

## ENZYME SCIENCE AND ENGINEERING

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LECTURE – 3

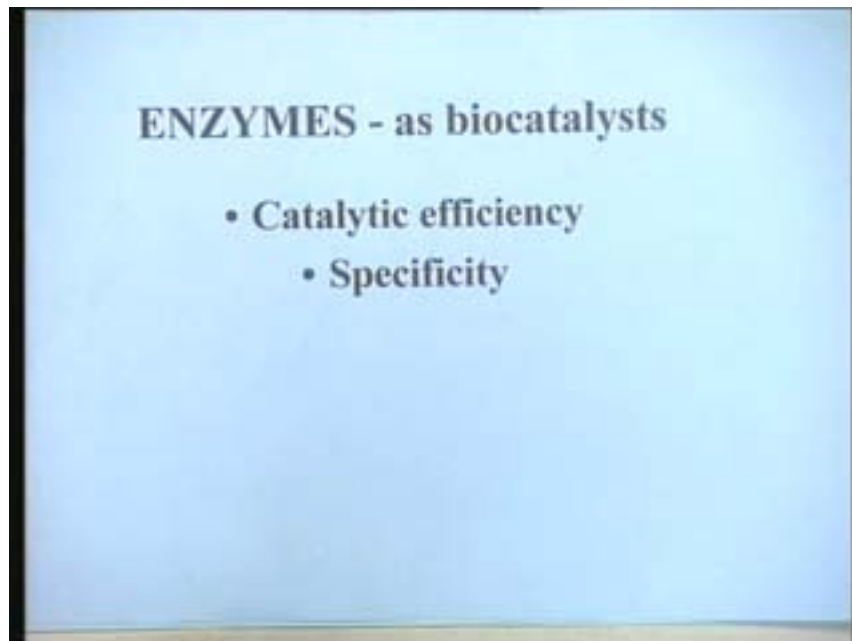
### **ENZYMES AS BIOCATALYSTS**

**\* CATALYTIC EFFICIENCY**

**\*SPECIFICITY**

Having discussed some of the characteristic features of enzymes in the light of their chemical nature, today we will discuss the functional nature of the enzymes and as most of you are familiar catalysis is the universal property of enzymes.

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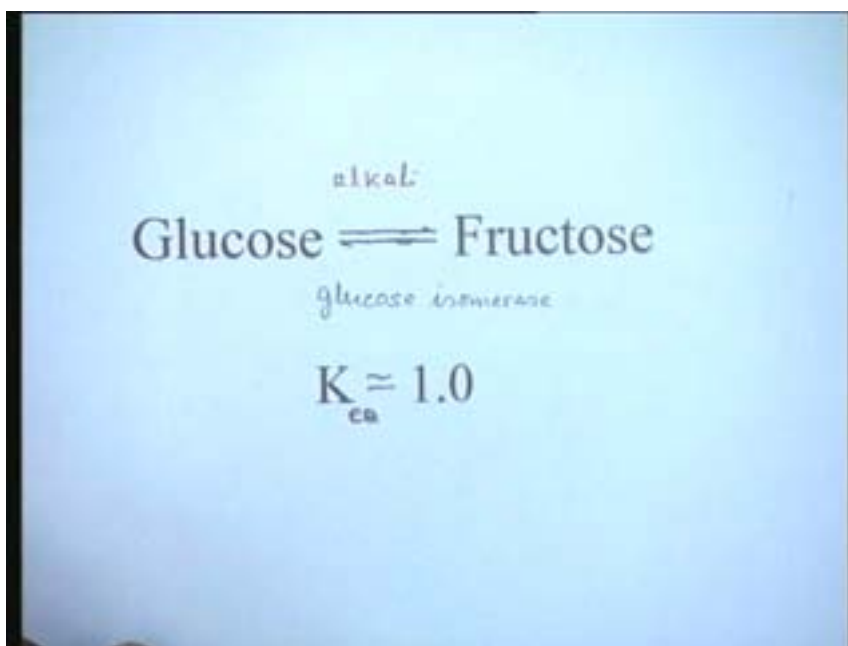
The essential characteristic of a catalyst is to accelerate the rate of reaction that it is supposed to catalyze but in the process it is not used up in itself and in an ideal situation it must be possible to recover the catalyst at the end of the reaction. As a biocatalyst in the living systems two very significant characteristic properties of enzymes are noted that is the catalytic efficiency. As you will notice, as we go further, that the enzymes provide a much more efficient catalytic system than their chemical counter part. There are basic

intrinsic mechanisms and the confirmation of the chemical nature of enzymes is the responsible factor for providing the catalytic efficiency. We look in to the basic factors that provide the catalytic efficiency.

When I say catalytic efficiency I normally mean the magnitude by which it is able to increase the rate of reaction compared to an uncatalyzed reaction or the turn over number of the enzyme that means number of moles of substrate converted to product per unit time per mole of the catalyst. The other feature which is very important is their specificity which is again not attributed to chemical catalyst and the enzymes provide a very specific catalytic system specific to the extent that we can recognize even the isomers they can distinguish. Therefore it will be interesting to note how they catalyze the reactions such that these properties are built in, in the system.

Another feature which I think must be important to note is that an enzyme like any other chemical catalyst does not influence the thermodynamics status of the reaction. That means whether it is the free energy change or the equilibrium constant of the reaction it remains unaltered during the process. It only accelerates the reaction rate by an order of magnitude. Now just as an example if you look at isomerization of glucose to fructose, the reaction can be catalyzed by an alkali say NaOH. You can get isomerization. You can also carry out isomerization by enzymatic route using the glucose isomerase.

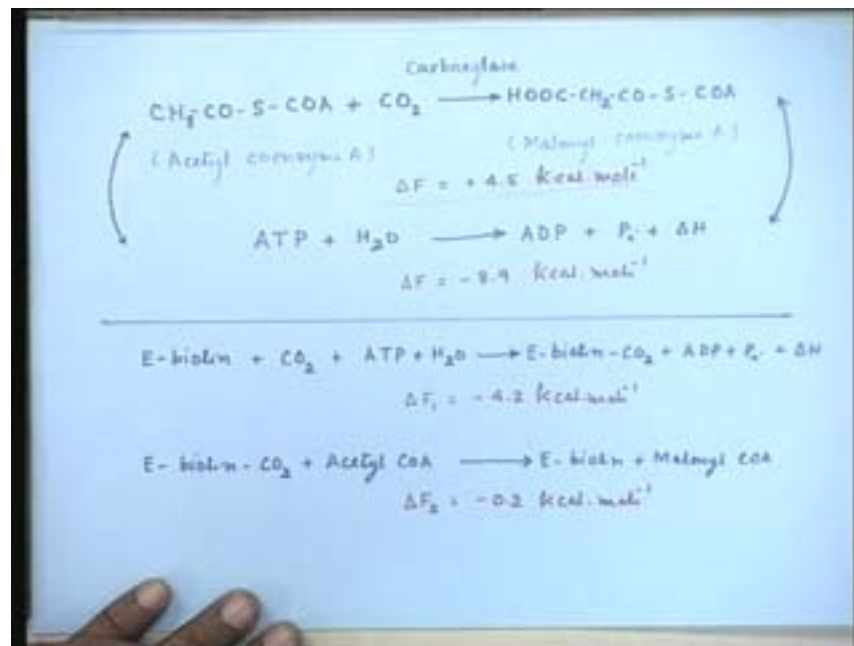
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In both the cases the equilibrium constant of the reaction is approximately equal to one. So whether you carry out the reaction by alkali or by the presence of glucose isomerase, the reaction will end up under equilibrium conditions at an equimolar mixture of glucose and fructose and that remains unchanged. The only difference, lies by the virtue of the presence of a catalyst or an enzyme in this case, will be that you can reach to the

equilibrium status much faster than in the case of an uncatalyzed reaction. Besides the equilibrium conversion or equilibrium constant of the reaction, the catalysis or the enzyme catalysis also follows all thermodynamic concepts that are applied to chemical reactions. Some times it may look in the case of living systems that certain reactions that are catalyzed are in totality have a positive free energy change. An example could be cited of conversion of carboxylation of Acetyl coenzyme A to Melonyl coenzyme A. The enzyme carboxylase catalyses the reaction. The reaction has a free energy change of +4.5 kilo calories per mole.

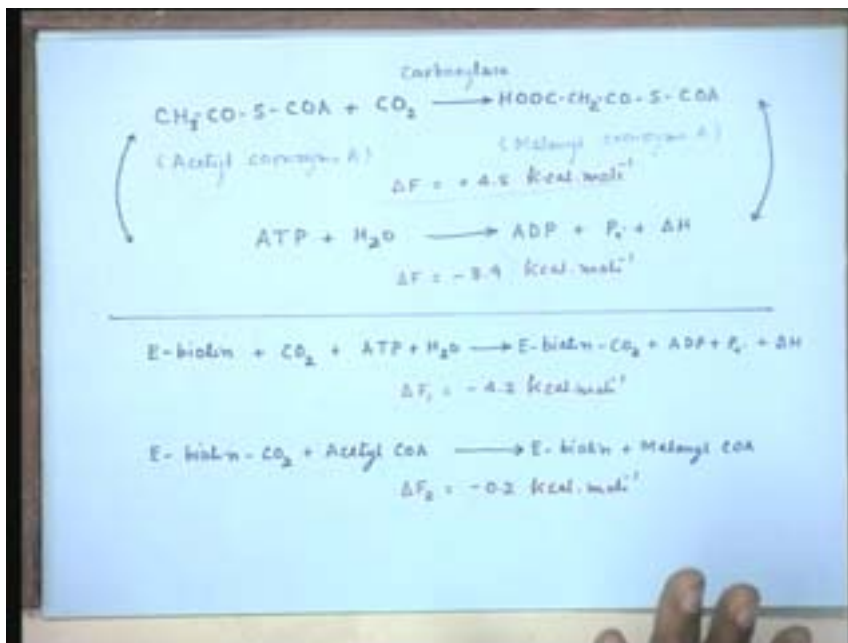
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The reaction must be non-spontaneous, should not take place as per the normal thermodynamic concepts. But we all know that this conversion does take place in the living cells. Apparently it may look as if enzyme is doing something which is not allowed by a chemical thermodynamics but in practice it is not so because the reaction is catalyzed in association with another hydrolytic reaction which is energy yielding, which has a much higher negative free energy change and that is hydrolysis of ATP to ADP and inorganic phosphate. The free energy change of this is highly negative – 8.9 kilo calories per mole. While the reaction takes place as a coupled reaction of the two systems, the net reaction is a coupled reaction where the acetyl coenzyme carboxylation is coupled to hydrolysis of ATP.

But in practice the reaction does take place in a living cell as shown in the second half. That is the enzyme which uses biotin as a cofactor activates the carbon dioxide at the expense of energy of hydrolysis of ATP and you get activated carbon dioxide with a negative free energy change which is a spontaneous reaction and this activated carbon dioxide then carboxylates the acetyl coenzyme A to melonyl coenzyme A, an enzyme along with the cofactor biotin is released free.

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The second reaction is also a negative free energy change and therefore it is also carried out and the net reaction is the sum total of the two reactions, what we have shown here. Therefore another feature is that the enzymes often show certain behavior which can be explained on the basis of coupling of more than one enzyme reactions where by we are able to produce certain net results which may look apparently to be non feasible but otherwise they follow all the laws of chemical reaction kinetics as well as thermodynamics.

The catalytic behavior of enzymes can be probably best illustrated by chemical reaction rate theory. One of the most accepted theoretical interpretation of chemical catalyzes can be considered from point of view of chemical reaction rate theory which illustrates that molecules can react only if they come in contact with each other. That means the molecules do undergo certain collisions. Therefore for different molecules of reactants to react collisions must take place. Any factor or any parameter which increases the frequency of these collisions between the molecules will tend to increase the rate of reaction.

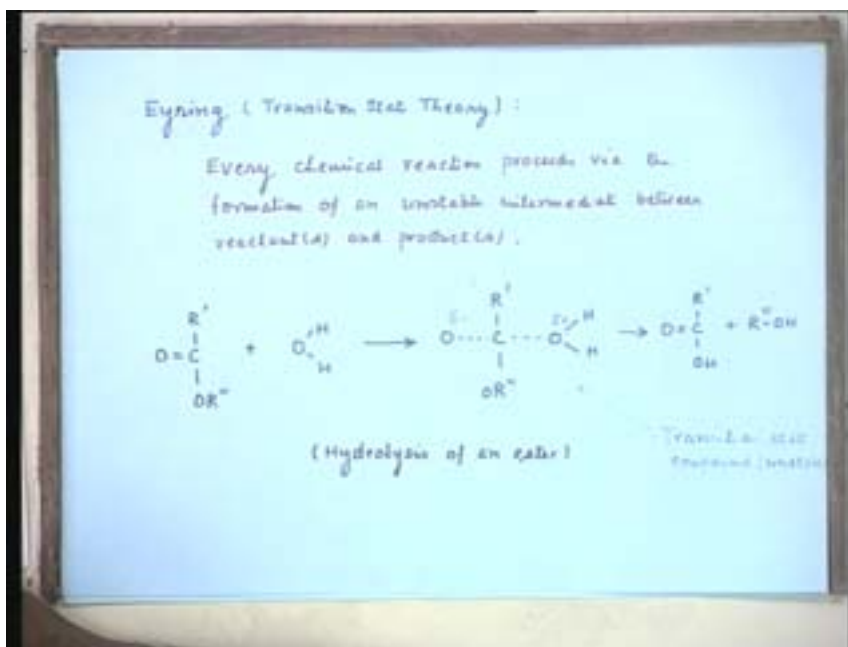
Of the two of the most sort after parameters, one is temperature. We know that if we increase the temperature of a reaction system the frequency of collisions will increase and there by the rate of reaction. Another is if you increase the reactant concentration again there also the frequency of collisions will increase and therefore rate of reaction will also increase. However while collisions among the molecules might lead to product formation as per this chemical reaction rate theory, it is also understood by virtue of some of the studies on the distribution of molecules which undergo reaction that not all the molecules that collide will result into the product. Only certain molecules which have sufficient energy to undergo reaction will result into the product formation and it will be only a

fraction of the total molecules that participate in the collisions process are able to yield the product.

If you consider two molecules in a binary reaction, bimolecular reaction that is A and B and both of them are in their ground state keeping in view all their electronic, vibrational and rotational ground state but possess translational energy which makes them to move for the collisions to take place. If they are to react when they collide the rearrangement of the electronic confirmation in each molecule must take place. Consider two molecules say for example A and B which are supposed to react in the presence of a catalyst or without a catalyst. If the reaction has to take place, then they must start colliding with each other.

Initially they will be at a ground state of their electronic, vibrational and rotational ground states but they will posses the translational energy which can make them move and collide. They can only react when the rearrangement of the electronic distribution in each molecule perhaps by transfer of atoms from one molecule to the other takes place. It is clearly pointed out by Eyring in his transition state theory that every chemical reaction proceeds via formation of the unstable intermediate between reactants and the products.

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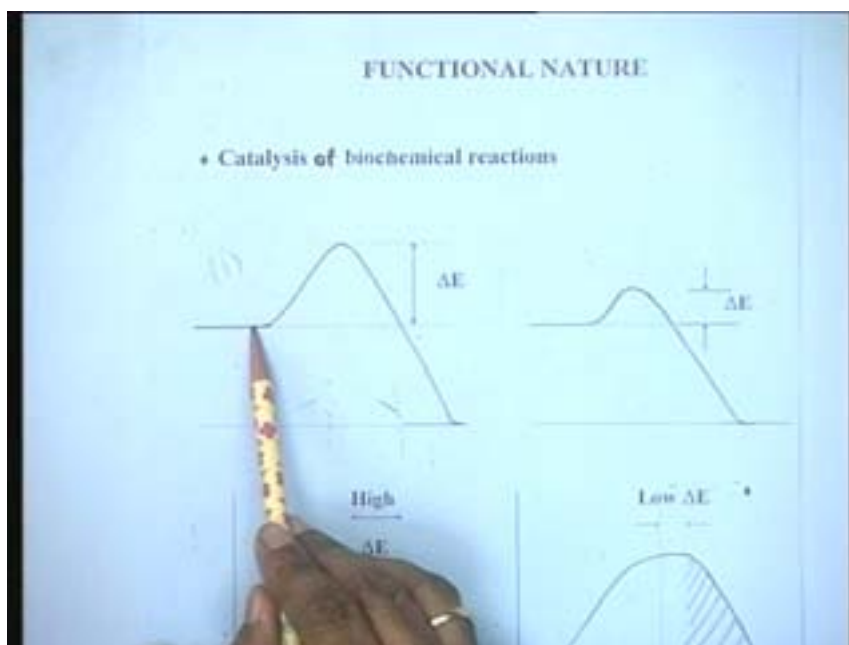


Take the example of hydrolysis of an ester, a very common reaction even occurring in the case of enzymatic reaction. To the ester molecule a water molecule is added. This addition of water molecule results into an unstable transition state compound, which has partial bonds, partial negative and positive charges at the molecule. This is a stable molecule which has much higher free energy change than the reactants and will break down to give the corresponding acid and the alcohol.

Eyring's transition state theory states that for each reaction to occur it must pass through a transition state which is an unstable state of the molecules or unstable state somewhere in between the reactants and the products and which has much higher energy than possessed by the reactants of the ground state. This energy requirement is considered as the energy of activation. That means the free energy required for the reactants to reach the level of transition state compound is considered as the energy of activation. The transition state compound will ultimately break down to give you the product or in other words the energy of activation acts as a barrier. Unless the reactant molecules cross the barrier they cannot get converted to the product.

From the energy diagram, consider the first stage which is an uncatalyzed reaction. In the case of this reaction you see the reactants are at a ground state.

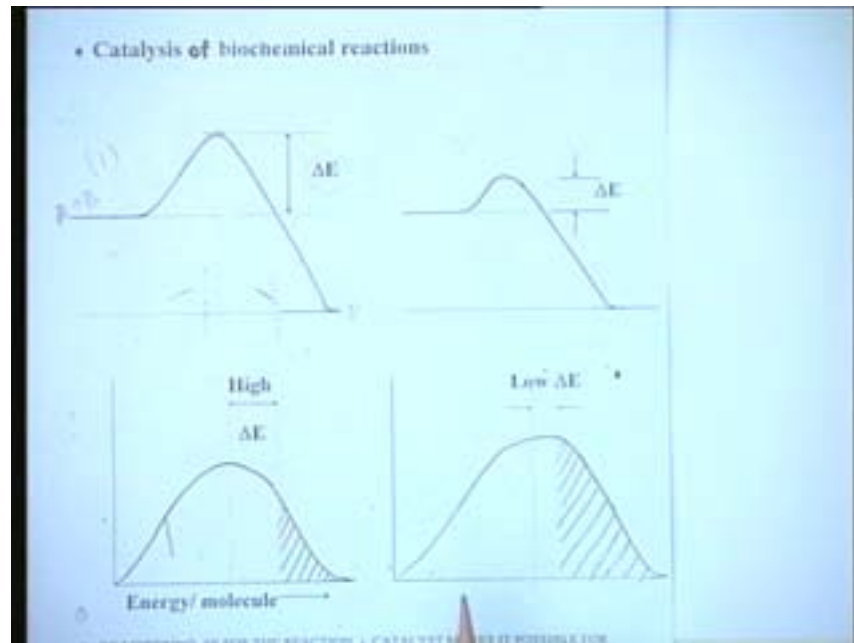
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The X-axis denotes the extent of reaction the Y-axis is the energy content and then the molecules have to undergo an increase in energy till the point where a transition state is reached and then this transition state will break down, will undergo reduction in energy and lead to the products. These are the reactants and these are the products. The difference between the ground state and the maximum level of energy at which the transition state is generated is called as energy of activation. If you look at the energy content of the molecules it will show you a Gaussian distribution. That means energy content per molecule and the number of molecules in a system will have a Gaussian distribution and only those molecules that possess sufficient energy which are showed here by a shaded portion, only that fraction of molecules will undergo chemical transformation. That means the transition state will be able to break down and form the products. Rest of the molecules which have much lower energy content will not be able to lead to product formation.

On the other hand when we consider the second area where we use a catalyst the main role of a catalyst as we will also further confirm is to reduce this energy of activation by some means.

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Whether it is chemical catalysis or enzymatic catalysis the primary function or the primary route by which a catalyst functions is by reduction in this activation energy. You see that the energy of activation from here is reduced to this level. The number of molecules, the fraction of total number of molecules has increased, which possess sufficient energy for the transition state molecule to break down into product, and large number of molecules can then take part in the reaction and result ultimately into the product.

So in conclusion by lowering the energy of activation for the reaction, a catalyst makes it possible for substrate molecules with a smaller internal energy to react. Even at a smaller internal energy level the product formation can take place. You can see the related concentration of the shaded portion in the two cases without the catalyst and in the presence of catalyst. That will illustrate the point. Here it is the low energy of activation as a result of catalyst and this indicates the high energy of activation in the absence of any catalyst.

If we look deeper into the transition state, the formation of transition state can be described as a very easily studied, as an ordinary chemical equilibrium process. As I mentioned earlier the reactant molecule has to pass through a transition state. The formation of transition state starting from A and B can be considered as a simple

chemical equilibrium process. That means A and B combining to give you an activated complex  $AB^*$  with an equilibrium constant of  $K_{eq}$  or you can put it as say  $K^*$ .

$$K^* = [AB^*] / [A] [B]$$

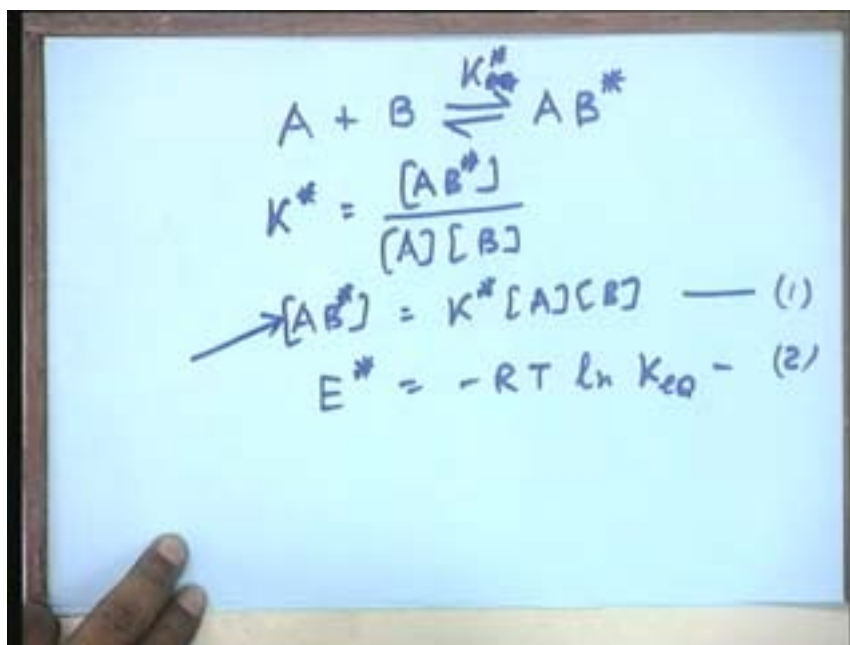
$$[AB^*] = K^* [A] [B] \quad (1)$$

This chemical equilibrium follows the other relationships. That is the free energy of activation which is required in this whole process can also be given by

$$E^* = -RT \ln K_{eq} \quad (2)$$

Rate of reaction between A and B mind that we have not undergone the whole reaction process. We have only come to the transition state complex and the rate of reaction will depend upon the concentration of  $AB^*$ .

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The larger this concentration of the transition state complex, the higher will be the rate of reaction and this rate will depend upon the frequency at which the activated complex will decompose and this rate of decomposition will depend upon the vibrational frequency of the bond that is breaking. From transition state some bonds have to break some has to form. Ultimately the transition state complex is somewhere intermediate between the reactant and the product. Some bonds are partially formed, they will be completely formed. Some bonds are still in the formation stage and therefore the vibrational frequency of the bond that is breaking will determine the rate of the reaction. This frequency can be arrived at from the theoretical treatment from two routes, by quantum mechanics as well as from classical mechanics.



Consider the frequency of energy of excited oscillator by quantum mechanics you get

$$E = h\nu$$

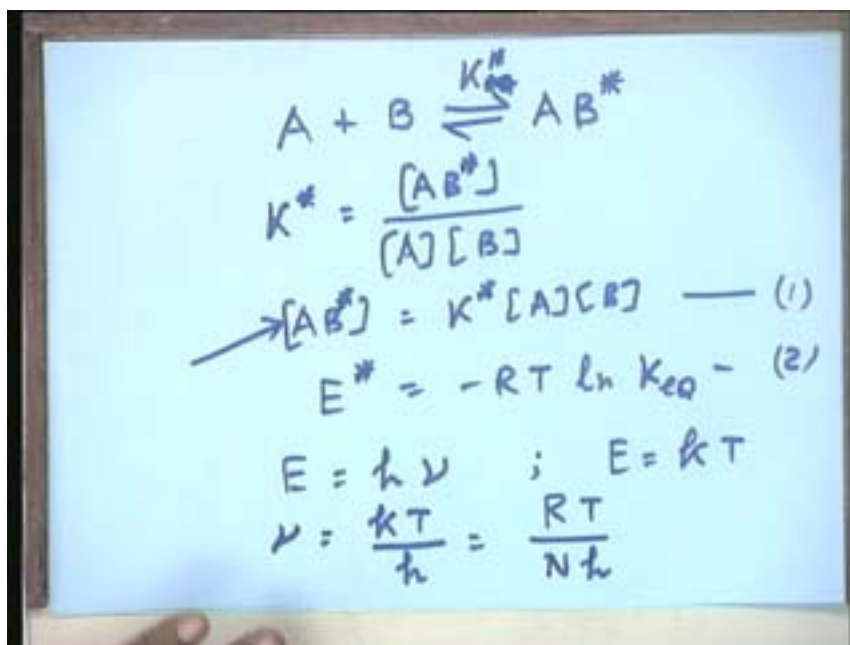
and in classical mechanics you get

$$E = kt$$

$h$  is the planck's constant,  $E$  is the energy, the  $\nu$  is the frequency. Similarly here the  $k$  is the Boltzmann constant and  $T$  is absolute temperature. Therefore

$$\nu = kt/h = RT/Nh$$

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You are familiar with all these terms. That is  $R$  is a gas constant,  $T$  is absolute temperature,  $N$  is the Avogadro's number and  $h$  is the Planck's constant. The frequency at which bonds in a transition state complex will break will be determined by these factors which can be obtained by equating the energy of an oscillator by two treatments.

We had as we mentioned earlier the rate of the reaction  $r$

$$r = \nu [AB^*] \quad (3)$$

The rate of reaction will depend upon the concentration of the transition state complex and the frequency of the break down of this transition state complex can be written as

$$r = RT/Nh \cdot K^* [A] [B] \quad (4)$$

The expression we got for the concentration of the transition state complex from the chemical equilibrium process. This term  $\frac{RT}{Nh} \times K^*$  is the second order rate constant. Let us say we define  $k_2$  as second order rate constant for the reaction of A and B. It becomes

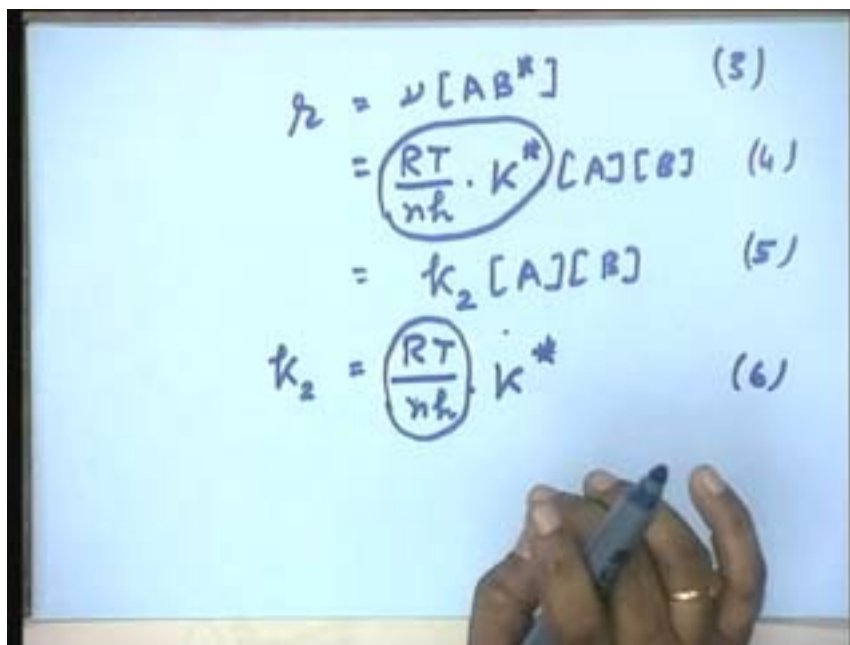
$$k_2[A][B] \quad (5)$$

or in other words the second order rate constant will be nothing else but

$$k_2 = \frac{RT}{Nh} \cdot K^* \quad (6)$$

Again you notice a significant fact here that the rate constant is directly linked. All these are constants. Temperature is a constant. If the temperature is constant these are all constants. So the rate constant is dependent upon the equilibrium constant of the transition state process, the conversion of the A and B into the transition state complex and this also gives you a relationship between the rate constant and the  $K^*$ , equilibrium constant of the formation of transition state complex.

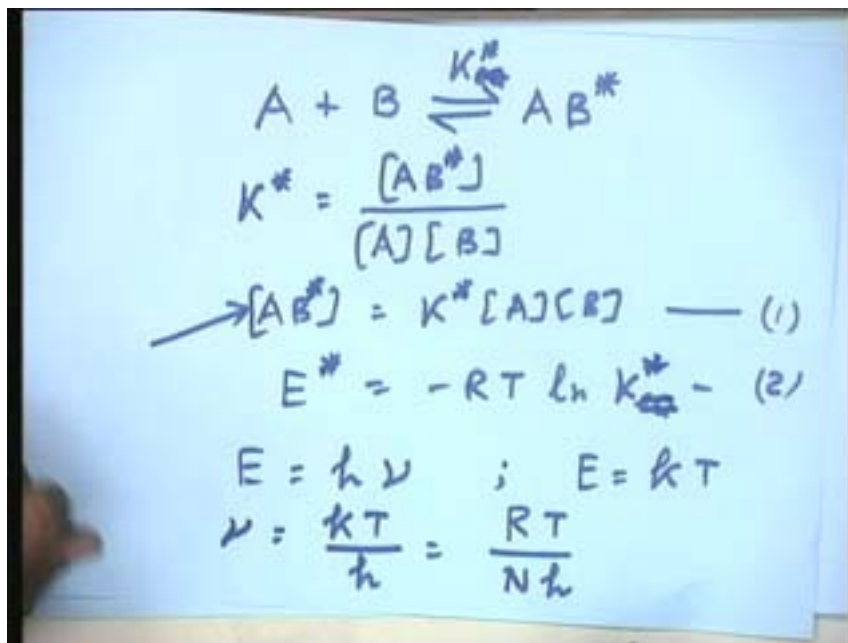
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It will imply or it will also be a very logical conclusion that the faster reactions, if the reaction has to go very fast rate, then they must have larger value of  $K^*$ . That means at a very fast rate the reactants must go into the transition state or they must have higher concentration of  $AB^*$ . This equation will also allow you to calculate the value of  $K^*$  and hence the value of activation energy during your earlier equation that is your  $E^*$

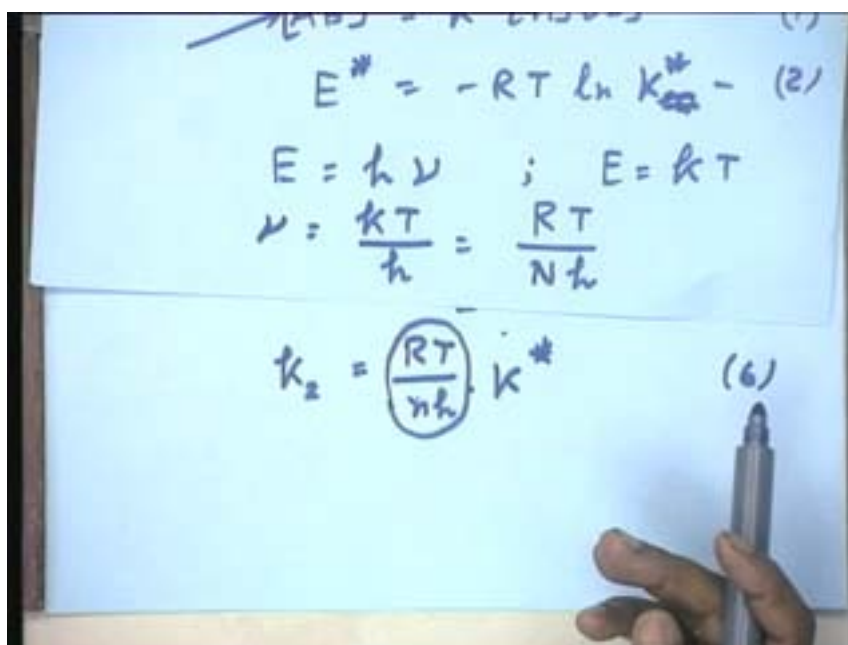
$$E^* = -RT \ln K^*$$

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If you use the equation number 6 and 2 you can calculate the value of energy of activation for a given system.

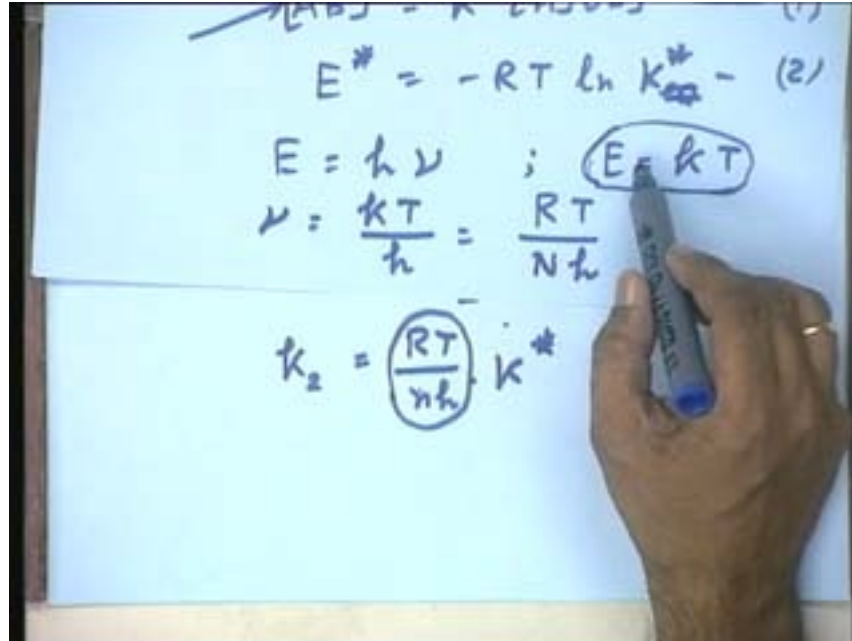
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You can also conclude that the faster reactions will have a smaller value of  $E^*$ .

No. Energy of activation is not equal to  $kT$ . This is the energy of the oscillator, energy term for the oscillator based on the classical mechanics.

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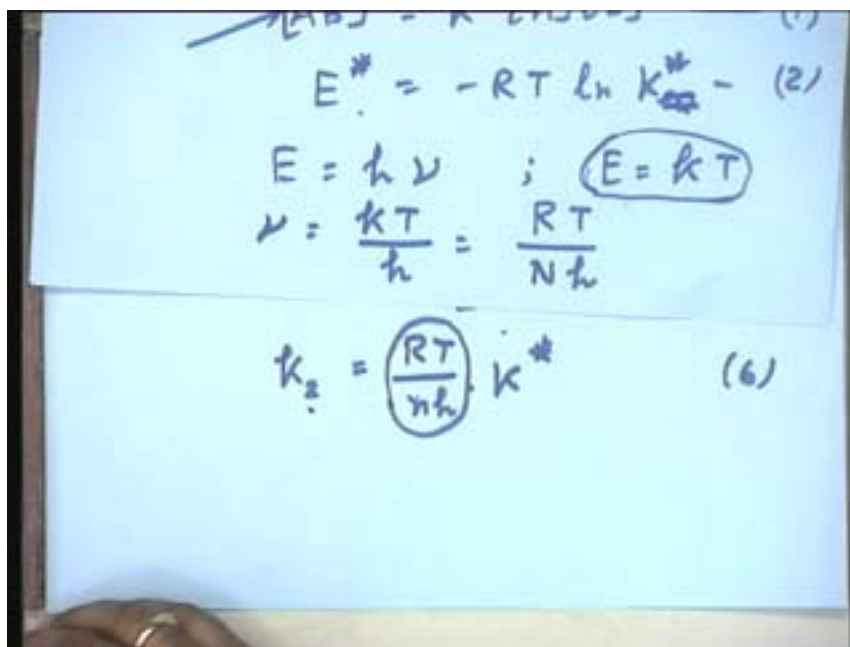


Handwritten equations on a whiteboard:

$$E^* = -RT \ln K^* \quad (2)$$
$$E = h\nu \quad ; \quad E = kT$$
$$\nu = \frac{kT}{h} = \frac{RT}{Nh}$$
$$k_2 = \left( \frac{RT}{nh} \right) K^*$$

This is not energy of activation.  $E^*$  is energy of activation. Energy of activation can be calculated from here which will be related to  $K^*$  and also can be related to  $k_2$  and the two conclusions which we can make from this analysis is that the fast reaction will have a higher value of  $K^*$ , equilibrium constant for the conversion of reactants into the transition state or in other words the fast reactions will have higher concentration of  $AB^*$  at equilibrium. The second is that the first reaction will have smaller energy of activation. If you combine equations 6 and 2 energy of activation for faster reaction if the  $k_2$  is high that means lower energy of activation.

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So these two conclusions do arrive from the theory of reaction rate which is now well accepted world over. If you look at what we talked about

$$kT = \frac{RT}{nh} \times K^* \quad (6)$$

you can also write  $k_2$  as

$$k_2 = \frac{RT}{nh} \times \exp(-E^*/RT) \quad (7)$$

Now this as all you know we call it as frequency factor which is often written as  $A$ . The frequency factor  $A^*$  you can say because  $A$  is the reactant.

$$A^* \exp(-E^*/RT)$$

which is a very common expression what we all know as Arrhenius' expression.

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$$\begin{aligned} r_2 &= \nu [AB^*] & (3) \\ &= \frac{RT}{nh} \cdot k^* [A][B] & (4) \\ &= k_2 [A][B] & (5) \\ k_2 &= \frac{RT}{nh} \cdot k^* & (6) \\ A^* &= \frac{RT}{nh} \cdot \exp\left(-\frac{E^*}{RT}\right) & (7) \end{aligned}$$

The term  $E^*$  can again be separated into both enthalpic and entropic contributions.

$$E^* = \Delta H^* - T\Delta S^*$$

from second law of thermodynamics.

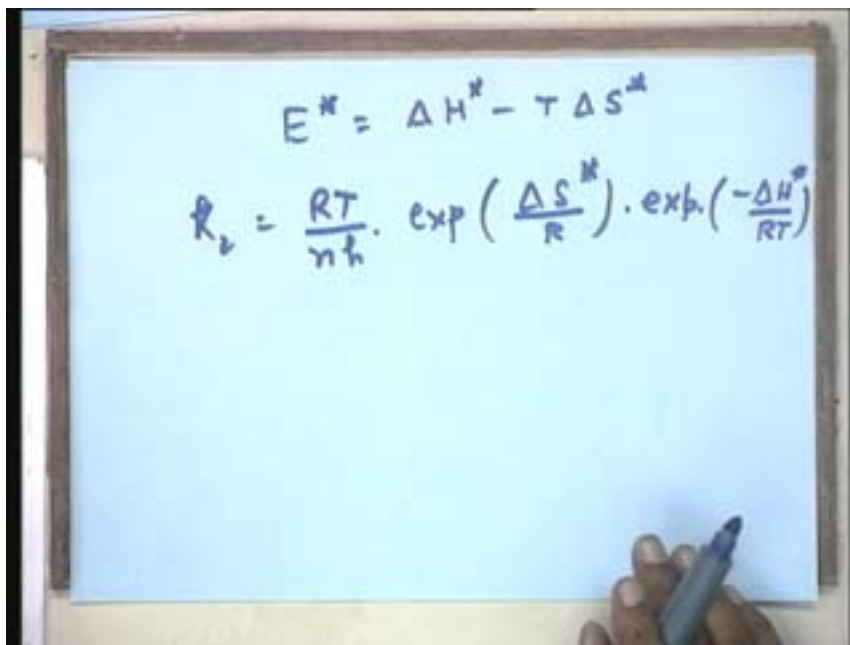
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$$E^* = \Delta H^* - T\Delta S^*$$

That means the energy of activation can also be considered both in terms of entropic contribution as well as enthalpic contributions and if you separate into it then you can write

$$k_2 = RT/h \cdot \exp(\Delta S^*/R) \cdot \exp(-\Delta H^*/RT)$$

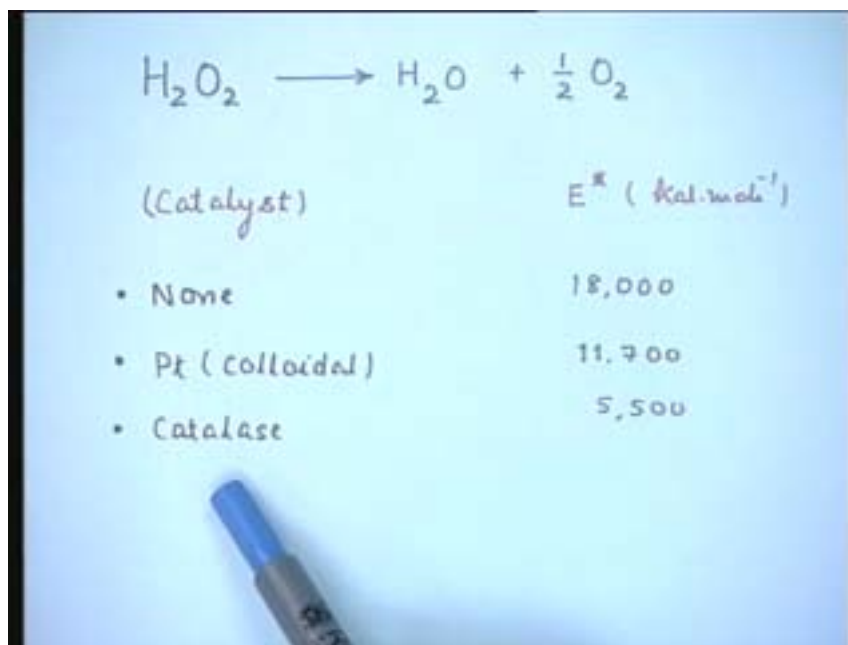
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The final conclusion is that for a catalyst or an enzyme in our case as we are talking about to be able to include the value of  $k_2$ , it must be able to reduce the energy of activation or increases the  $K^*$  value that is equilibrium constant. This conclusion will apply commonly to a chemically catalyzed reaction or an enzymatic catalytic reaction.

If you look at the performance of some of the enzymatic catalyst as compared to chemical catalyst, you will notice that enzymes provide very efficient catalyst. Simple example where I can compare the reaction which can take place both by chemical catalyst as well as by enzymes is hydrolysis of hydrogen peroxide that is break down of hydrogen peroxide into water and oxygen, a very common reaction that takes place in living cells by the enzyme catalase.

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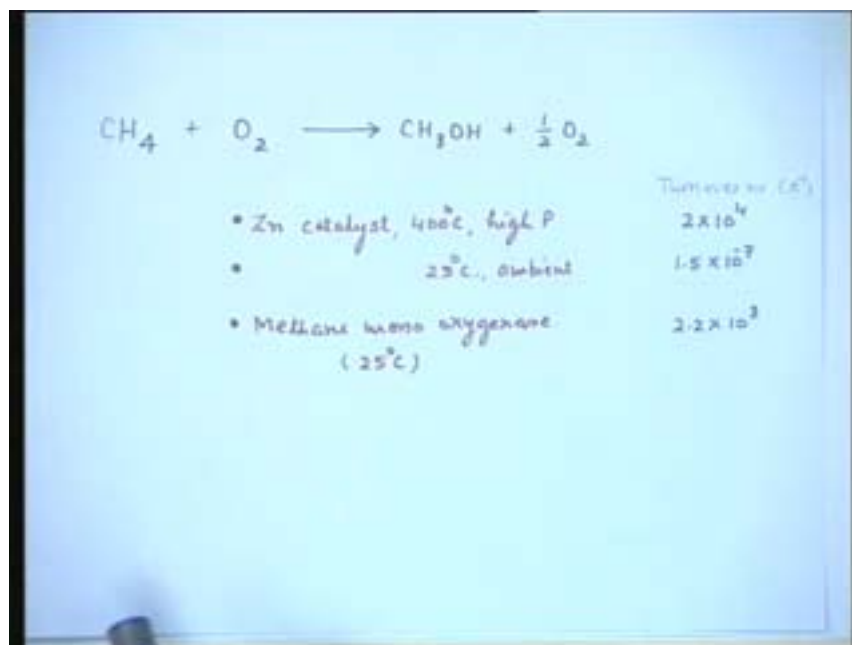
It can also be carried out by colloidal platinum. The reaction is also feasible under normal conditions without any catalyst. That means not a very non spontaneous reaction. It is possible to carry out the reaction. The corresponding energy of activation for this reaction without any catalyst is 18,000 kcal.mole<sup>-1</sup> as against in the presence of colloidal platinum as catalyst it is 11,700 and for the catalase it is 5500.

This is just an illustrative example but a similar example can be cited from a variety of reactions that can be carried out both by chemical catalysis as well as by enzymatic catalysis and the conclusions are almost same. The enzymes are able to bring down or reduce the energy of activation to a very large extent compared to chemical catalyst and we will again see how this is done.

Another interesting reaction in the living systems is on oxidation of methane. The methane mono oxygenase enzyme oxidize methane in to ethanol spontaneously in the living cells particularly those organisms which can survive or consume methane as a carbon source. This is the very first reaction which they carry out when the methane is used as carbon source. This reaction can also be carried out chemically using a chemical catalyst, like zinc catalyst at 400°C and high pressure, almost few hundred atmospheres, and the turn over number is of the order of  $2 \times 10^4 \text{ s}^{-1}$ , that means number of moles of methane oxidized per second per mole of the catalyst.

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The same reaction if you carry out at ambient conditions, 25°C and ambient atmospheric pressures the turn over number is as low as  $1.5 \times 10^{-7}$ , almost negligible. In the presence of enzyme, methane mono oxygenase at 25°C and obviously at ambient pressure the turn over number is  $2.2 \times 10^3$ , a much higher value although it is lower than the one which is obtained at 400°C and few hundred atmospheres. But compared to ambient conditions and the same catalyst, the catalyst here is zinc and the value is much higher.

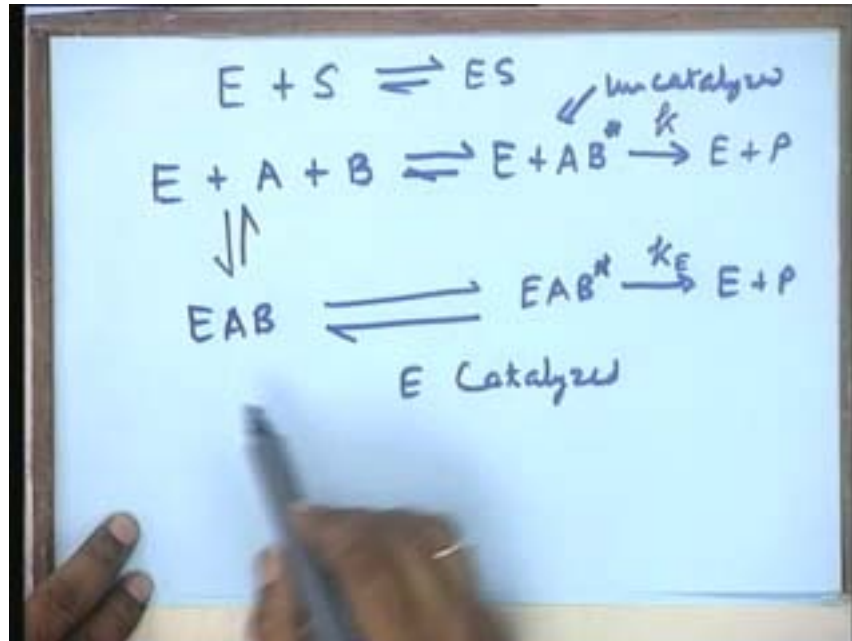
Very often a comparison of the first and the third case is not logical because the reaction conditions are different and comparing the turn over number may not be a very reasonable proposition. But if you compare under the same conditions the enzyme as a catalyst will provide you much more efficient system and one should look at how the enzyme provides this kind of efficiency compared to a chemical catalyst because the ultimate goal in both cases is the same, to reduce the energy of activation. But the enzyme is able to reduce the energy of activation to a large extent as compared to chemical catalyst and also its turn over number is much higher.

Let us see what happens when we consider an enzymatic catalysis. Now in the case of enzymatic catalysis we must bear clearly in our mind that one of the characteristic features is that enzymes has a certain ligand binding sites and more specifically substrate binding sites. That means an enzyme can bind to substrate forming enzyme substrate complex. This enzyme substrate complex then again provides a system which will undergo the energy of activation, undergo the transition state before the product is formed.

Consider an enzyme catalyzed system and an uncatalyzed system. If you consider let us say  $E+A+B$ , same as what we are talking about reaction between A and B. Consider two routes. One is E, the enzyme does not take part in reaction, an uncatalyzed reaction and it goes to E plus P. In the second case we can also have that enzymes binds the substrate A

and B and forms a enzyme substrate complex EAB. It also undergoes a transition state  $EAB^*$  and then it goes to enzyme and product. Let us consider the rate constants  $k$  here  $k_e$  here. The lower route is catalyzed one, enzyme catalyzed. The upper route is uncatalyzed. That means the enzyme has no role to play here.

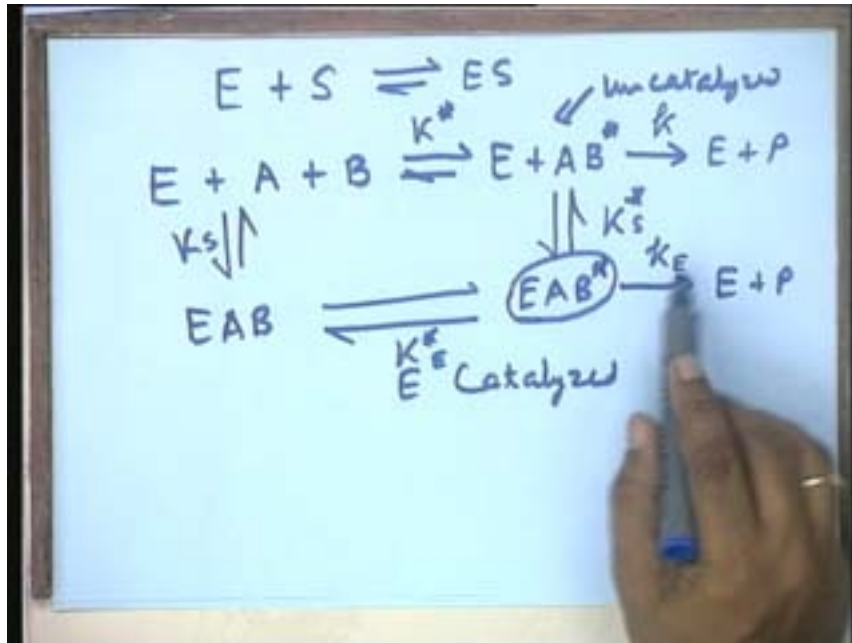
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The enzyme binds to the substrate and then undergoes transition state and then forms the product. In the middle case from the enzyme and transition state AB you can also form the EAB activated complex.

If you consider the equilibrium constant of all these equilibrium reactions let us say this is  $K^*$ , this is  $K_S$ ,  $K_E^*$  and  $K_s^*$ . Thermodynamically we can notice that ultimately we are interested in this. As a catalyzed reaction, we are interested in the  $EAB^*$ , which is the transition state complex which will give you a product at much faster rate.

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$K_e$  is much, much higher than  $K$ , the lower being a catalyzed reaction. Catalyzed reaction will have a much higher reaction rate and so to really get the reaction at a high rate we want a larger concentration of  $EAB^*$ .

If you look thermodynamically, this product  $EAB^*$  can be obtained by either of the routes. Either it can go this way and come here or it can also come this way and reach to this side. From either way under equilibrium conditions, it is possible to arrive at this complex and if you consider the two routes then your

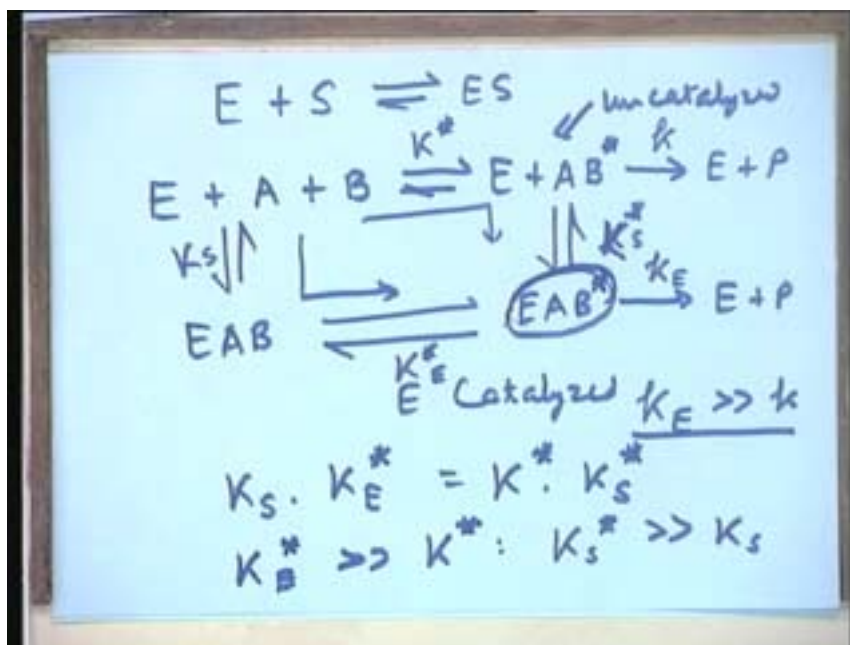
$$K_S \cdot K_E^* = K^* \cdot K_S^*$$

Multiplication of equilibrium constant of these stages should be equal. As I said earlier  $K_E$  is greater than  $K$  and also

$$K = RT/nh \cdot K^*$$

Therefore the  $K_E^* \gg K^*$  and it will imply that  $K_S^*$ , equilibrium constant is much, much greater than  $K_S$ . This means that the binding of the enzyme to the AB complex, uncatalyzed AB complex is much, much larger than the  $K_S$ , the binding of the enzyme to A and B.

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This implies that the enzyme binds  $AB^*$  more tightly. The higher value of  $K_S^*$  means the enzyme binds  $AB^*$  more tightly than the substrate molecules. The general concept that the enzymes have certain substrate binding sites on molecule but conformationally it is more complementary to the substrate transition state and that was the contribution which was stated in very clear terms by Linus Pauling that enzymes are molecules that are complementary in structure to the activated complexes of the reactants they catalyse.

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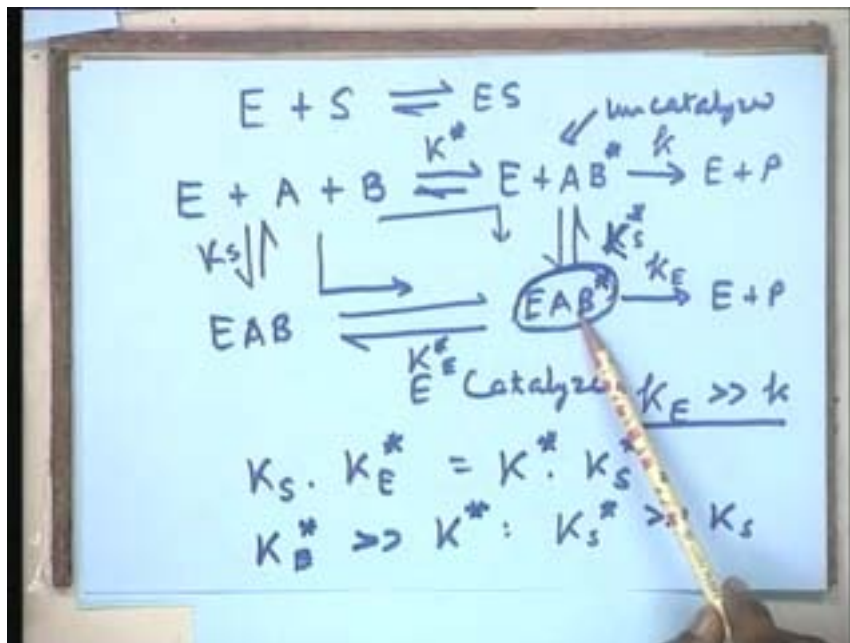
**L. Pauling:**

*Enzymes are molecules that are complementary in structure to the activated complexes of the reactants they catalyze. The attraction of the enzyme for the activated complex would thus lead to a decrease in its energy and hence decrease the energy of activation for the reaction and to an increase in the rate of reaction.*

The attraction of the enzyme for the activated complex would thus lead to a decrease in its energy and hence decrease the energy of activation for reaction and to an increase in rate of reaction. The enzyme is in structure conformationally which is very much complimentary to the transition state of the substrates and by binding that it stabilizes those transition complex. Stabilization of transition state means that the enzyme because of its confirmation which is complimentary to the transition state, binds and stabilizes the transition state complex. So at any given equilibrium the concentration of the  $EAB^*$  that is the product of this binding is much, much higher than as if enzyme does not take part.

If suppose this enzyme was not a bio catalyst. Suppose this E would have been a chemical catalyst this binding would not have been feasible because there would have been no complimentary catalytic function. The catalyst might be able to perform the catalytic function by any other mechanism: by acid base catalysis or covalent catalysis which we will discuss later but the contribution made by stabilizing the transition state of the substrate will not be possible to be provided by the chemical catalyst which enzyme is able to provide and there by it increases the stability of  $EAB^*$  and thereby reduces the energy of activation and leads to the formation of the product.

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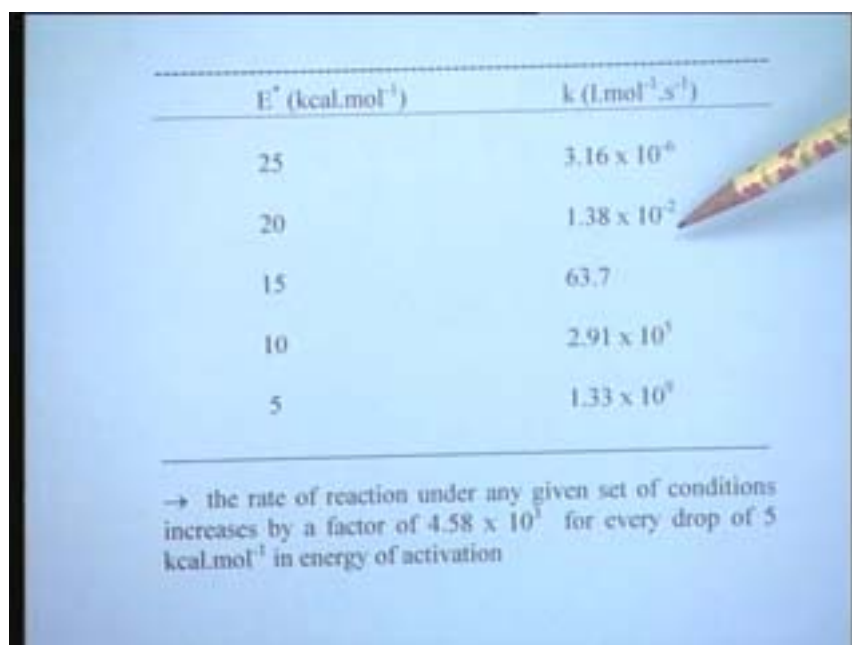
That was a major contribution in the understanding of the enzyme functions made by Linus Pauling.

For the unimolecular reaction also as I mentioned for any reaction whether it is unimolecular or bimolecular the substrate or the reactant has to undergo a transition state. The product cannot be formed without undergoing a transition state. As a matter of fact practically there are hardly any reactions, very few reactions, which are single molecular. Apparently hydrolysis may be unimolecular reactions but it is not so. Even in the earlier

example we discussed the hydrolysis of esters, it is an addition of water molecule to the ester which leads to transition state. So most of the reactions undergo or involve either cofactor or water molecule, excepting certain isomerases which are truly unimolecular. But even in that case the molecule has to undergo a transition state otherwise the catalyst reaction is not feasible according to reaction rate theory.

Coming back to the role of the energy of activation on the reaction rate constant, if you take arbitrary value of energy of activation, say from  $25\text{kcal mol}^{-1}$  downwards to  $5\text{kcal mol}^{-1}$ , a decrease of activation energy results in a very significant increase in the reaction rate.

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$E^*$ (kcal.mol <sup>-1</sup> )	$k$ (l.mol <sup>-1</sup> .s <sup>-1</sup> )
25	$3.16 \times 10^{-6}$
20	$1.38 \times 10^{-2}$
15	63.7
10	$2.91 \times 10^3$
5	$1.33 \times 10^9$

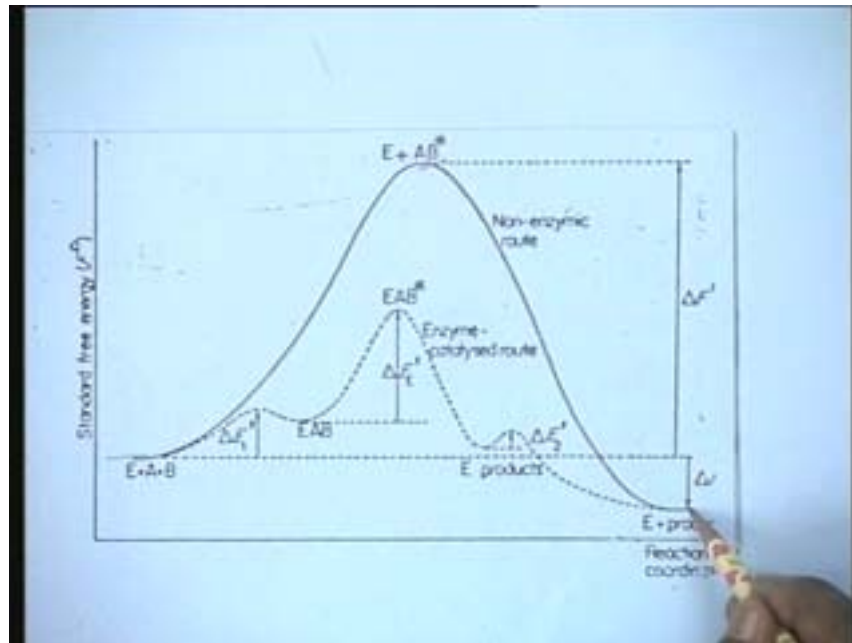
→ the rate of reaction under any given set of conditions increases by a factor of  $4.58 \times 10^3$  for every drop of  $5\text{kcal.mol}^{-1}$  in energy of activation

The magnitude at every step of reduction of energy of activation by  $5\text{kcal mol}^{-1}$  you will see that starting from  $3.16 \times 10^{-6}$  at a energy of activation of  $5\text{kcal mol}^{-1}$  you will go up to  $1.33 \times 10^9$ . That means an increase of magnitude by  $10^{15}$  fold of the rate constant.

It is a very, very significant increase in the reaction rate constant and it will give the rate of reaction under any given set of conditions. That means if you maintain same temperature and pressure and substrate concentration increase in the reaction rate constant by a factor of  $4.58 \times 10^3$  fold can be achieved for every drop of  $5\text{kcal mol}^{-1}$  which is a very, very large. So therefore even a decrease of the energy of activation by  $5\text{kcal mol}^{-1}$  can lead to an increase of the reaction rate by a factor of  $4.5 \times 10^3$ . Very significant number and in case of most of the enzyme the decrease is of the order of about  $15\text{-}20\text{kcal mol}^{-1}$  in the case of energy of activation. Therefore one can achieve a very high catalytic efficiency in the case of enzymatic catalysis and as you notice that this efficiency is primarily achieved by two distinct factors as we saw today which are unique compared to the chemical catalyst.

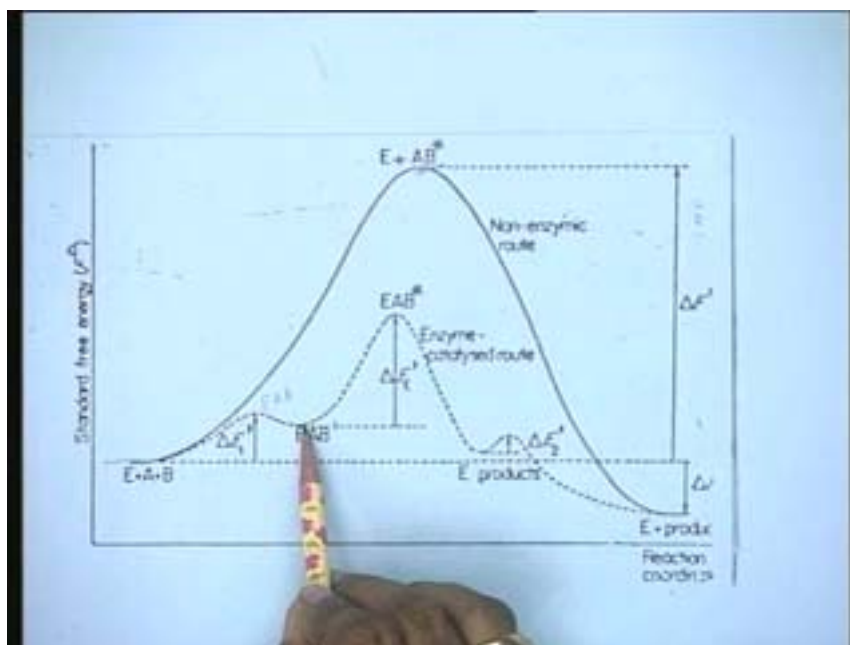
One is that enzymes have binding sites for substrate molecule. The second is the enzyme conformation itself is complementary to the enzyme transition state complex thereby it can stabilize the transition state and increase the concentration of transition state in any given reaction at equilibrium. Now if you look at the whole picture of the enzymatic catalyses again, in the energy diagram we notice the bold lines which go here from the ground state  $E+A+B$  uncatalyzed reaction that is the non enzymatic reaction is this one. The molecules have to reach to this state of the energy so that the transition state can form and then it can be broken down to form the enzyme and the product.

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What happens in the presence of an enzyme molecule? The enzyme binds to the substrates A and B, forms EAB and this process also undergoes energy of activation. That means some amount of energy is released by this binding and the enzyme substrate complex instead of starting from the level of ground state of A and B now has reached to a state little higher than ground state by binding process and from there it goes to the bound state.

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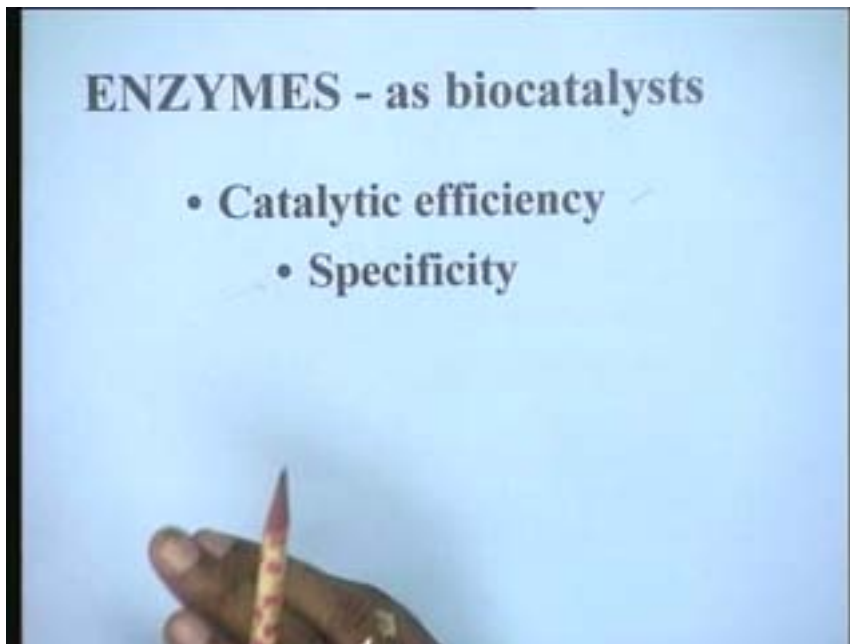
Even the binding process will undergo the transition state and for this binding process EAB is the product. Then from here EAB has to go to transition state  $EAB^*$  as I showed in the earlier comparative figure and this  $EAB^*$  is much, much smaller than the energy level of  $AB^*$  for uncatalyzed reaction. So you have an advantage. This  $EAB^*$  is reduced by virtue of the complementary nature of binding of the enzyme molecule to the  $AB^*$ . The concentration of  $EAB^*$  is increased by significant amount and at lower energy level this equilibrium can be achieved. Two factors which contribute to the reduction in activation energy or the efficiency of the enzymatic catalyst are: one is binding of the substrate and other is complementary of the enzyme molecule to that of the transition state. The same process follows on the reverse of reaction that means  $EAB^*$  under goes break down into products whereby instead of product directly being formed it forms EP, the enzyme bound to the product, the enzyme product complex and then this EP again breaks down to the next level of your product which is same as of the uncatalyzed reaction. Therefore one is by binding process the enzyme processes started at much high energy level then the ground state as applicable in the case of uncatalyzed reaction and the other is the complementarity of the enzyme conformation to that of the transition state complex. These two factors put together give you much higher catalytic efficiency as we noted in the case of one of the examples which I have illustrated to carry out the conversion of reactants into product. The dotted line here indicates the process. In fact I have shown here only one step just for sake of clarity and removing the congestion whereas this process might undergo more than one humps.

For example binding of E to A, the EA further binds to B in different sequences depending on the nature of catalysis. But there can be more than one processes undergoing here and thereby the binding process can provide you a large fraction of the activated energy to be compensated during binding process and reducing the energy of activation. Such a process therefore leads to a very efficient catalytic system.



In my first presentation I mentioned about the specificity. Coming back to the specificity the presence of ligand binding sites on the enzyme molecule are responsible for specificity.

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The so called ligand binding sites and when we consider ligand binding site along with the catalytic sites what we consider as active sites on the molecule they constitute for the specificity and we will discuss that in our subsequent lecture. So today if you recall we have considered in general the basic theoretical considerations behind the catalysis as it applies to any chemical process. Secondly how enzyme under the same background is able to provide much larger reduction in activation energy and provide a more efficient catalyst compared to chemical catalyst. With this we will stop.