Introduction to Biomolecules
Prof. K. Subramaniam
Department of Biotechnology
Indian Institute of Technology - Madras

Lecture – 10
Enzymes (Part 4/5)

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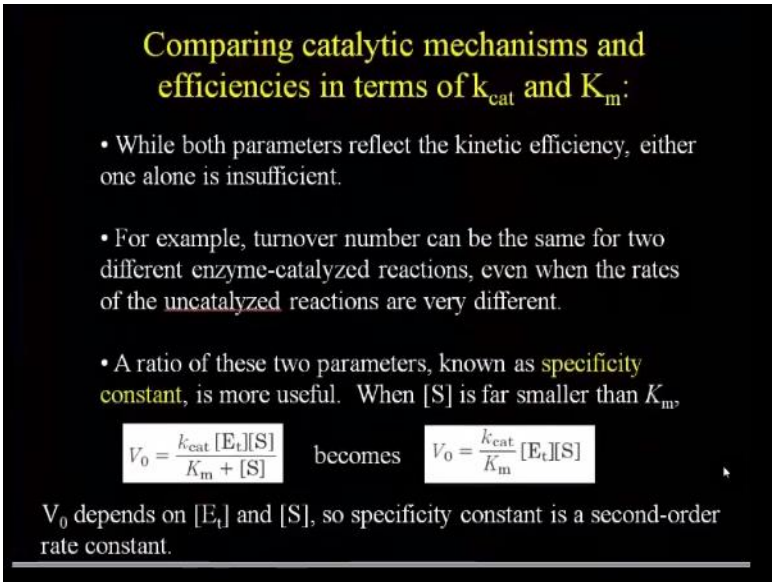

So yesterday I think around the time I was explaining about this k cat that is the rate constant for the rate limiting step. When we think of enzyme catalyzed reaction, these reactions have intermediate steps like for example S does not become P, S combines with the enzyme to become S and that is an intermediate step. And when the ES becomes EP that is another intermediate step. Then you have the EP dissociating as E + P.

So, among these intermediate steps whichever is the slowest becomes the rate limiting step and the total rate of the reaction would depend on that particular rate of that rate limiting step. So, the rate constant for that kind of a rate limiting step is what we call as k cat and the k cat is also known as the turnover number because it basically indicates the number of substrate molecules converted into product per single molecule of enzyme when the enzyme is saturated with the substrate concentration.

So now what we are going to do is we are going to consider how well k cat and K m tell about the performance of the enzyme which is technically called the catalytic efficiency. So, our goal is to have a parameter that accurately reflects the performance of the enzyme. So,

meaning how well the enzyme functions as a catalyst. So that depends on what is the rate acceleration over the uncatalyzed reaction okay.

So that is not indicated by k cat, k cat is simply the turnover number. So, the main point here is the turnover number for two enzymes can be same. Meaning k cat can be the same but one enzyme may be catalyzing several orders more than the uncatalyzed reaction compared to the other one. So, which means the first enzyme in this example is doing better than the second one but that is not indicated by the k cat, k cat is simply the turnover number.

And similarly, when you look at K m, many enzymes or most enzymes their K m value depends on naturally is the given substrate low abundant or high abundant in the cellular environment. So, the enzymes acting on substrates that are low abundant in the in the cell usually have lower K m. Otherwise they are not going to be useful for converting that substrate into product compared to the enzymes that work on high abundance substrates.

Where the K m is relatively higher and that difference in K m does not reflect on actually the performance of the enzyme because K m may be higher but then the substrate is also abundant. So, it does not matter that the K m is higher. So due to this neither k cat nor K m accurately reflects on the ability of the enzyme as a catalyst okay. So therefore, we use a ratio of these two called the specificity constant which actually more accurately reflects the enzymes' performance.

So, if the catalyzed reaction if the turnover number is high then you are going to have a high k cat and at the same time the same enzyme can work at a low substrate concentration that is at a low substrate concentration itself if it can reach half V max then that would be K m. So then the ratio will be higher. So higher the ratio better the performance of the enzyme and this reaches a maximum that is limited by the rate at which molecules diffuse in the cellular environment.

Like for example the rate at which the substrate diffuses within the cytoplasm and become available for the enzyme that becomes the limiting step. So that is something on which enzyme cannot do anything. So environmental factors like the diffusion rate becomes a limiting. So, if an enzyme's specificity constant is at the limit of the diffusion rate, then you say the enzyme has actually achieved perfection.

So here as given by this equation V 0 depends on the concentrations of these two this k cat by K m is a second order rate constant.

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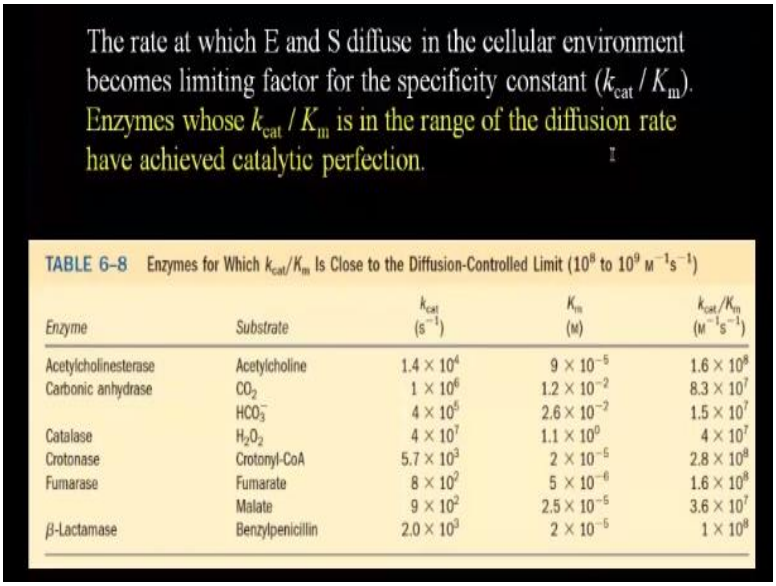

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

So here are some of the enzymes and their substrates and the corresponding k cat, K m and the ratio are given and if you look at it the diffusion control limit is in this range and some of these enzymes are already there. So, most enzymes are actually limited only by the diffusion rate within the cellular environment indicating many enzymes actually have achieved catalytic perfection.

So, this is not surprising given the very long time the life has been around and the evolution has perfected many of these enzymes.

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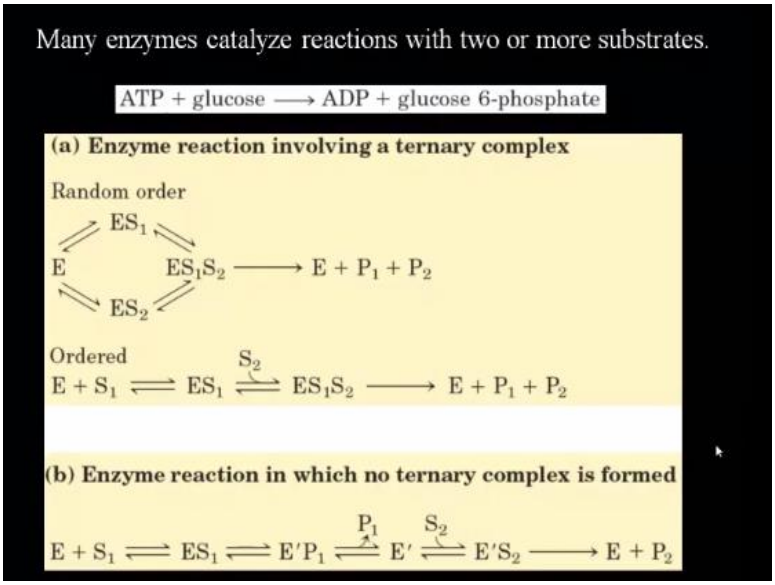

Okay so after that we discussed about the fact that most enzymes work on two substrates. So far, we are looking at a single substrate to understand the steady state kinetics, so now we are going to look at not in detail about the kinetics of these bi-substrate reactions we are just looking at what are the different reaction and mechanism possibilities exist okay. One where the enzyme combines with the substrate in either order okay.

S 1 could bind first or S 2 could bind for the second and then you have the ternary complex formation or instead of being this random like S 1 or S 2 can bind first and the other one binding second it may be ordered where one substrate binds first and then only the second substrate binds to form the ternary complex. This is called ordered reaction. So, these are the two ways random order and ordered ternary complex formation happens.

And some of the enzyme reactions involve no ternary complex where the substrate 1 binds and gets converted and then you have the first product released, then the second enzyme binds, sorry second substrate binds, then that gets converted and then product release happens. So yesterday I used this ADP glucose itself an example to explain this. For example, S 1 you imagine is ATP.

Then enzyme ATP complex then the terminal phosphate gets transferred to the one of the active site amino acid on the enzyme. For example, serine hydroxyl group can be phosphorylated. So now the phosphate is temporarily on the enzyme and as a result the enzyme is now altered. It has an additional phosphate group so you call it E prime and the product 1 is the ADP okay. Phosphate is transferred to the enzyme.

So, it became E prime. Then ATP has become ADP and that is what is P 1. Now that is released, now to this enzyme phosphate you have the glucose binding. Then the phosphate group from the enzyme is transferred to this, so E prime now becomes E and because S 2 gets phosphorylated it is no longer glucose the substrate, instead it is glucose 6-phosphate the product and the product is released.

So, it can be like this with no ternary complex and fortunately the difference between whether it involves a ternary complex or not can be readily recognized using the kinetics that we saw so far.

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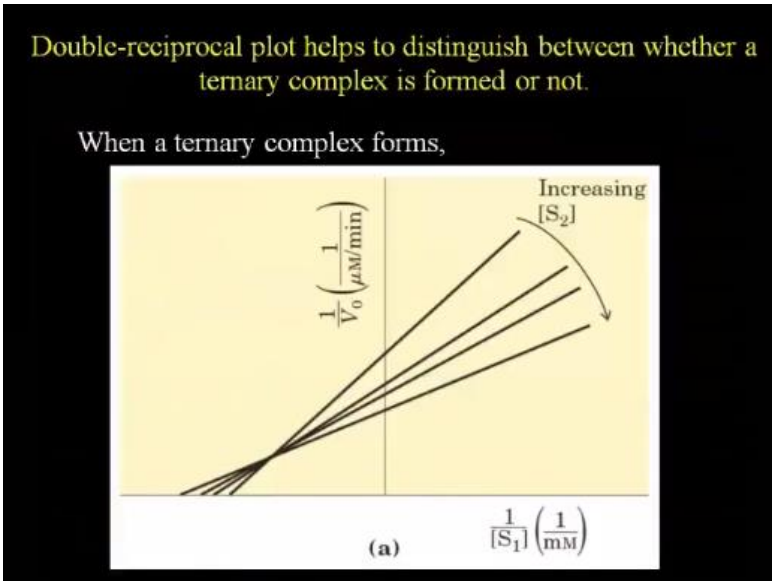

So, this will be the way the double reciprocal plot or the line we will plot looks for an enzyme that involves ternary complex formation. So here what actually has been plotted is each individual line you already understand that is reciprocal of the substrate and velocity are plotted. The additional lines are because we have increased the substrate 2 concentration. So, you take one particular concentration of substrate 2 and then vary substrate 1 and then you have one line.

Then you increase the substrate 2 concentration, then you have the second one okay and so on. So, each of these lines correspond to one particular concentration of the substrate. In this particular direction the substrate two concentration has been increased okay. So, increasing the substrate concentration has reduced the slope here. Meaning K m by V max which is the slope, you know K m by V max equals the M in that equation that we saw 2 days ago.

So, indicating that presence of the second substrate affects the K m as well as the V max because this also changes okay and these non-parallel lines indicate ternary complex formation.

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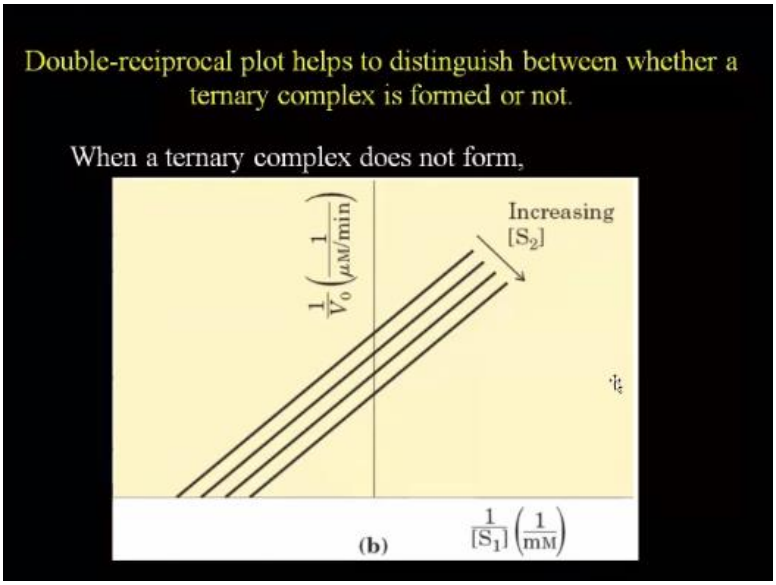

And in a case have this situation where presence of substrate 2 will have no consequence on E binding with S 1 because only E prime can bind S 2. So, S 2 concentration is not going to affect this and due to this you get parallel lines for the situation where there are no ternary complex okay. The K m by V max does not change at all. So only the velocity itself like this alone changes V max individually changes because V 0 changes.

That is because this availability can drive this reaction this way, so therefore it can speed up the ES breaking down okay. So due to that V max decreases okay. So, the main point here is by looking at these lines whether it is like this or like this one will be able to tell whether ternary complex is involved or not. So here you are not really doing any molecular characterization of the protein.

Simply by measuring the rate of the reaction you are able to get an idea of what kind of a complex formation happens. So that is the goal of doing kinetics. So, this is all we are going to learn about the enzyme kinetics and the basic introduction about how measuring rate of the enzyme catalyzed reaction can tell about some of the aspects of the enzyme itself. So next we are going to consider kinetics of enzyme inhibition.

So, the inhibitors are molecules that inhibit the rate at which an enzyme converts a substrate into product and many of the pharmaceutical medicines that we take they are actually enzyme inhibitors. Most of them that are currently in the market are all enzyme inhibitors. For example, the ubiquitous painkiller aspirin is an inhibitor of an enzyme called cyclooxygenase that prevents the formation of prostaglandin biosynthesis, hormones involved in the immune response in that particular context.

So many drugs that we take are all inhibitors of enzymes. So therefore, kinetics of enzyme inhibition is an important branch of enzymology and it is a good idea for us to learn at least an introductory level. So, let us look at what kind of inhibitors are there? And how they can be distinguished kinetically?

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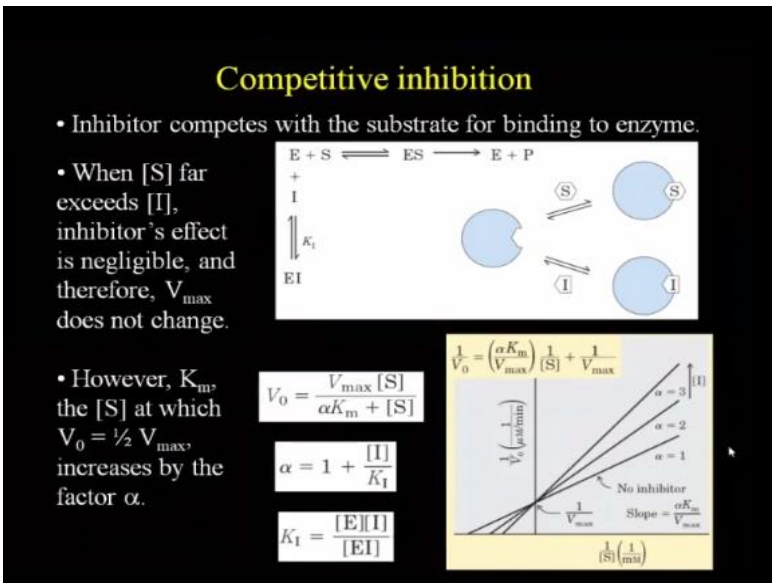

The first one we are going to look at is competitive inhibitor. So, the competitive inhibitors compete with the substrate to bind to the active site. So, if an enzyme is bond with substrate, then it does not bind a competitive inhibitor because both of S and I are competing to bind with E at the same place that is the active site okay. So due to this if you have a certain level of substrate concentration and if you increase the I, then ES formation gets decreased.

Conversely if you increase the substrate to saturating amount that is what you will do to get to V max, V max is the maximum velocity that is attainable. At such a high concentration of substrate, the inhibitor gets competed out. So due to this V max does not get affected by competitive inhibitor. So when you look at the graph non-changing V max indicates this is a competitive inhibitor.

On the other hand, the inhibitor competing with a substrate to bind the enzyme in a sense reduces the affinity between the enzyme and the substrate, which means K m is going to increase. You need lot more substrate or otherwise more substrate to bind with the enzyme when the velocity is half V max in the presence of inhibitor than in the absence of inhibitor. So as a result, K m is going to increase and because K m is going to increase slope is going to change because slope is K m by V max.

So, as you increase the inhibitor, the slope is going to increase indicating that the K m is increasing. So, this slope changes due to K m increase but the ordinate intercept not changing indicating V max not changing is a hallmark of competitive inhibition. So, this is how simply plotting the line we were plot at different concentrations of the inhibitor we can get an idea of whether it is a competitive inhibitor or not okay.

So, a good example is if someone, nowadays this is quite common in the newspaper in many states alcohol is freely not available. So due to that people drink all kinds of solvents that are usually containing methanol. So, methanol is usually added to industrial alcohol to make sure people do not drink them because methanol is poisonous while ethanol is not, so the commercial spirit for example.

If you go to a medical store and ask for a surgical spirit to clean a wound or to clean up anything to disinfect something if you buy it that comes not as pure ethanol, instead it will have methanol added a little bit so that it is spiciness and people do not drink okay. So, let us say someone drank that kind of a spirit and they come to hospital, so how do you treat them? How do you get the poison out of them?

So, the way to do is so why is methanol poisonous in the first place? So, methanol is acted upon by alcohol dehydrogenase and enzyme present in our cell particularly in the liver that converts methanol into formaldehyde and formaldehyde actually crosslinks proteins and therefore our cells will not function. So, it is a preservative. In your biology lab in schools, you would have seen biological specimens preserved in a clear liquid and that clear liquid contains formalin.

Formalin crosslinks proteins and preserves the shape, but of course when you cross link

everything covalently no enzyme, nothing is for its function, so therefore nothing is going to degrade and it is going to be preserved. So due to this production of formaldehyde methanol is poisonous. Now alcohol dehydrogenase normally in our system works on ethanol. Ethanol is usually converted into acetaldehyde by alcohol dehydrogenase and methanol and ethanol compete as substrates for the same enzyme that is alcohol dehydrogenase.

So now when someone has drunk methanol and the enzyme is attempting to convert that into formaldehyde and if you want to inhibit it you provide that person ethanol. So, you put that person on a bed and try to perfuse a certain amount of alcohol into the bloodstream over a long period of time okay so that the ethanol acts as a competitive inhibitor and methanol's conversion to formaldehyde gets slowed down okay.

So, this is a good practical example of competitive inhibition. So here by providing ethanol let us assume S is ethanol. You prevent inhibitor methanol binding to enzyme and when you do this for a while this eventually gets cleared by our detoxifying part of the metabolism and once methanol is cleared below a certain level like you are actually buying time, you are slowing down this formation or you basically detoxify and recover the person.

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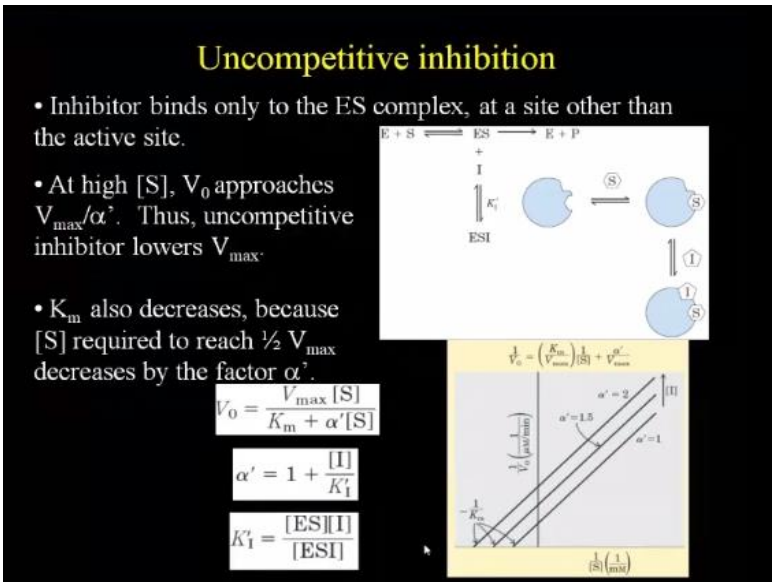

So next inhibition we are going to see is uncompetitive inhibition. So uncompetitive means it is not competing with the substrate okay, instead it binds to enzyme somewhere else but not to the free enzyme, only when the enzyme is combined with substrate the conformation change of the enzyme is such that now a site where this particular uncompetitive inhibitor binding is made possible.

Only when the substrate binds first enzyme, then substrate, then the inhibitor binds. So, inhibitor binds to ES. So, by doing this this is going to affect the rate at which this combination is going to happen as well as it is also going to affect the K m okay. So, at a high substrate concentration V 0 approaches V max by this factor. So this factor exists in the previous one also, I kind of ignored it.

So, the K m increases by a factor of alpha and alpha equals 1 plus concentration of the inhibitor divided by the equilibrium constant for enzyme inhibitor formation and then the K i can be calculated as usual by the reactant by product concentration. So alpha K m is what the K m is modified by a factor of alpha in competitive inhibition whereas here you introduce two terms, sorry only one in this, next one we are going to have two.

So alpha prime okay and that is going to affect the V max. So, this is primarily because as inhibitor increases it is going to drive the forward reaction here to combine with ES because you are removing ES. So, this forward reaction like k1 rate constant this one will be enhanced and due to that the V max is going to decrease by that factor. And which means K m also is going to decrease because the substrate required to reach half V max is also going to decrease.

So due to that K m and V max they both change by the same factor that alpha prime. So, the slope remains the same, slope does not change. At increasing concentration of the inhibitor slope does not change, but then the V max decreases okay. So yesterday I told you this is we are plotting reciprocal. So, this direction means it is actually decreasing and this direction actually means decreasing. So, this shape tells you that it is an uncompetitive inhibitor.

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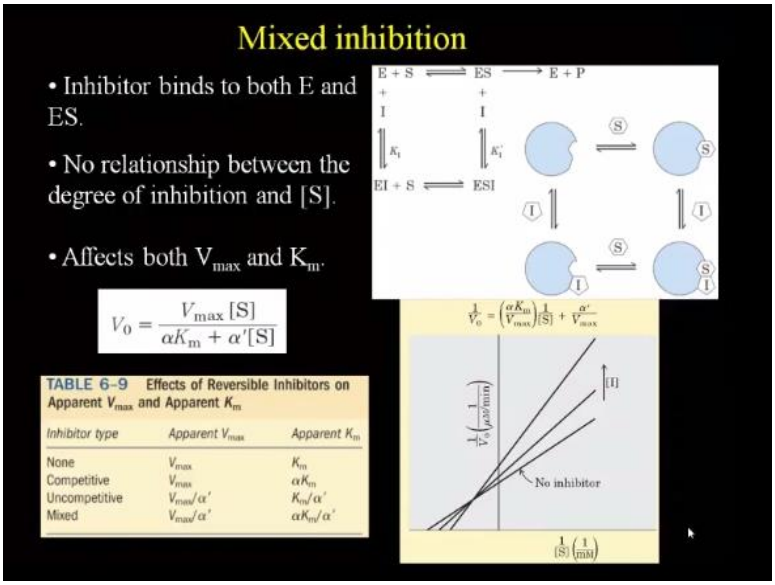

Then you have a situation where both are changed. They are called mixed inhibitors, meaning they can bind with the free or the substrate bond enzyme. It can bind with E as well as ES and therefore both of this come into play. So, you have the competitive situation that is alpha K m as well as you have the uncompetitive situation that is the alpha prime. So, actually full depth of this kinetics is quite complex.

But we are not getting into all those details because it is an introductory course so we are just familiarizing that there are certain factors by which these components of this equation, these terms are going to change, these parameters are going to change and that will affect the shape of the reciprocal plot and the characteristics shapes indicate the type of inhibition. So that is the main point we are getting here.

So here you do not get parallel line, but at the same time the slope changes that indicates it is mixed inhibitor okay. So, this is all we need to understand. So, if you want to get into good details you can try to dig further in the references given in Lehninger in this chapter to get an understanding of like how these terms come to be about divided by alpha prime or alpha K m divided by alpha prime.

All those things you can try to work out the equations and the conditions by going through the back references if you really get very excited about learning about enzyme inhibition in detail. But for this particular course what is here in the slide is sufficient.

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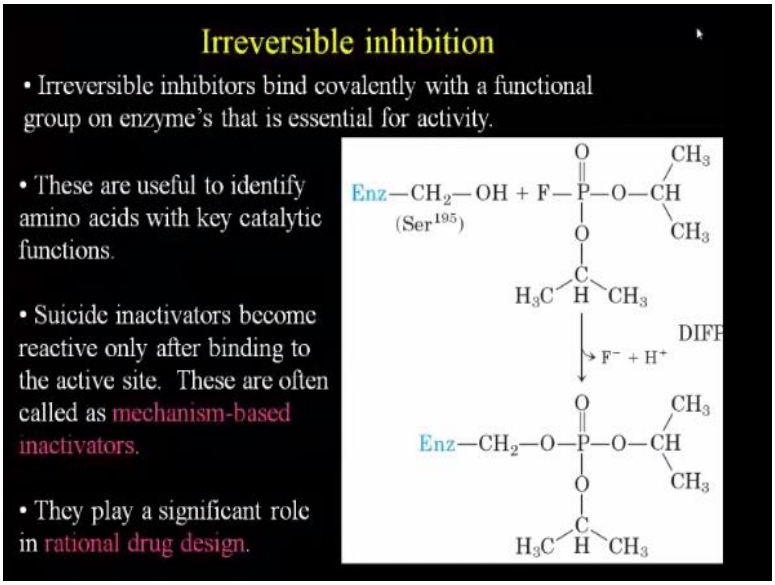

So, you need to mentally visualize these situations multiple times to get an idea of why the V max changes, why K m changes all those things. So now we are going to look at, so far what we were looking at were all reversible inhibitions where the binding is reversible going by these equations you see here if the forward as well as reverse both are possible. So, these are all reversible reactions, so these are reversible inhibitions.

And there are irreversible inhibitors. They covalently modify residues on the enzyme oftentimes the active site residue, the residue that actively participates in catalysis and they are useful to identify what residues are in the active site. For example, if you take this chymotrypsin, serine hydroxyl group, serin 195 on the enzyme, 195 indicates the amino acid position from the N-terminus this is the 195 th amino acid.

N-terminus meaning the end of the molecule where that amino group is not in any peptide bond with another amino acid okay, while its carboxyl group is in peptide bond linkage with the amino acid number 2 and 3, 4 and so on. This is the 195 th amino acid in chymotrypsin and that is in the active site. So, this binds to the active site, this is 2 isopropyl groups so di-isopropyl fluorophosphate.

So, when this molecule binds to this, this is highly reactive and it covalently binds to it. And once it binds, this is irreversible and this is not getting cleaved and the enzyme is permanently inactivated okay. So here you are not going to be able to reverse or change the kinetics by increasing the substrate okay. So, these help us to get at the mechanism of the reaction. For example, this helps in identifying that the serine is the residue or the active site

participating in the reaction.

And there are some versions of these inhibitors they are um called mechanism-based inactivators, so what they actually and they are also called suicide inactivators that is because they become reactive only when they bind to the active site. Remember all that we learnt about our transition state, induced fit like the enzyme and substrate both undergo conformational changes such that the transition state of the substrate and the induced fit state of the enzyme are the perfect fit.

And these suicide inactivators undergo that kind of distortion and when they undergo to that distortion then they become reactive and then they kill the active site and that is why they are called suicide inactivators. And since it is mechanism dependent, the mechanism of the enzyme catalyzed reaction dependent inhibition they are also called mechanism-based inactivators okay.

And understanding of these helps in rational drug design okay. So, drugs I told you are actually inhibitors of the enzymes and that is an understanding of what kind of distortions they undergo and what readily binds to the active site irreversibly, etc., that knowledge helps in designing the drugs. So, this is one of the reasons why people crystallize and solve the structure of the enzyme.

So that they know all the active site residues and the surrounding amino acids and then you go ahead designing a drug that will fit there properly like a substrate, but then will inactivate the enzyme activity okay.

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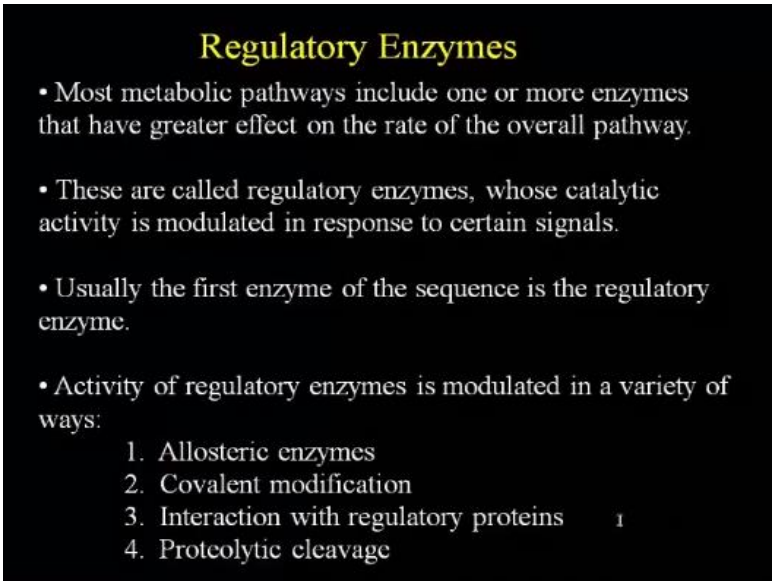

Okay so now we move on to another topic, with this we will be actually be done with enzymology and this topic is how the enzymes are regulated and some of the enzymes are constituting. So far whatever we saw they are all constitutive. If they have substrate, they are going to act and produce the product. So, their activity itself is not modulated or altered okay and that happens for some enzymes and such enzymes are called regulatory enzymes.

Most of the enzymes in our metabolic pathways where conversion of one starting molecule through a series of individual steps to a final product. For example, producing energy out of glucose involves a series of modifications to glucose. Glucose becomes glucose 6-phosphate, fructose 6-phosphate, then fructose 1, 6 bis phosphate and so on, finally to pyruvate and the pyruvate enters the TCA cycle to produce energy.

And that sort of long pathway, each step is catalyzed by an enzyme and these enzymes are regulated based on whether the substrate is in abundance and product is in short supply or vice versa. If you have too much of product, there is no point in converting the substrate again or if a downstream step is going slow no point in going faster with an upstream step. It is like an assembly line.

You want to regulate based on the supply demand and therefore regulation becomes very important in biochemical reactions in metabolism and how the enzymes are regulated is what we are going to consider here. So, they are regulated by 4 different ways listed here. A set of enzymes called allosteric enzymes have a characteristic regulation. We will consider them in some detail and some of them are covalently modified to be active or inactive.

And some interact with the other proteins that regulate them and some undergo proteolytic cleavage before becoming active. So first let us look at the allosteric enzymes they are really important.

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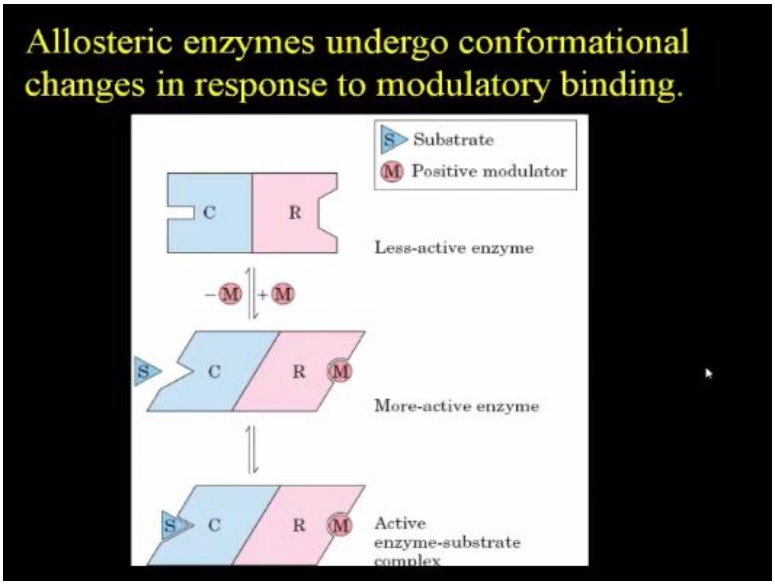

A good example of allosteric enzyme is actually a known enzyme, hemoglobin. Many of you already know hemoglobin and hemoglobin is the molecule. It is a protein, it is a tetramer, so you have 4 subunits, 4 polypeptides interacting together to make the full complete protein and that has a prosthetic group which is the heme and it also contains a metal cofactor like iron ion present there and this binds oxygen and transport oxygen from lungs to the tissues via our RBC's in the blood okay.

So, in hemoglobin you have 4 heme and therefore 4 oxygen molecules can bind and binding of oxygen to one subunit of these 4 subunits protein induces changes in the conformation such that the remaining subunits more readily bind oxygen bind at a higher rate and that is allosteric effect okay. So, the allosteric regulator in this case the substrate itself which is oxygen so binding to one unit activate the substrate binding that is oxygen binding on the other subunits.

So, with respect to let us say you are going to consider the subunit to which oxygen first binds as subunit 1, now for subunit 2 the subunit 2's activity is enhanced by the binding of oxygen to subunit 1. So, meaning the activator binds somewhere else other than the active

site okay. Here we are considering the subunit 2's active site with respect to that oxygen binding in subunit 1 is somewhere else in the protein.

And somewhere else is what is this word allo, steric is the position other than the active site. So, the modulator which may be activator. In the case of oxygen and hemoglobin, oxygen is the activator because oxygen binding to one subunit activates oxygen binding in other subunits and that sort of factors called the allosteric activators and to consider it could be activator or inhibitor, we call allosteric modulator okay, allosteric regulator and that binds elsewhere.

And these proteins like hemoglobin, in hemoglobin multi subunits are there but though for one subunit the other subunits active site is like the regulatory subunit and the regulatory molecule allosteric binding site but otherwise it is an active site. So, if you leave out hemoglobin and take a normal allosteric enzyme, they usually have like hemoglobin multiple subunits but each subunit may not be converting a substrate into product.

And that kind of a thing hemoglobin kind of thing we call it as homotropic allosteric enzyme because one subunit's active site acts as the regulatory subunit and the regulatory site for another subunit's active site based on active site driven reaction. So, in a heterotopic of allosteric enzyme, one subunit acts as a regulatory subunit okay, it does not do any catalysis. And there the allosteric regulatory molecule here integrated by M binds.

And that binding induces the conformational change in both the subunits such that in the catalytic subunit where you have the active site the shape changes such that now substrate readily binds. This kind of a situation very commonly we see when we learn about cell cycle regulation. So probably in a later course when you learn cell biology you will learn about how cell division is regulated.

There you will find a set of proteins called kinases are important for regulating on the various events of cell division. And these kinases bind to another set of molecules called cyclins and the cyclins act as the regulatory subunits. So, cell division dependent kinase CDKs they are like the active site like the C and the cyclins are the regulatory subunit like this R and the cyclins regulate the activity there.

So that is a real-life example for this cartoon, but the primary point here is allosteric enzymes have multiple subunits and if we consider two kinds among those subunits one set of subunits are the regulatory subunits where the regulator binds that induces confirmation change in the active site such that now a catalytic subunits activity is increased okay. On the other hand, a negative regulator will do the opposite.

When it binds the conformation changes such that the substrate does not readily bind okay. So, both are possible, positive regulation, negative regulation. In a situation like hemoglobin where substrate binding to one subunit activating substrate binding to another subunit, I told you these kinds of enzymes are homotropic enzymes. So, this homotropic situations when the effect is positive we call that as positive cooperativity because there is a cooperation among the subunits.

When the substrate binds or oxygen binds to one subunit, it increases oxygen binding in the other subunits meaning the subunits are cooperating in a positive manner because the binding is enhanced because here the function is actually binding oxygen and transporting. So, we call that as positive cooperativity. If the opposite happens binding in one reduces the binding in other subunits, then you call that as negative cooperativity okay and for these enzymes okay.

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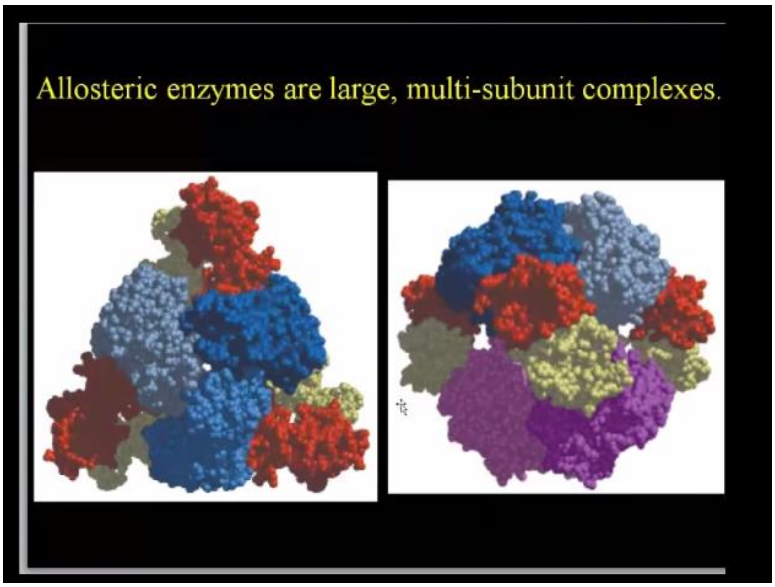

So before I get to the kinetics, we need to consider this. So, these enzymes are large multi-subunit complexes. So, an enzyme molecule if you take each color here is one subunit like if I am going to take this in a strong pink color subunit in the right image, so an individual

subunit itself is a lot of amino acids, it is a very big molecule when you consider molecules in normally encountered in organic chemistry.

So, these molecules are large in individual enzyme, polypeptide is large, primarily to ensure all those various interactions that we learnt about when we learnt binding energy, the idea of binding energy and how binding energy contributes to reducing activation energy. And for that sort of non-covalent interactions and orienting the substrate and desolvation and so on.

To bring about all of that in a very stable manner you need large molecules that is why enzymes tend to be large molecules. These allosteric enzymes are an order of magnitude larger because they have multiple subunits because some act as regulatory subunits, some act as catalytic subunits.

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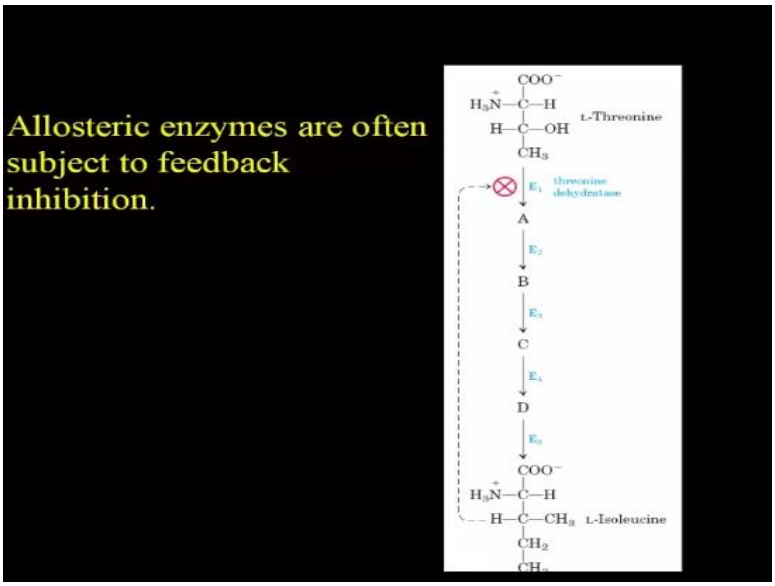

And allosteric enzymes are often subject to feedback inhibition. So, this is an important concept. So, remember metabolic pathways we are right now studying the property of individual enzymes, but in our body enzymes function in a series of reactions okay. I keep telling this glucose getting converted to pyruvate through a series of steps. So, this is what you are seeing here.

This is an amino acid biosynthesis, threonine becoming isoleucine through these intermediates and each intermediate is catalyzed by one enzyme. So, in the beginning of the enzymes, we learnt about intermediate steps those are different. Those are actually from this

to A or A to B in one reaction, the E 2 converting A to B might involve multiple intermediate steps and that is the intermediate steps we were learning initially.

Now we are looking in a metabolic pathway, one set of molecules going through a series of intermediate stable molecules that are present in the cytoplasm stably and which could enter into other pathways meaning these can be cross linked. On those sorts of intermediates, here A diffuses out from E 1, so that E 1 could have had multiple intermediate steps within its active site converting this to A and that is the kind of intermediate steps we learned earlier.

Now we are looking at a biochemical pathway where one product is one substrate is converted into a sub product which is released out of that enzyme okay, which can go into multiple different pathways and in this particular direction then E 2 acts on A to make B and so on. So, in this sort of a multi-step biochemical pathway, oftentimes the very early step is catalyzed by an allosteric enzyme and its activity is often subject to regulation by the ultimate product not the immediate product of that enzyme.

So, the purpose of this entire pathway is to produce this. If isoleucine is present in large excess like you ate food when digested it produce a lot of isoleucine, so the cell need not make isoleucine using its reserved threonine. So, when you have a lot of isoleucine, then this entire step can be inhibited, so usually the first step, you know it makes sense why make A if you are not going to use A to make B to C to D to isoleucine.

So, you stop here itself, close the tap at the very first step and that sort of end of the pathway product acting as a negative allosteric effector of the enzyme catalyzing the first step is a common thing and this we call as feedback inhibition because this is providing a feedback to this pathway okay. And allosteric enzymes often are subject to feedback inhibition. Usually, the first step involves an allosteric enzyme and that is usually negatively regulated by the end product of the entire pathway.

So, with this I will stop today's class and we will continue to for the first set of molecules now we are going to see in detail are going to be carbohydrates, so that is what we are going to move to. So, we had a brief introduction to the molecules. Then we had some good look on the enzymes, the biocatalysts. Now we really move on to understanding the reactions in the

cell and before getting into those reactions let us familiarize in some detail about the molecules.

And as well as principles that govern energy transformation in the body and then we will go to the actual reactions that is the order we are going to go. So next we will move to carbohydrates in some detail in next class. So, if you have questions, please ask, otherwise I will see you next Monday. **Professor – student conversation starts."** Sir. Yeah go ahead. So what kind of substances are positive modulators made of?

What kind of substances like, are they like sedatives or something like that the positive modulators? Okay, positive modulator in a pathway. So, you are wondering why would for example in this case threonine stimulate its own conversion to A isoleucine, right? So, this negative feedback you readily understood. That is if I have a lot of isoleucine then I do not need to do this, so negative feedback I understand.

So, your question is what kind of situation will need positive activation? So sometimes what happens is the substrate is in great abundance and that needs to be quickly metabolized and in that situation that high concentration of the substrate if that itself can induce its conversion and that helps, so that is fast positive feedback inhibition. So right at this moment I only think of the oxygen and hemoglobin.

But an actual metabolic enzyme we will learn when it comes on our way because there are many that will come. So that is the situation usually the substrate itself activates the enzymes activity. Okay, yes, I got it. Thank you. Okay. Any other question? Let me look at this. There is one question on the chat. Yes, I got your question. Will the middle enzymes be affected? Yes, it depends on what else can happen to B.

If there is a branching, if B can be used in some other pathway, then it is actually okay to convert threonine A and move on and to address this question I need to evoke another concept called committed step. So rate limiting step you understood okay. The rate of the that particular step limits the overall rate. There is something called committed step, let us say formation of A from threonine is only for the purpose of making isoleucine.

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And A has no other fate that is the word we commonly use in the cell that is A is not useful in any other manner in the cell. In such a situation making A is useless when you have a lot of isoleucine and that kind of a step is called a committed step. Meaning by doing this reaction you are committing this threonine to making isoleucine and usually that is the step that is regulated allosterically and in this particular case usually committed steps are rate limiting also.

But let us say in a pathway, A can actually participate in many things, then A may not be E 1 may not be subject to regulation, instead E 2 or E 3 may be regulated. So therefore, the feedback regulation maybe on the other enzymes like E 3 or E 4 or any of them. Did this address your question? Yeah, yes sir. Thank you. Okay. **"Professor – student conversation ends."** Alright, see you guys Monday.