Introduction to Biomolecules
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Lecture – 8
Enzymes (Part 2/5)

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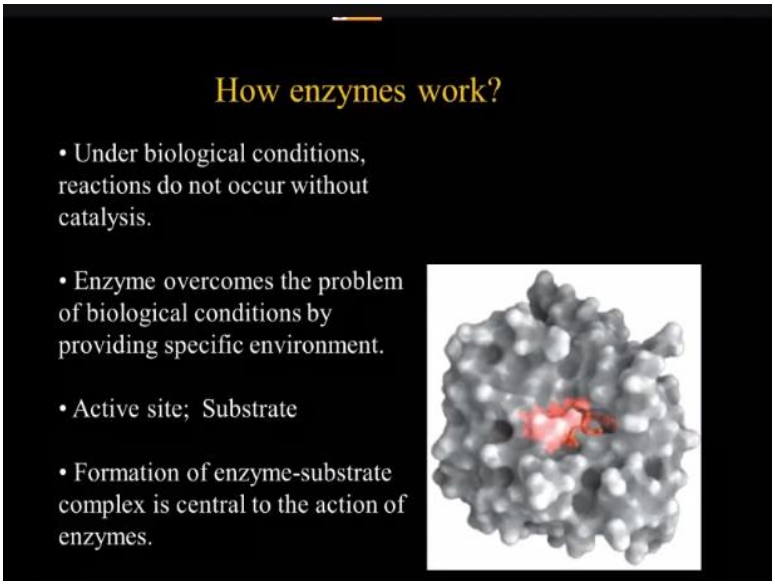

So, in the last class we were discussing the a part of our enzymes topic. So, this slide I have already explained.

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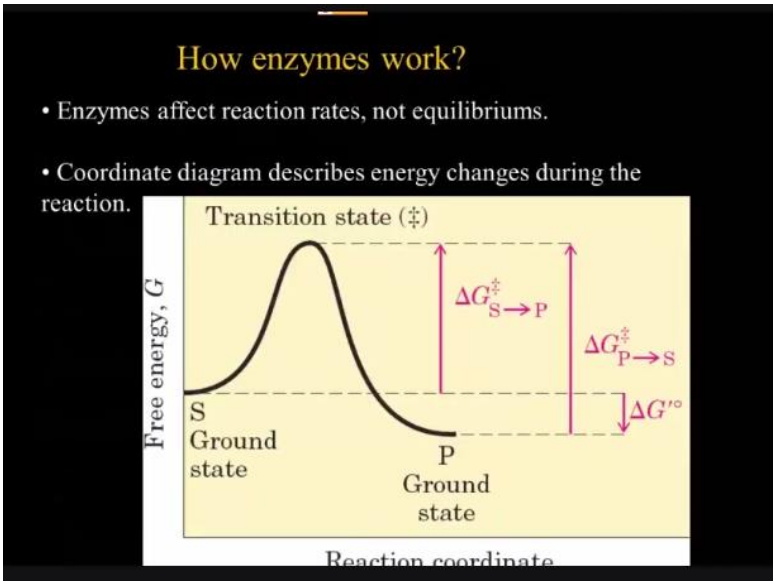

And then we went through this.

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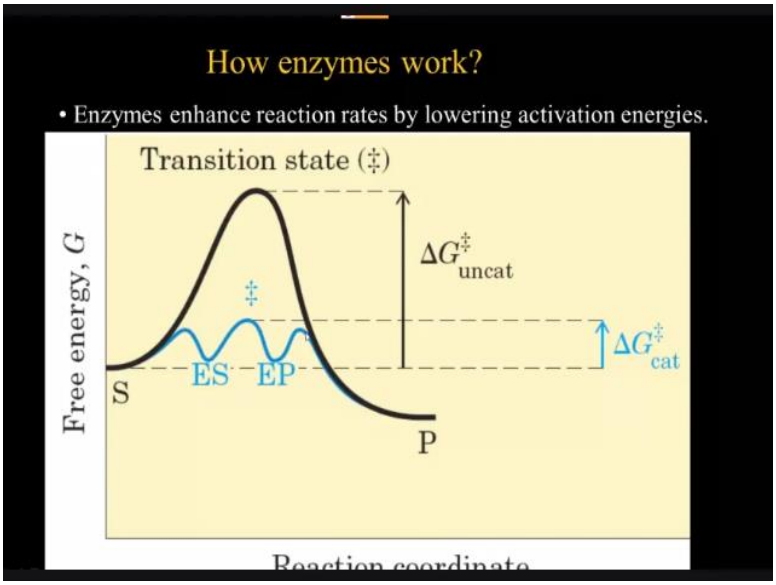

Then we saw this that enzymes reduce the activation energy and they have multiple intermediate steps.

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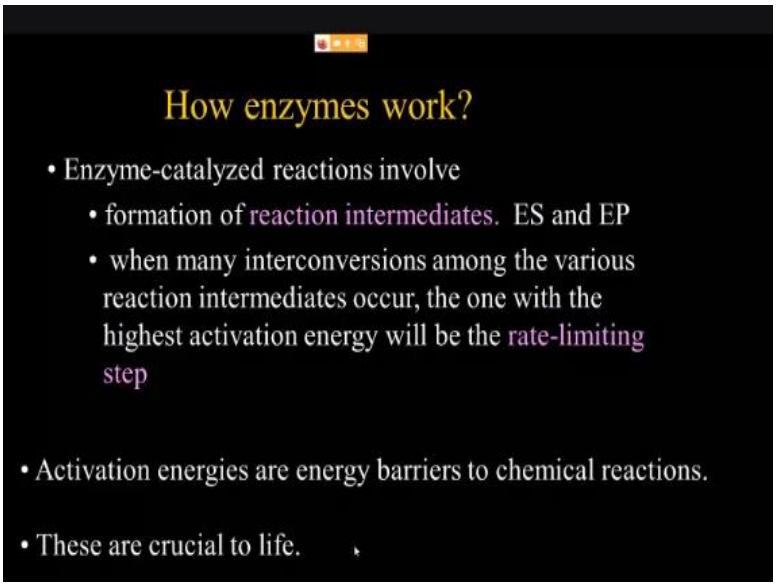

Then we also saw the reaction intermediates, rate limiting step and the activation energy barriers being crucial for life. So up to this we discussed in the last class. So, we will continue from here.

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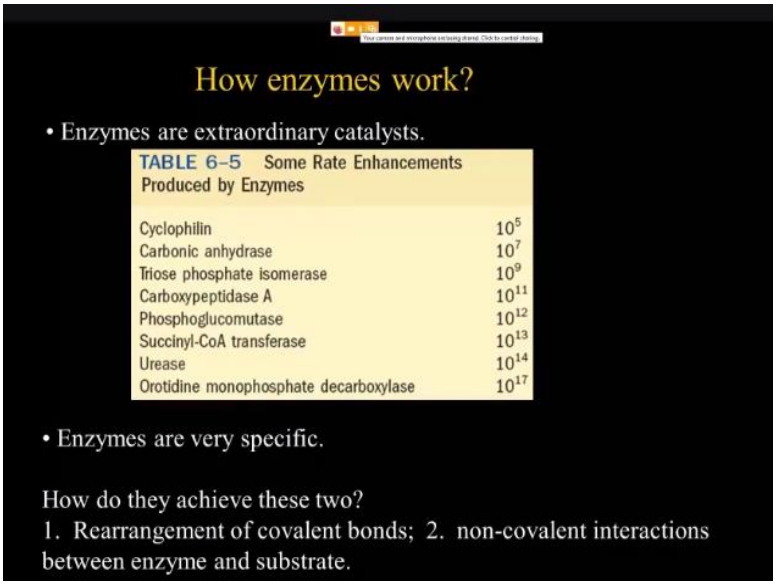

So, enzymes are extraordinary catalysts. You know it is not like a little help like you put a spoon into a tumbler having sugar in your tea and mixing it, and without that spoon if you just shake with the hand also it will get dissolved and that helped by the spoon is not that great. It is some catalyst but not great catalyst, but enzymes are not like that. They accelerate the rate of the reaction like several orders of magnitude as listed in this table.

So if you take the last one this orotidine monophosphate decarboxylase. It accelerates 10 power 17 times that of the uncatalyzed reaction. So, this is typical like 10 power 5 is kind of on the lower ones. Most enzymes are in this order like 7 to 11 orders of magnitude they accelerate the rate of the reactions. So, they are extraordinary catalysts and they are very specific. This I have highlighted multiple times.

An enzyme that is phosphorylated glucose at sixth position will not do so for characters at the sixth position. And I think the last time I used the example of digestion of starch versus cellulose. The enzyme in our body will hydrolyze starch into glucose, but not cellulose into glucose. So, enzymes are very specific. So, two important points here shown in these two bullets. One they are extraordinary catalyst and they are very specific.

Both of these features are accomplished using very similar mechanisms. S,o they achieve these two by these two features listed here. One rearrangement of covalent bonds in the reacting molecules and second several non-covalent interactions between enzyme and substrate. When the substrate binds to the enzyme, multiple non-covalent interactions hydrophobic interactions among hydrophobic groups.

For example leucine, isoleucine or aromatic amino acids like phenylalanine, tryptophan and so on. And you have polar groups available, hydrogen bond forming possibilities, you have NH 2 group and then you have OH. So, you have multiple electronegative atoms with the hydrogen bonded to them and as result hydrogen bonding possibilities. So, you could have covalent interaction as well among groups like serine hydroxyl group, threonine hydroxyl group, cysteines thiol group and so on.

So, these interactions many of them are non-covalent and therefore individually the strength of such interactions are very low, but then they are in large numbers, so they all add up. So, these non-covalent interactions between enzyme and substrate is really the key point when you talk about enzyme-catalyzed reactions. And why am I elaborating on these non-covalent interactions is because they are primarily responsible for fulfilling these two that is extraordinary catalytic power as well as specificity.

So if there are going to be a lot of such interactions, then specificity is going to increase. The right groups are to be at the right place and otherwise the interaction is not going to happen. And the very same interactions that are responsible for specificity, the energy provided by those bindings those bond formations all of them individually low, but collectively they add up and that binding energy helps in reducing the activation energy and increasing the catalytic power okay.

So, both of this is achieved by the same feature of the enzyme that is multiple non-covalent interactions between enzyme and substrate. And rearrangement of covalent bonds also happens and that we will see with specific examples as we go along.

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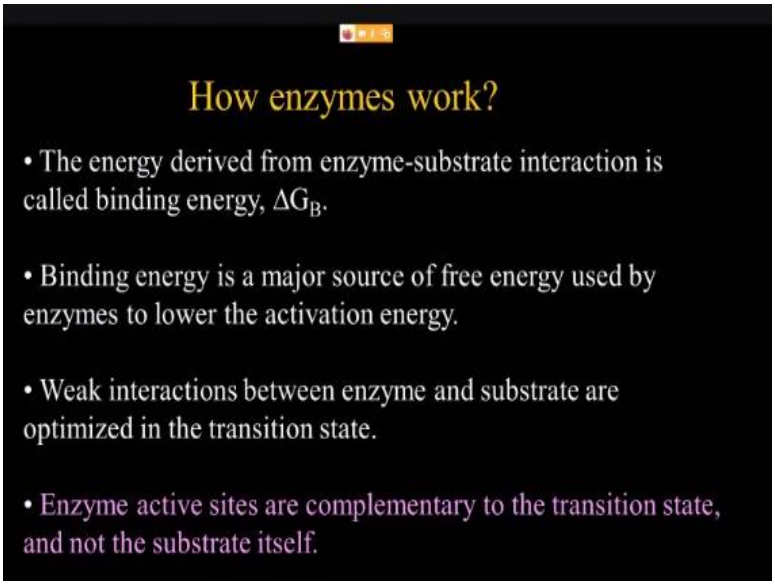

So, the energy derived from enzyme substrate interaction is what we call as binding energy and this binding energy is a major source of free energy used by enzymes to lower activation energy. So, this is something you need to remember. This is characteristic of all enzymes. So, the covalent bond formations are specific to certain enzymes and certain substrates that is not going to be there in every enzyme.

Whereas these non-covalent interactions multiple of them is common to all enzymes and therefore their contribution to reducing the activation energy is something that you need to remember as the primary hallmark of an enzyme. And these interactions are optimized in the transition state. So, this is something we need to specifically think about. So, the substrate binding to the enzyme is not like the way key fits into the lock.

So here the substrate at the ground state fitting into the active site would be same as key fitting in a lock okay. The key outside our key inside it is the same. It has not undergone any change in its structure. Whereas when the substrate binds to the enzyme active site to fit into the active site multiple groups are specifically oriented in the substrate such that some of the bonds are even distorted.

And those specific distortions happening in the substrate that helps in fitting in the enzyme leads to the transition state of the substrate. So now the substrate is at a level where it can actually become product. So, this transition state is what is the enzymes active site is actually complementary to this transition state. So this is an important concept. And similarly,

interactions with the substrate leads to reorientation or rearrangement of groups in the enzyme active site as well which is called the induced fit.

The substrate binding actually makes the enzyme also undergo certain conformational change such that the active site is now catalytically lot more powerful than the one before the substrate bound. So by the time these two bind, already they have undergone changes such that they are catalytically poised for the reaction okay. These are very important things to remember about enzymes.

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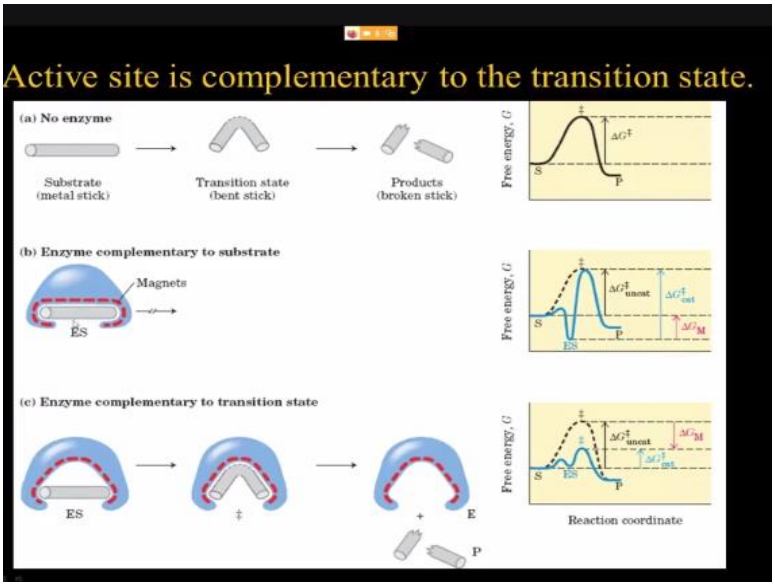

So, in a cartoon way it is depicted here. Suppose let us say you have a long metal stick and you want to break. So, if you want to go from here to here, this intermediate step is readily understandable. This is how you would have held both ends and given pressure in the middle such that it bends and the bend form can easily break into two. Without this intermediate step you do not imagine this breaking like this unless otherwise you are using a knife-like thing to cut it.

And if this is the transition state for this substrate becoming this product, then if you have a device that fits in this manner it is not going to break into this. So, the activation energy barrier will remain really high, but on the other hand in the process of this fitting into this device if the device is shaped like this and to fit it is going to bend like this. This is what we call as a transition state.

So enzyme active site, the active form of active site is complementary to the transition state and not to the substrate. This is something very important you have to remember. And this process is what reduces the activation energy and here you are making an important assumption that is the reason enzyme substrate complex forming. So today for the rest of the class I will keep on reiterating this idea of ES enzyme substrate complex formation.

And this is critical to explain the enzyme kinetics, the measurement of the rate of the reaction catalyzed by enzyme and how that rate gets altered when we change experimental parameters and those observed effects on the kinetics can only be explained by assuming the formation of an ES, enzyme substrate complex. And so this cartoon very clearly illustrates why enzyme active site is complementary to the transition state of the substrate and not to the substrate itself.

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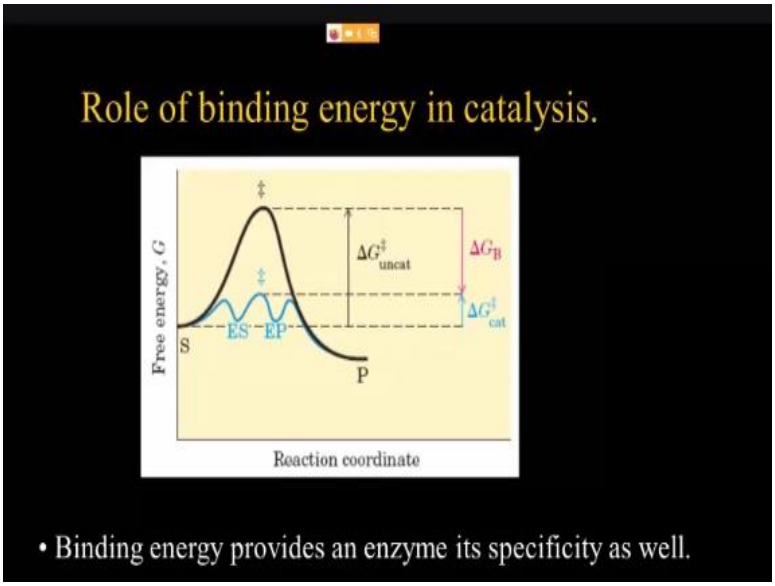

So, this I have already explained in the bullets like binding energy greatly contributes to the reducing activation energy and as well as the requirement of so many multiple bond formations ensure specificity as well okay. So, both extraordinary catalysis and extraordinary specificity comes from several non-covalent interactions between the enzyme and substrate.

And those interactions lead to substrate reaching into the transition state as well as enzyme active site undergoing conformational changes such that it fits the transition state and that process of enzymes active site change is what we call as induced fit. The fit is induced here by binding of the substrate okay.

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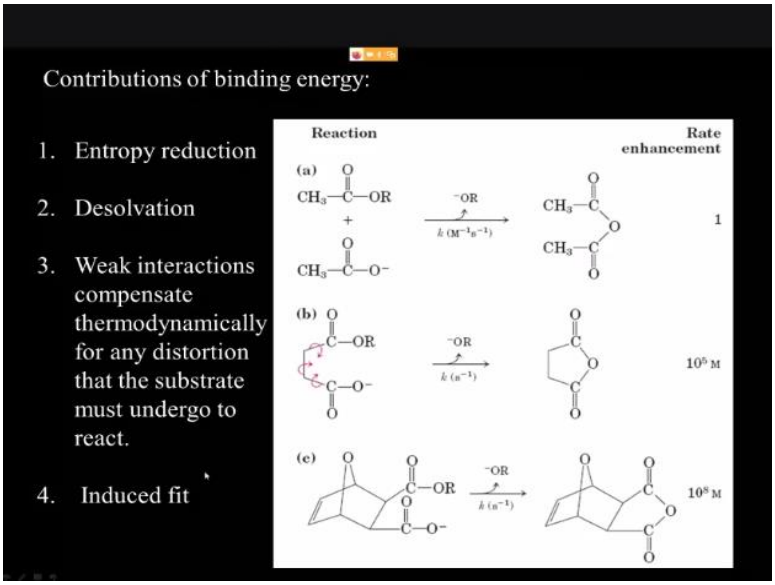

So, this simple organic chemical reaction illustrates how these things help. So, for example if you take this you have an ester bond getting hydrolyzed and then you have an anhydride bond formation okay. So, this is acetic acid forming an anhydride bond with another acetic acid. So here you have two reactants and to use our terminology two substrates. So therefore, it is a second order kinetics where the rate is dependent on the concentration as well as indicated here in this k times moles per second.

And if you take this as equal to 1 like the rate enhancement if you arbitrarily take it as 1 and when you link these two functional groups that are going to interact to make this molecule in this manner, now the reacting groups are so next to each other that it is an intramolecular reaction because they are connected. And since it is intramolecular reaction happening only within one it is first order kinetics and it is not dependent on the concentration.

And by virtue of that itself from this to this you have at five orders of magnitude the catalyst is increasing the rate increases. But even then as indicated by these pink arrows, you have bond rotations possible in this. So, these two groups need not be just rigidly placed next to each other due to these rotations. Now if you constrain these rotations by adding additional structure to it and these are rigidly held.

Then they more readily react and that is another three orders of magnitude reaction increasing. This is the sort of thing that happens in the enzyme active site. The first thing binding of the two reacting groups in the active site converts from an intermolecular reaction to an intramolecular reaction. All those reacting groups may be on two different molecules,

but now in the active site when they are bonded, they are virtually converted into a single molecule and therefore it is like intramolecular reaction.

And then further interactions with the other active site groups in the enzyme probably orients the functional group such that they do not even have this much flexibility and due to that the reaction more readily happens okay. So, this is the entropy reduction okay. So here the entropy is very high reduced further reduced. And the second one the substrate all enzymatic biochemical reactions in our cell or in our body if you are talking only about a human, all happen in an aqueous environment.

It is all water based. So, the substrates are well in interaction with water, they are hydrated. So, you have a hydration shell around them. So, if you have an amino acid the carboxyl group as well as the amino group and if the side chain has polar group they are all happily interacting with water. When they are happily interacting with water, they are not going to interact with someone else, they are not even seeing someone else.

So, getting the molecule out of water actually is important for the reactions to happen. So the binding at the active site takes the substrate away from water and that is why it is desolvation as an attribute for rate enhancement. Then these weak interactions thermodynamically compensate for the distortion okay. Normally in free solution those distortions are not stable, they are not thermodynamically stable.

They are like somebody walking on a rope few 100 feet above the ground, they are going to fall down. But instead, these interactions make sure that that person has a belt and a handrail around and therefore he is not in danger of falling down kind of situation. So, these weak interactions make thermodynamically stable those distorted structures and induced fit is something I have already explained in detail. So, these are 4 means by which enzymes actually enhance the rate of reaction.

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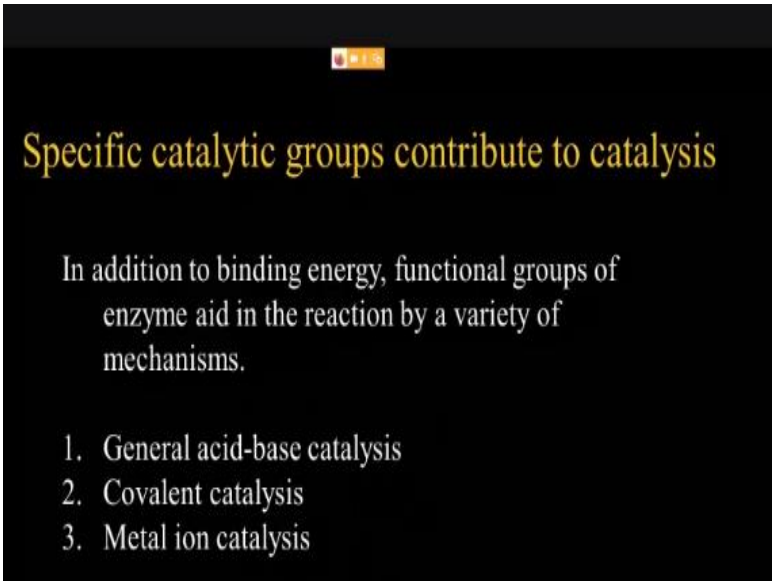

And in addition to this so here we saw how the enzyme might orient to the reacting groups at the active site. And in addition to this, enzyme active site provides additional groups for specific catalytic mechanisms, 3 of them are listed here. These are the most common ones. So, they act as general acid-base catalysis. So here I will explain this with an example as we move on.

So, this is one mechanism and another one is covalent catalysis, another one is metal ion catalysis. So, these are the 3 mechanisms that the enzyme active site can actually provide in making sure the reaction happens. So let us look at one by one.

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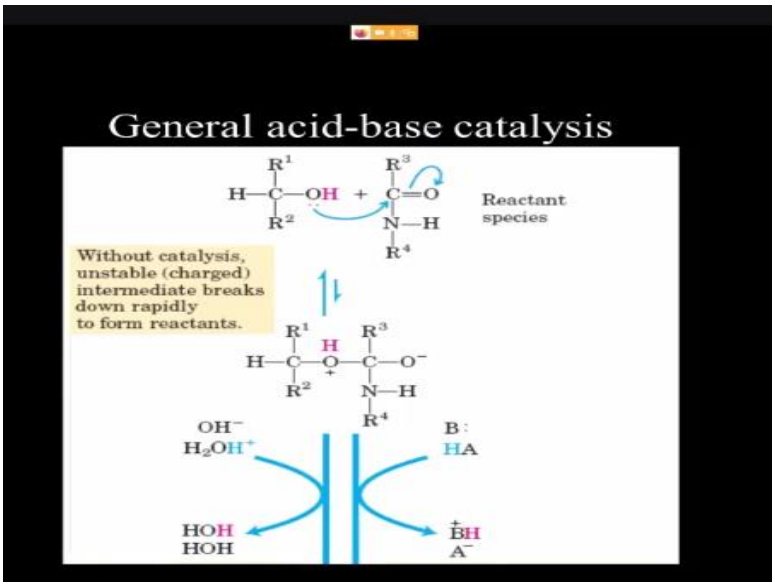

So, let us take acid-based catalysis. So, acid-base catalysis means for a reaction to happen temporarily if a proton needs to be abstracted and the resulting charge is unstable, if the

intermediate there is a resulting charge is unstable and if it is going to readily breakdown back into substrate then the reaction is not going to happen. But on the other hand, if someone can hold on to that proton or someone can balance the charge in the intermediate, then the intermediate will become stable and it could go towards the product.

So therefore, proton acceptors and proton donors can actually be very helpful in making sure certain reactions happen simply because the transition state intermediate is probably an unstable charged structure that normally tends to break down back into the substrate. So, they do not cross the barrier. They climb the mountain, but then they constantly slip back, they do not reach the peak to go to the other side.

And there if someone stands on the peak and holds the hand then this molecule can get there. So, there is that sort of an analogy. Since you know these reactions happen in aqueous phase, water itself can do it. So, we have seen that water can ionize into hydronium ion and hydroxyl group that one water ionizing the proton gets hydrated and that is why you have hydronium ion. So, water itself can do this.

So, if the electron transfer or rather the proton transfer to water is faster than actually breaking down to substrate, then the reaction will happen on its own in water environment okay. So in this example what you are seeing is without catalysis the unstable charge intermediate breaks down into the reactants. But on the other hand if the speed with which this proton can transfer to water, then the reaction can go forward with water acting as the proton acceptor okay.

I am ignoring temporarily for simplicity the proton in a donor part. So, I am only talking about the acceptor part. So, water itself will do it and when water does that you call it a specific acid-base catalysis because it is specific to water doing the activity.

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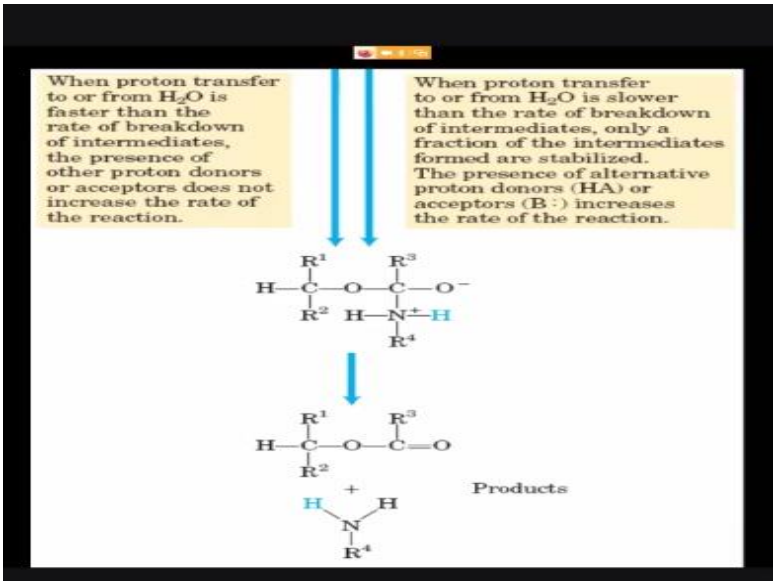

So, I just said this I just want to read it once more for clarity when proton transfer to or from water that is acceptor or donor situation is faster than the rate of the breakdown like from here going back. If this is slower than transfer to water, then it readily happens in water itself, you do not need to have any other proton acceptor or donor okay. And their presence does not help because the rate of transfer to water is faster and water is there plenty.

So, you are not going to have something that can do better than water. But on the other hand if the proton transfer to or from water is slower than the rate of breakdown okay, so that means this happens a lot faster than the water taking care of this charged intermediate, then very little gets stabilized in this structure. So, they mostly remain like this. Under that circumstance if you have one more group that can very readily like accept proton or donate proton faster than the transfer to water, then that helps in the catalysis.

So, when they provide a proton then you call it as acid catalysis, when they accept then you call base catalysis okay and this is what you call as general acid-base catalysis where the proton donor or acceptor is not water but something else and that you call as general acid-base catalysis. So here the catalyst by temporarily accepting a proton or temporarily providing a proton stabilize an unstable intermediate structure and that then it can move forward the group.

If you follow the electron flow as well as the color of the atom from where the proton comes, then you will understand how the reaction is made possible. Here what you are seeing is an amide bond cleavage okay which is common in protein. So, if you want to break the peptide

bond between two amino acids, then you are actually breaking this bond okay. So here this is what we are seeing and this is the one amino acid broken down.

And here this is attached to this and these intermediate steps are balanced because acceptor or a donor help in stabilizing the intermediate step. And this is readily possible in an enzyme active site because we have amino acids with side chains that can donate or accept protons. So, this you have already seen when we were looking at the amino acid structures.

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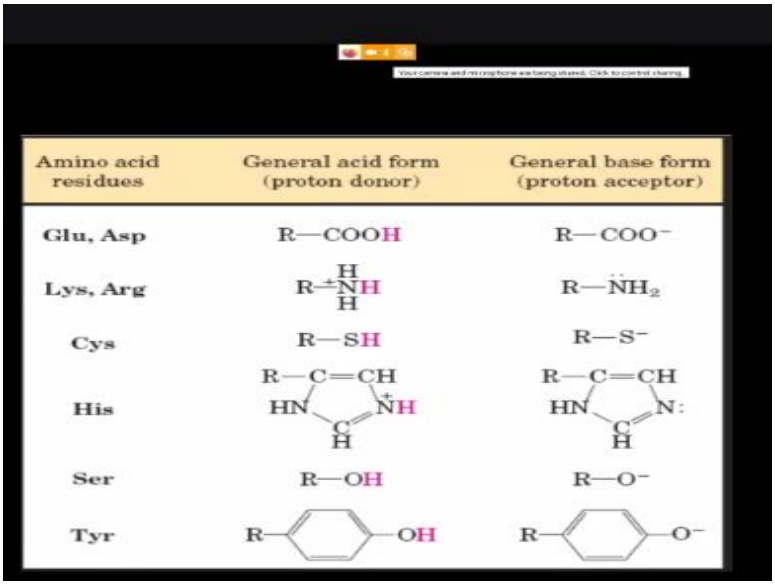

We will see that once more here. Like for example here if you take glutamic acid and aspartic acid, they have a carboxyl group in the side chain. Even when their amino group and carboxyl group are in peptide bonds on either side, the side chain carboxyl group can act as a donor and the dissociated form can act as an acceptor. Whether it exists in this form or this form depends on the pH. So as a result, the enzyme activity is highly dependent on pH.

So, this is something we have already seen. So, lysine and arginine they have the basic group this amino group and that can be protonated or in which form it can act as a proton donor or it can act as proton acceptor when it is not protonated. And thiol group of cysteine and histidine's imidazole group can act like the way lysine and arginine do. And the serine and the serine's hydroxyl group, tyrosine this is hydroxy phenyl group, so this group.

Similarly, threonine also has a hydroxyl group, so they can all I have participated in the acid-base catalysis. This is one of the common mechanisms of enzyme-based catalysis. So, acid-

base catalysis is not specific to only certain enzymes, many enzymes use this mechanism okay.

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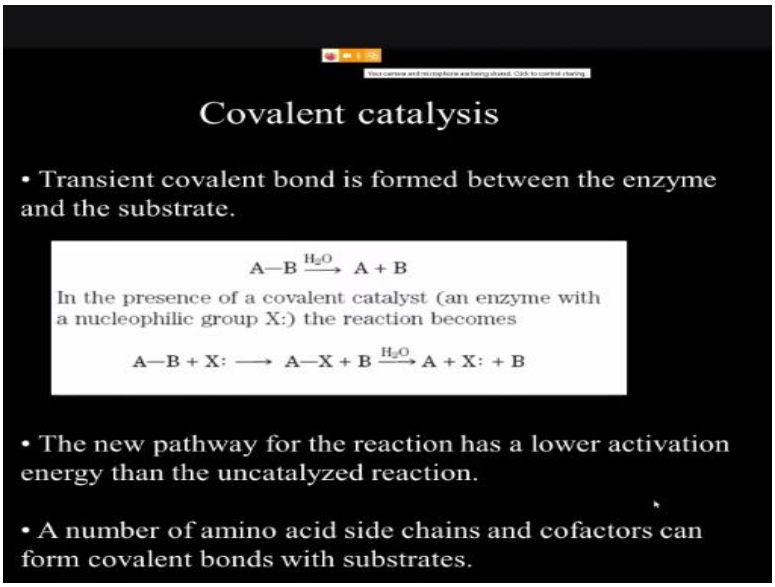

So, the next one is covalent catalysis. So, where an enzyme's active site group participates temporarily in a covalent bond with a moiety in the substrate. Here for example A B getting cleaved into A + B. You can take that amide bond earlier example itself where peptide bond is getting cleaved. So, two amino acids getting hydrolyzed into individual amino acids A and B. So, you can think of that situation or you can take sugar getting cleaved into glucose and fructose.

So, there are many situations where you have this after we are talking about polymers right like nucleic acids, proteins, carbohydrates they are all polymers, so this sort of reaction is very common. So, in the presence of a covalent catalysis, so in this particular case a nucleophilic group like this if it is an amino acid side chain then that can readily attack this bond and get attached to A.

So, this intermediate can more readily form than these two forming directly from this. This is equivalent to that metal rod in bend the position and now this hydrolysis readily happens regenerating this active site group back and substrate leaving the active site, the hydrolyzed first part of the substrate. So, this sort of a covalent bond formation in the active site is what you call as covalent catalysis.

This happens quite often with these kind of enzymes like cysteine, serine, threonine, tyrosine where this hydroxyl group, this alcohol group or this thiol group participate in covalent intermediate formation. And when these sorts of things happen, the new pathway for the reaction, this is one pathway, this is a new pathway instead of going from A B to A + B, this goes via A X + B and then A + X + B.

So, this new pathway has lower activation energy because this bond formation requires lot less energy like this transfer from this covalent bond from B to X requires lot less energy than breaking this out right. And similarly hydrolysis of this requires less energy and so this is how this new pathway reduces the activation energy compared to the uncatalyzed reaction. And I just told you this, the number of amino acid side chains.

Cofactors we have not had gone into, although we have learnt initially at the very beginning of the enzyme discussion that metal ions and vitamins function as cofactors and coenzymes and when they are tightly bonded, we call prosthetic groups. So, they can also help in this covalent catalysis.

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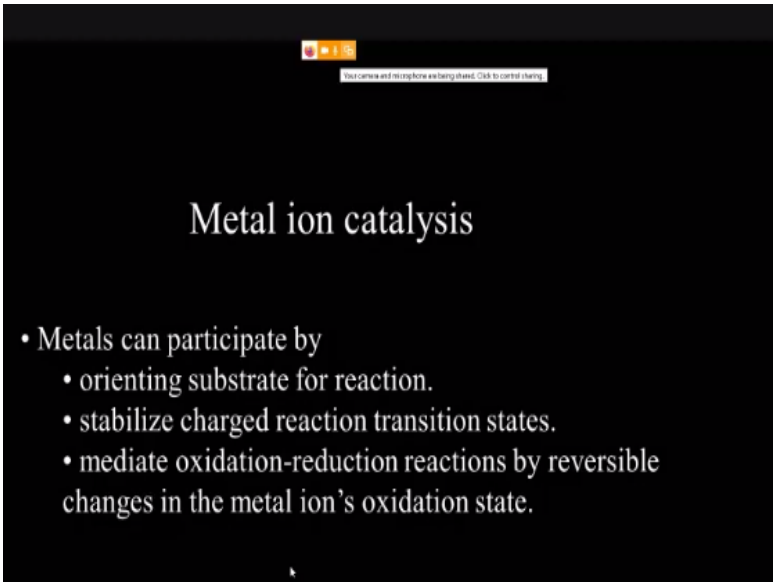

So, the last one is metal ion catalysis. So here metals bound to the enzyme active site participate in catalysis. So, through their charge they could interact strongly in an electrostatic manner with the substrate such that the substrate is specifically oriented for the reaction. And again, by virtue of the charge on them they could stabilize the charge reaction intermediate. Sometimes these metal ions do lot better than the amino acid side chain simply because the charge on them is higher.

And due to that they can stabilize the transition state better. And they can participate in oxidation reduction reactions by reversible changes in metal ions oxidation state because many metal ions exist in multiple oxidation states. We will see one great example when we are going to talk about how in photosynthesis for example water is split into hydrogen and oxygen and that exploits the multiple oxidation states of the manganese ion in that particular enzyme.

Many ions have multiple oxidation state. For example ferrous, ferric, cupric, cuprous. So, like that you have multiple metal ions that have multiple oxidation states and by reversibly going from one state to the other state they can help in electron transfers, oxidation and reduction, lose electron and accept electron. So, these are the ways by which metal ions help in enzyme catalysis.

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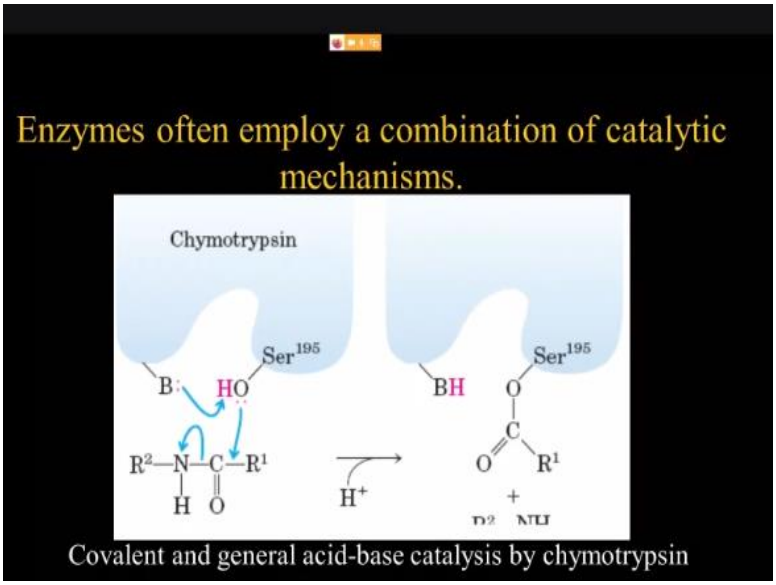

So, we saw these acids, these are all separate mechanisms. It is not that an enzyme that uses acid-base catalysis does not simultaneously use covalent catalysis or does not benefit from the presence of a metal ion. Many enzymes use the combination of these catalytic mechanisms. So, shown here is a protease chymotrypsin which hydrolyzes a polypeptide chain into amino acids. They cleave at the C-terminus of lysine or arginine.

And this is an amide bond cleavage, it is very much like the one that we discussed a few slides ago. So, in its active site you have a serine hydroxyl group that participates in covalent catalysis you see serine's this alcohol group in linkage, it is in an ester linkage with the

carboxyl group of this amino acid with R1 group. And just before that step another amino acid side chain in the active site acts as a base catalysis okay.

It acts as a proton acceptor temporarily. So, like for example the proton leaving from this is stabilized by this and due to that this becomes a reactive oxygen that more readily reacts with this carbon. This carbonyl carbon is going to have charge imbalance here because this is a strongly electronegative and this is going to have a partial charge and due to that this very readily reacts with this and intermediate covalent structure forms.

So initially base catalysis followed by a covalent bond formation. So, this is an example from chymotrypsin. So, the main point here is enzymes often employ a combination of catalytic mechanisms. So, we have seen three catalytic mechanisms. One is general acid-base catalysis where the active site group either donates or accepts a proton and by doing that it stabilizes an otherwise unstable intermediate.

And the second one we saw is covalent catalysis where a covalent bond is transferred from a substrate temporarily with an active site residue in the enzyme and that is energetically more favorable than converting directly into product. And then the third we saw metal ions. So here this is an example where we find the first two being used in the single reaction, but there are some enzymes where all three are also used. So, this brings us to the end of a general introduction to the enzyme catalysis.

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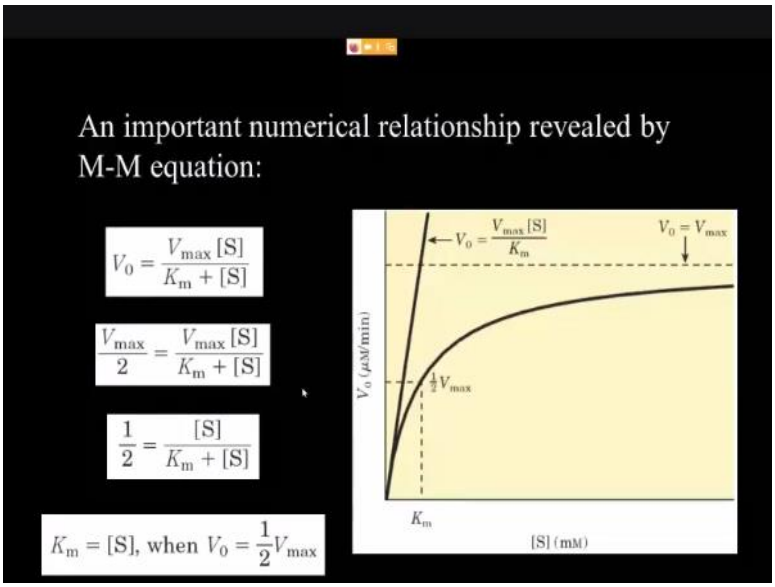

Now we are going to specifically talk about how do we get to the enzyme mechanisms okay. Like how do I find out how what specific groups are involved in a particular reaction and how it is really bringing about the conversion of the substrate to product, the reaction mechanism essentially the well detailed electron flow diagram. So that is a very challenging question in enzymology.

And in modern times what we do is we really use multiple techniques to identify the active site residues and their orientation. So, for example we purify and crystallize the enzyme and using the crystal you determine the three-dimensional structure of the enzyme such that you know what groups are positioned in what way at the active site and that helps in understanding mechanism.

And we also can mutagenize meaning in the enzyme we can change different residues by combination of methods we call side directed mutagenesis. By replacing the different residues and looking at the structure and the rate of the reaction we can really get to understanding of the reaction mechanism. And the other method which is historically more ancient and still very powerful is measuring the rate of the reaction and manipulating the rate by changing experimental parameters.

So, what kind of experiment manipulation of experimental parameters affect the rate in what way that sort of a measurement helps in getting at the mechanism. And that is what was readily doable before these sophisticated structural biology techniques came and this is what we call as enzyme kinetics okay.

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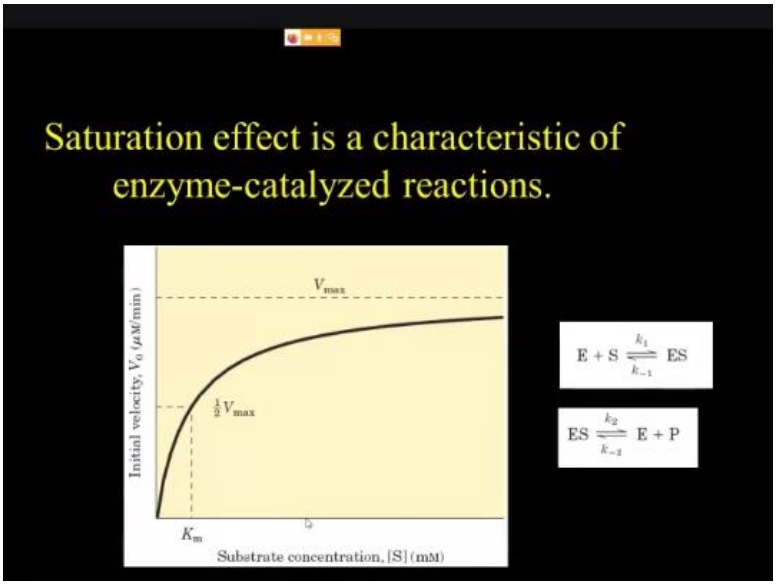

So, I was worried that I missed slides. So, this is the first slide here. So, the enzyme kinetics means you are measuring the rate of the reaction okay and you manipulate the experimental parameters and see the consequence of such manipulations on the rate of the reaction doing well as enzyme kinetics and this has been the bedrock of getting at the enzyme mechanisms for a long time and it continues to do so.

Now the structural biology and site-directed mutagenesis go hand in hand with rate measurements in finally getting at the enzyme mechanism. Okay so let us now take you know small steps in understanding enzyme kinetics. So, this is a very introductory course. We are not going to get into the kinetics in great detail. So, we will only see the preliminary part to get an idea but to get a flavor of the how enzymes function.

So, the first one is illustrated by this graph. So here you have the rate of the reaction plotted against change in substrate concentration. So, as you increase the substrate concentration, the rate of reaction increases. But this increase the rate of increase keeps changing, initially it is steep increase as the substrate increases, but after a point it starts to slow down, so the rate of the reaction does not increase any more.

And when you reach the substrate concentration at a certain level at a certain substrate concentration, the rate no longer increases. It has reached the maximum possible rate and that we call as V max. This graph is intentionally drawn with this actual plot not getting to the V max simply because in practical measurements you never reach V max but you get very close to it and that is why this dotted line here showing the V max point.

So, this is a characteristic of enzyme catalyst reaction and this plateauing of this rectangle shape curve happens purely due to what we call a saturation effect. And the saturation effect results from this formation of enzyme substrate complex. Remember a few minutes ago I was telling this I will keep repeating this enzyme substrate complex formation is critical in understanding enzyme catalyzed reactions.

And this readily forms and from this breaking down to E + P that ES breaking down is slow and that is why when you reach a concentration of substrate in which the definitive concentration of enzyme that you began with. So, in this entire process enzyme concentration is kept constant. So, you will reach a point where all the enzyme is in ES because this readily happens, but then this breaking down to this happens slow.

So, therefore this k 2 reaction becomes rate limiting and due to that you reach a point where there is no free enzyme available anymore to combine with substrate and that is what we call as a saturation effect and that is the reason why the graph plateaus off okay. So, this plateauing off or the saturation effect is a characteristic of enzyme catalyst reaction. This does not happen when you put the spoon into the tumbler with the sugar and mix it okay.

So, you do not see this in other chemical catalysts. So, this is very characteristic of only enzymes and that is primarily because of ES formation. So, in this sort of a reaction if we are going to call let us say V 0 we are looking at the initial velocity. So, the reason we are looking at initial velocity is as the reaction happens the substrate concentration changes because it is getting converted into product.

And therefore, measuring the rate in terms of the substrate concentration at later stages is not readily possible, primarily because ES is not easy to measure because we do not know what is ES and how to measure its concentration. It is an assumption, we try to understand this is what it happens, but of course ES complexes can be crystallized now and we can know their structures but even then while measuring the reaction as the reaction is happening measuring ES is not easy.

So, we try to always focus on the initial stage where the substrate change is very little that it can be actually ignored. And this V 0 is what we focus and the initial velocity V 0 will be a

function of ES and since measuring ES is not easy people had to derive an equation where it can be defined alternatively where the components of that equation can be readily measured.

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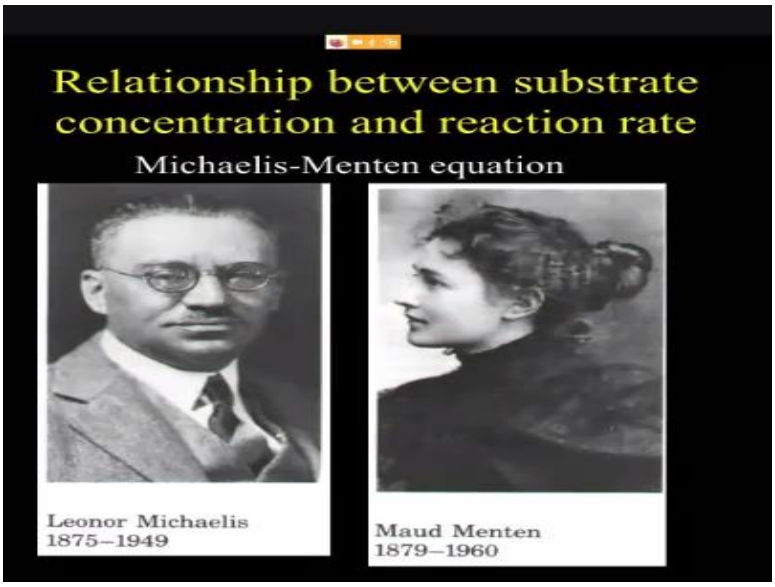

And that was accomplished by two famous scientists Michaelis and Menten. They did this work more than 100 years ago, 1913 they put forward their equation and more than 100 years later it is still the way we measure the rate of reactions okay. So forever we are going to remember Michaelis-Menten equation regardless of which branch of biology you are going to learn. So essentially what Michaelis-Menten equation does?

It relates the reaction rate with substrate concentration. So, by measuring the substrate concentration I can get an idea of the rate of the reaction, so that is the equation they provided us.

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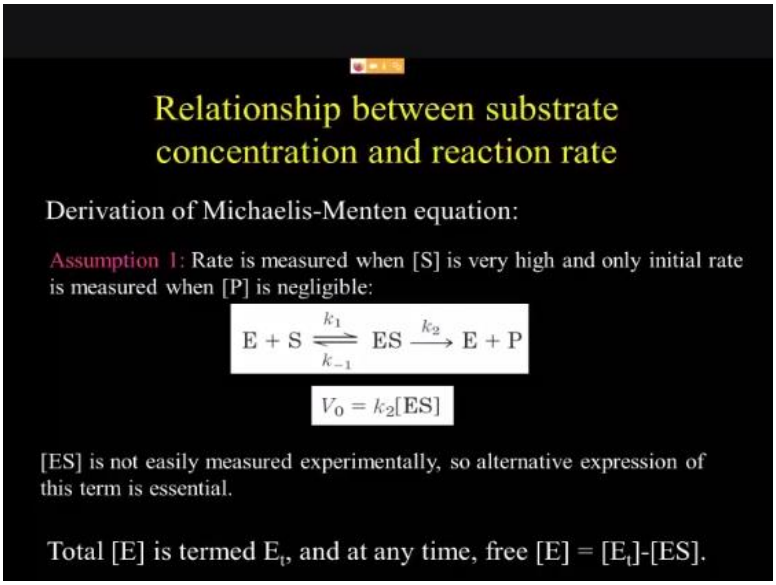

So, let us look at how to get to that equation. So their equation in modern times is very simplified. So, we have a simple way of deriving this equation and we make couple of major assumptions and we make ourselves aware of two important facts in the process of the reaction. So, the first assumption is that we are measuring the rate of the reaction very early okay, the initial rate. So, we have two stages here.

There is one thing called pre-steady state meaning before reaching the steady state, I will explain steady state as well. First the pre-steady state is very initially ES is just forming, ES is just accumulating that is pre-steady state okay. So, there was not enough ES and only ES is forming. So even this is not happening only k1 is valid and after some time still very initially in terms of the amount of substrate getting converted into product you reach steady state.

Meaning you reach a state where ES concentration remains constant, meaning the ES breaking down either side like going back to E + S or E + P like k –1 or k 2. Now k –1, k 2 these two reactions, the rate of these two reactions are equal that is what we call as the steady state that is when ES concentration is going to remain constant. So, the first assumption is we are measuring it very early such that the substrate concentration is very high and the product formed is negligible.

And as a result, this k –2 equation this reaction we are going to ignore that is assumption number 1. Assumption number 1 is that we are measuring very early at the point where the concentration of product is negligible, therefore we do not consider this reaction. This reverse

reaction of E + B giving ES and that is the reason k –2 is not shown in this equation. It is not because this is an irreversible reaction.

Under this condition, the rate of the reaction will be equal to, so this is the rate of the reaction ES becoming E + P so that is what will be actually S having become P. So, this is the rate limiting step, so that is the one that is going to define the rate of the overall reaction. This we saw at the very beginning. And therefore, it is proportional to the concentration of the reactant, so here that is ES and that proportionality constant is k 2.

So V 0 = k 2 ES, so this will be the rate of the reaction under this assumption. And we cannot readily measure ES, I just told you and due to this we need to rearrange this and that is what Michaelis and Menten did. So before going into assumption 2, we are going to remind ourselves of two important facts here. One is we are going to call the total concentration of the enzyme as E t. So enzyme can exist as free unbound enzyme or bound enzyme as shown here.

So E t will therefore be equal to ES + E okay, so this is unbound enzyme or rather if you want to always talk about the free enzyme unbound enzyme then that will be E t – ES shown here. So, this idea of E t we are bringing into our attention here. And the second one is we are assuming that when the ES concentration is very low compared to substrate concentration, remember we are measuring initial rate this is the major assumption here.

So, the amount substrate bound to the enzyme is going to be negligible, so we are going to actually ignore that. In that sense the k 2 will actually be equal to E t, E t meaning the total enzyme. So, you need not worry about that point for now, but you just aware that initially the fraction of the substrate existing in the form of ES will be negligible okay.

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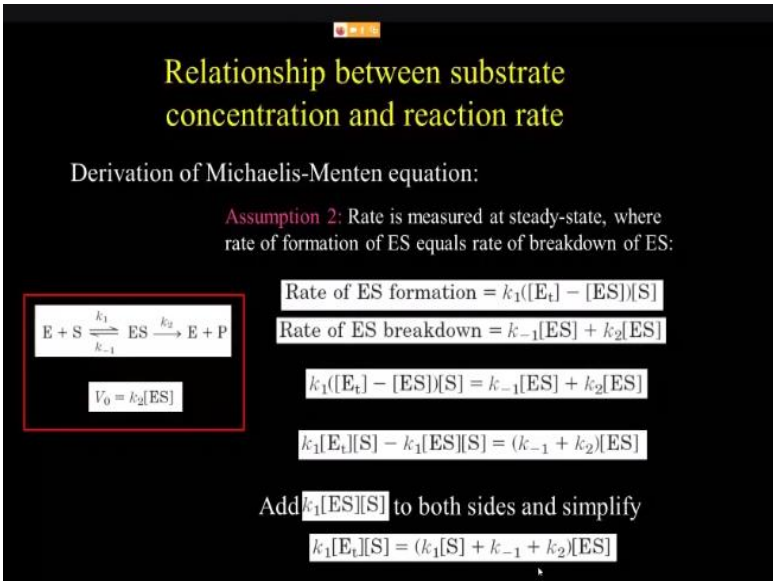

So, with those two in mind and with that assumption one that we are measuring initial rate, we go to the second assumption. So, second assumption I kind of mentioned already while defining the pre-steady state, I had to define steady state. So, the steady state is what is assumption 2. So, we assume the reaction is at steady state where the ES formation and breakdown both are equal okay.

So this is the major assumption of the Michaelis-Menten kinetics here. So now let us look at the rate of ES formation. Rate of ES formation will be dependent on the concentration of these two and then we have already defined E = E t – ES, so that is introduced here instead of calling k 1 = E times S, we are calling it as E t – ES times S and rate of ES breakdown therefore is this you know one is ES breaking down.

So therefore it is k –1 times S + also k 2 times ES, so breaking down in this direction. So if it is steady state then this equals this and that is stated here. Then you multiply this and you get this then you rearrange and simplify then you get this. So, in the interest of time as well as assuming that these are all very simple algebraic equations and you have done lot more complex ones in Math.

I am not taking time to derive the equation myself and instead I am directly showing it in Power Point, I hope it is not a problem for you to understand. So now to further simplify what we are going to do is we are going to add this factor to both sides and when you do that and when you do this to this side and it becomes just k 1 E t S and this side if you add and then rearrange then you get this.

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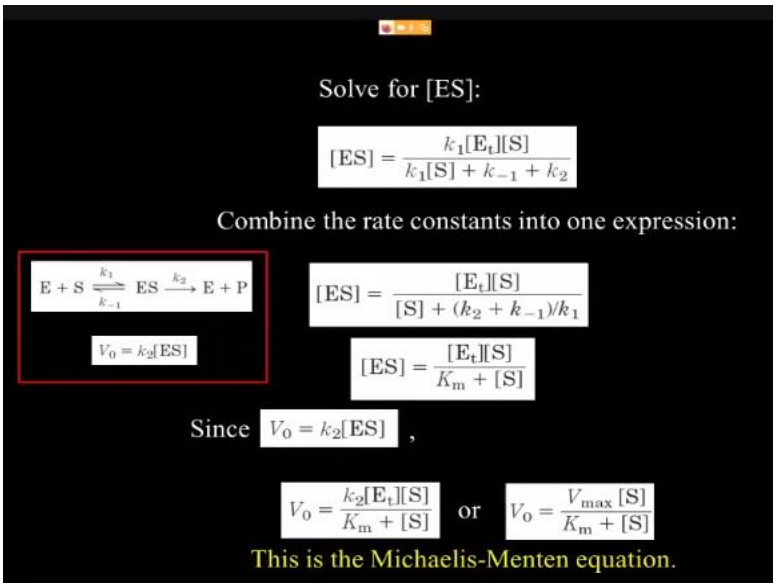

And when you try to bring all these constants together and you derive the equation solve it for ES like you take this ES out, so it will be k 1 E t S that is what is this side and then divided by this. Now you rearrange such that all the constants are together then you get this equation okay. And all these constants together we call them as Michaelis-Menten constant or K m which is an important parameter of enzyme catalyst reactions okay.

And now our goal we know that ES cannot be readily measured so it has to be rate of the reaction. So, we are going to go further with it and since V 0 is just to remind I have it here k 2 ES. So, therefore now ES is this that means you multiply this with k 2 to get V 0 going by this relationship. So V 0 = k 2 E t S divided by K m + S. Now we know E t is the maximum enzyme available. So, V max will directly be dependent on E t.

At that point when the substrate constant is very high you are going to ignore the ES component there and therefore k 2 E t is what will be V max. So you substitute this k 2 E t with V max then you get the Michaelis-Menten equation which is V0 = V max S divided by K m + S okay. So, this is the major equation for all enzyme catalyst reaction. So here each of these parameters are readily measured one.

Second this is consistent with observed experimental results. Even if you look at the theoretical points, you will readily understand from that parabolic curve the saturation curve that these relationships are met. So, let us try to look, I guess I am running over time, so I will stop here with the derivation of the Michaelis-Menten equation. So, in the next class we will

look at the implications and usefulness of Michaelis-Menten equation in actual enzyme catalyzed reactions.