby they will break down. In fact a large number of hydrolytic enzymes follow such a behavior including proteases, particularly …. (41.38) proteases and lipases or tannase or for that matter n number of other hydrolytic enzymes follow the covalent bond formation.

These are some of the major mechanisms by which enzymes reduce the energy of activation. There are some minor mechanisms also and among the minor mechanisms one is environmental or dielectric effect. The earlier mechanisms that I mentioned were more or less general in nature. Most of the enzymes involved in those mechanisms reduced the activation energy.

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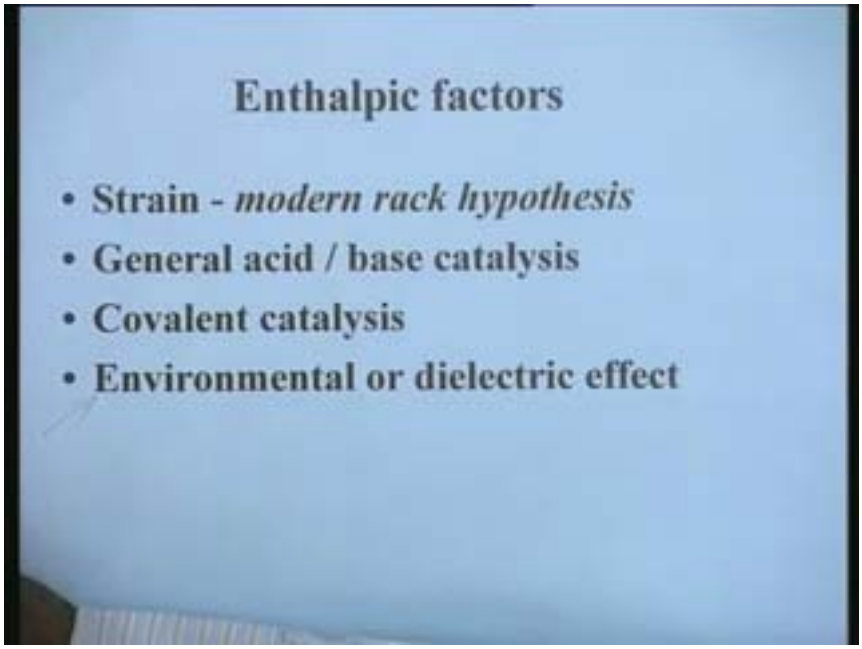

But the mechanism like environmental effect is provided by very specific enzymes only.

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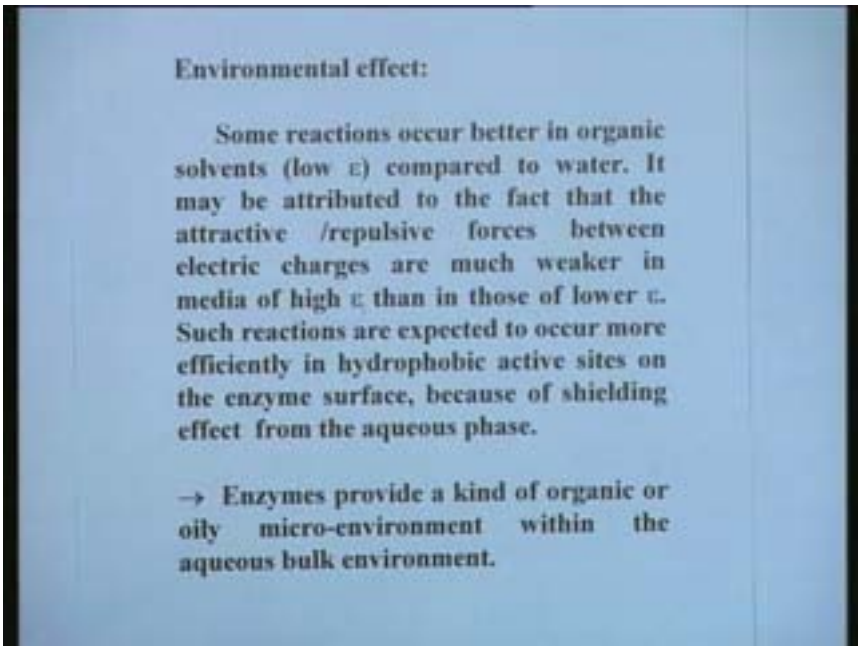

A not very frequently encountered effect for reducing the energy of activation is attributed to environmental effects. Some reactions occur better in organic solvent which have low dielectric constant compared to water and this may be due to the fact that some of the attractive repulsive forces between the electric charges are much weaker in the medium of high dielectric constant that is water than in those with lower dielectric

constant. Such reactions are normally likely to occur more efficiently in the hydrophobic active sites on the enzyme surface as it can shield them from the aqueous phase. Therefore in such cases enzymes provide a kind of organic or highly micro environment within the aqueous bulk environment. A situation something like that of micelle in which the substrate can be shielded from the bulk aqueous phase and still the hydrophobic environment can be provided to the substrate.

It is not a micelle. This is something analogous to micelle. Substrate is not entering here. The active site is predominantly hydrophobic in nature. It predominantly consists of some hydrophobic active sites, all the residues whereby the micro environment provided for the reaction is hydrophobic and really it is away from the bulk aqueous environment of the enzyme.

To summarize whatever we have discussed today, the mechanism where by enzymes lower the free energy of activation for a reaction they catalyze can be grouped under following major heads:

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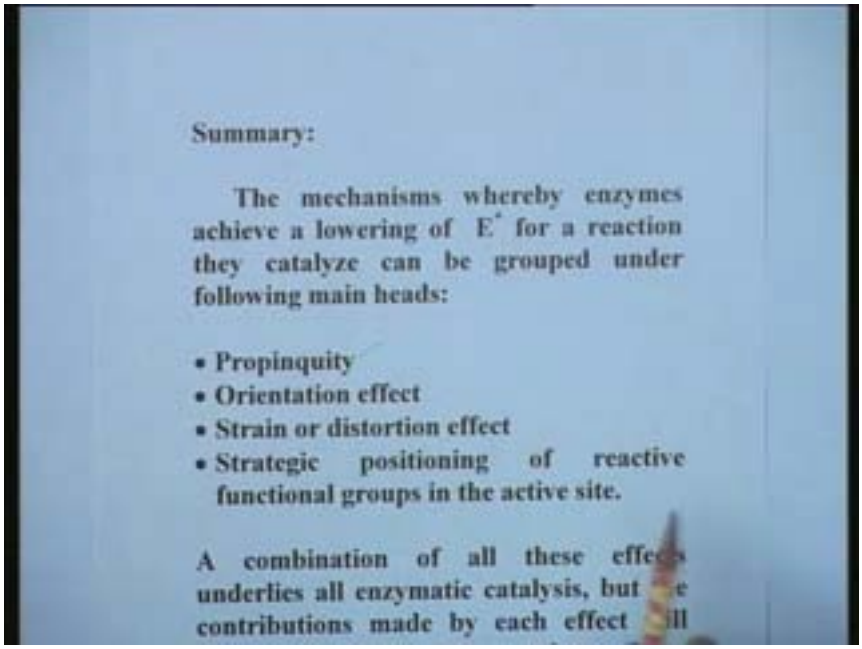

Propinquity or approximation, orientation effect so that they are built into a proper orientation either through aligning some of the functional groups or by orbital steering, then strain or distortion effect on the bond which is desired to be broken during the reaction or strategic positioning of reactive functional groups in the active site. This is primarily based on the acid base catalysis or covalent catalysis, a feature which is common to that of chemical catalysis. A combination of all these effects underlies all enzymatic catalysis.

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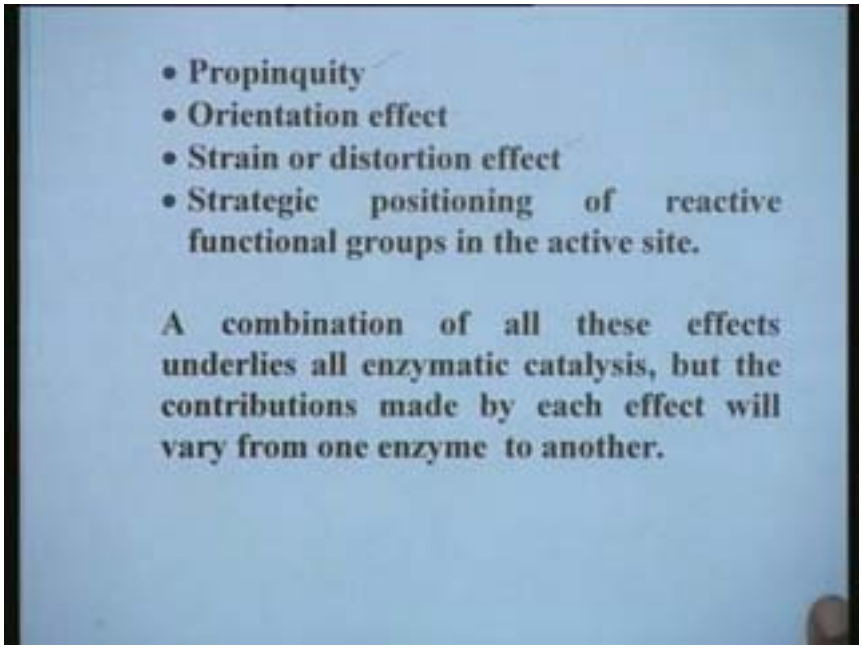

No enzyme follows a single mechanism. They all follow a combination of these mechanisms and the contributions made by each of these mechanisms will vary from enzyme to enzyme and if more than one mechanism is employed to reduce the energy of activation, the enzyme will be able to perform better catalysis in a more efficient way. So we will stop at this point today.