Overview and Integration of Cellular Metabolism

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Lecture 02: Regulation of Enzyme Activity

Hello everyone, welcome to the second lecture of the NPTEL course, Overview and Integration of Cellular Metabolism. In this class we will be learning about regulation of enzyme activity. So, in this class we will be covering the important concept of how enzymes are regulated allosterically, how feedback control of enzyme occur in the biological system, how covalent modification plays a role in enzyme regulation and how hormones play a role in covalent modification of enzymes. Now, we all remember this figure from the last class alright. So, what is enzyme? Enzymes are actually proteins that act as biological catalysts by accelerating chemical reaction ok. And we should remember that enzymes actually provide an alternative reaction path with lower activation energy.

I told you the ball was rolling from a higher point to lower point it was not finding the amount of energy to just go over the bump, but enzyme actually lowers the bump this bump is actually the activation energy. So, enzyme has lowered the activation energy now the ball can roll down. Looking at the structure of enzyme, enzyme actually reacts with see this is the reaction A plus B forms C and D or simply we can read A is getting converted to B ok whatever the situation is. So, here is an enzyme enzymes are there.

So, this for the enzyme the reactant is known as a substrate. Enzyme actually binds with the substrate to form an enzyme substrate complex which then dissociates to form a product. So, every enzyme there are multiple models of enzyme activity every enzyme has got a substrate binding site that is known as active site which exactly looks like a complementary to the structure of substrate. With this concept we will be able to easily understand how a enzyme is regulated. Now, the rate of enzyme catalyzed reaction are regulated.

So, when a compound is getting converted to B. So, enzyme E is converting a compound A to enzyme B the rate of the reaction. So, how fast A will get converted to B is in the hands of E. So, E will be determining whether A at all can be converted to B

right. So, naturally when the rate I mean the activity of E is very high more and more A will be converted to B right and so, who does it in biological system there is signal for everything.

So, in my body suppose we I need more amount of B right and A is getting converted to B with the help of E. So, when my body needs more and more B there are multiple signals by which E will be told will be signal to convert A to B and hence more and more amount of B will be produced. Now, when a lot of A has been converted to B my body does not need B anymore then E will be signaled by various mechanisms that we do not need anymore B. So, now, you check the reaction. So, the activity will of E will be altered in such a way that the action of E on A will be inhibited you get my point.

So, when we need more B enzyme reaction will be rate will be high when we do not need B rate will be controlled this phenomena is known as regulation. So, the control of the enzyme action is known as regulation and this can be regulated by multiple means one is allosteric, one is feedback, one is covalent modification and this lecture I will make sure we learn everything ok. So, allosteric enzyme what is allosteric enzyme the term allo means actually other. So, there is some regulator molecule. So, an x factor or some other thing that is not a substrate that will get attached to the enzyme at a site which is not this site some other site.

So, from there the concept of allosteric ok when it gets attached to an enzyme at some other site it alters the shape of enzyme in such a way or shape of the active site in such a way that the enzyme cannot bind to the substrate like it used to do in an earlier situation. So, with this illustration it is very clear you can see the substrate looks like this ok. The enzyme initially was not reactive with the substrate. So, what it does the allosteric regulator it binds at a site which is not the active site initially you see this is an example of positive allosteric regulation where a regulator is helping the enzyme to move forward with the reaction. So, it gets attached to the allosteric site it alters the active site in such a way.

So, that now it can easily bind the substrate and get converted to product ok. This this phenomena can happen in a reverse way. So, what is happening when a reaction is negatively controlled the allosteric regulator this in this case is the negative allosteric regulator allosteric inhibitor. Allosteric inhibitor is binding to the allosteric site and it enters or it alters the active site in such a way that it prevents the enzyme to bind to the substrate and now reaction cannot happen. So, this is the basic structural or conceptual phenomena of allosteric regulation of enzymes.

So, when we plot enzyme kinetic curve where the concentration of substrate that is

denoted by square bracket of S and the velocity of the enzyme concentration of substrate in x axis and velocity of the enzyme in y axis then what happens. Allosteric enzyme generally gives a sigmoid or an S shaped curve. What does it mean? It means the activity is very slows to start with it increases with the substrate concentration and ultimately when we keep on increasing substrate concentration at some point of time the activity will not be that much compared to the increasing substrate concentration. So, mind it this is a curve which is denoting the relationship of the substrate concentration and velocity of a reaction or enzyme reaction ok. V max is the maximum velocity half V max is half of the maximum velocity.

So, what happens when we add a positive effector to an allosteric enzyme the curve looks like this becomes hyperbolic almost ok and this is the curve when a negative allosteric inhibitor or allosteric inhibitor negative allosteric modifier has been added ok. So, this type of figure can be given in image based questions ok. So, some salient features regarding allosteric inhibition should be kept in mind the inhibitor is not a substrate analog this is very self explanatory because the allosteric effector inhibitor binds at a site that is other than the active site had it been a substrate analog it could have bound with the active site only. So, it does not it is not it does not look same structurally like the substrate. This type of regulation be it inhibition be it positive regulation can be reversed when excess substrate is added specifically inhibition we are dealing with allosteric inhibition.

So, allosteric inhibition can be partially reversed if excess substrate is added. Two changes I have mentioned K M is usually increased and V max is reduced again homework for you I I I will tell you K M is the Michaelis maintain constant and V max I told you in the previous part is the maximum velocity of a enzyme substrate reaction. So, it is for you we can discuss in the live sessions you it is up to you to find out what is the Michaelis-Menten equation and learn what is the K M and V max ok. The effect of allosteric modifier is maximum or at near substrate concentration that is equivalent to K M ok. So, let me tell you ok fine I hope those who are interested I have already pause this video and we have you have already learned in the background what is K M and what is V max ok for you I give a thumbs up.

So, let me tell you K M is actually the substrate concentration at which the velocity of the reaction is half of the maximum velocity. So, if we consider this reaction if this is substrate concentration this if we plot the maximum velocity over here if we drop down a tangent this value of substrate will be the K M for this reaction ok. So, what does allosteric modification inhibition does to K M it increases the K M and the V max is actually reduced you can actually see this from the plot right. Now, one more important thing about allosteric enzyme is they are multi subunit in nature. So, enzyme few

enzyme have got multiple subunit and generally binding of one substrate facilitates the binding of other subunits to a reactant or substrate right.

For example, aspartate trans carbamolus are 6 subunit pyruvate kinases 4 subunit these names might appear overwhelming to you, but believe me through the course of this overview and integration of cellular metabolism every reaction of every enzyme will be explained. So, these are few examples of allosteric enzymes I will be not reading them one by one you can easily pause the slide and note it, but do not try to memorize it at this point because again I tell I will be repeating this thing over and over again in the initial classes. The all the action of these enzymes will be repeated in subsequent classes. For example, allosynthase in heme metabolism aspartate trans carbamolus in pyrimidine synthase HMG choriductase cholesterol metabolism phosphofructokinase carbohydrate metabolism. So, I do not want to intimidate you at this point of time, but be slow and steady, but during those classes we will again refer to this slide ok.

At that slide the instructor showed you what was the allosteric inhibitor then you need to recall it. So, it is best that you prepare your own note and note this down so that you can easily refer as a handout when needed during the metabolic regulation of various pathways. So, we move into feedback control. So, what is feedback control? Feedback control is actually very easy to understand. Suppose the end product level is high as I gave the example.

So, if A to B so, my body has got a lot of B right. Now, I do not want A to B converted to B. So, this B the product the end product will act as an allosteric inhibitor ok will act as a allosteric regulator and this will bind to the allosteric site of the enzyme. So, that the product A can no longer bind to the active site get my point. So, some product reactant was getting converted to product ok instead of some other regulator here the end product acts as an allosteric regulator inhibitor in this case ok.

So, and what if my body now needs more and more A or more and more B sorry then the effect the effect of this allosteric inhibitor will be gone and the enzyme activity will be regained and all the products will be formed. Mind it in case of feedback inhibition specially if it is an end product inhibition what it does it actually inhibits the enzyme right at the start right at the beginning. So, since maybe A is getting converted to B via multiple steps there are multiple compounds X 1 X 2 and then it is getting converted to B it will inhibit the enzyme it will. So, there are two enzyme E 1 E 2 and E 3 it will inhibit when it is inhibiting E 1 all the intermediate product will not be formed right. So, this is an example of end inhibition.

So, this feedback control this is an example where the end product level is very high ok.

So, it is inhibiting. So, what happens when the end product level is very low absolutely same the allosteric regulator will give a positive feedback. So, that the negative regulator will then dissociate from the allosteric site and then the enzyme can bind to the active site again and the reaction can proceed. So, these two are almost similar the mechanism of action is reversed ok.

So, either the end product can be high or end product can be low depending on that the inhibition will be in effect or inhibition will be removed ok. So, this example this will come again and again I explained it to you in the first example where I explained about thermodynamic coupling ok. There purposefully I did not mention the name of the enzyme the name of the enzyme is hexokinase ok. Remember this you will need this a lot glucose getting converted to glucose 6 phosphate a lot right. So, what happens when glucose and ATP gets converted with the help of hexokinase to glucose 6 phosphate and ADP.

So, now, when there is an excess amount of glucose 6 phosphate in the body it will inhibit hexokinase. So, this is an example of immediate feedback inhibition. So, just suppose A is getting converted to B then it is converted to C so on and so forth because in biological cycles a lot of intermediates are formed a lot of enzymes are involved. Here what is happening suppose the first step is actually hexokinase glucose is getting converted to glucose 6 phosphate by the enzyme hexokinase. So, hex glucose 6 phosphate is inhibiting hexokinase right at the start.

So, that the further downstream processes will not happen this is an example of feedback control and as I told you end product inhibition where ultimately suppose this leads to production of say compound X. So, X is inhibiting hexokinase. So, if X is inhibiting hexokinase is an example of end product inhibition ok. So, some example of end product inhibition is CTP is the allosterically inhibiting the enzyme aspartate trans carbamoylase again and reaction of pyrimidine metabolism. The end product heme this is an example of heme synthesis we will be discussing in detail where the end product heme this is not the reaction this is the first reaction and ultimately this reaction will lead to formation of citrine triphosphate CTP.

Here also the reaction will ultimately progress to formation of heme. So, they will react to form heme and this heme will actually inhibit allosynthesis or deltamino-laflonic acid synthesis these are the regulating steps ok. These are just the examples might appear intimidating at the start, but believe me as you learn so many examples you will be able to answer each and every one of them for 5 or 6 examples spontaneously by the end of this course ok and I am confident about that. So, the this brings us to the next type of inhibition that is covalent modification. What is covalent modification? This is very

simple to understand that is covalent bonds are being formed in an enzyme or there is a enzyme is modified by formation of covalent bonds which actually dictates how the enzyme activity will be whether the enzyme will become active or the enzyme will become inactive.

This factor is dictated by formation of covalent bonds and this covalent modification of enzyme is reversible. Covalent bonding is might not be reversal, but this is do not confuse by this line we are dealing with covalent modification of enzymes this regulation of this regulation of enzyme by covalent modification is reversible ok. Prime examples are zymogens and proteins. So, what are zymogens and proenzymes? Zymogens are digestive enzymes these are the proteins the characteristically there are multiple hormones as well for example, insulin and clotting factor these are all proteins that are synthesized by the system in an inactive form. These are enzymes these are not active, but there are some covalent modification there are some might be there is a breakage of peptide bond ok might be formation of some covalent bond some transfer of some group.

So, in this case what happens they get activated by covalent modification. Why why do we need this phenomena? You see these zymogens are produced somewhere else and their site of action is somewhere else. So, this is the protective mechanism by the body with by which the organ in which they are synthesized or they are produced are not autodigested ok. So, they are activated you can see for example, chymotrypsinogen is produced in the pancreas and they are activated in small intestine ok. How they are activated? Mostly these most enzymes are activated by hydrolysis means water based degradation of some peptide chains.

How we can remember few example of zymogens you can see gen gen gen ok. So, chymotrypsinogen, pepsinogen, trypsinogen and what are their active forms that ends with IN chymotrypsin, pepsin, trypsin for example, right and regarding plotting factors they they start the name with pro. So, prothrombin becomes thrombin hormone like proinsulin becomes insulin ok. Nevertheless you can just remember these example for multiple choice question type purpose ok, but know this activation of zymogens and proenzymes are example of covalent modification. So, what happens in case of chymotrypsinogen giving a specific example this the peptide is cleaved in some areas ok.

So, you can see chymotrypsinogen is a large peptide and two area there are hydrolysis by hydrolase enzyme with the help of water the peptide is cleaved and then chymotrypsin after becoming short fragments it becomes active then it can exert its action produced in the pancreas acting in the small intestine. Before activation they are transported to the site of interest then they are converted and then they are activated using covalent modification ok. Again insulin another prime example where the

hormone insulin is an in an inactivated state in the form of proinsulin the degradation of or hydrolysis of the whole peptide chain occurs where ultimately the fragment it gets separated and it leads to the formation of active insulin. So, this is the inactive insulin in the form of proinsulin this is active insulin and this peptide act is C peptide this has got some biological importance let me tell you C peptide does not have any function in the body it is inert, but analysis of C peptide gives us an idea about insulin secretion in our body. So, it gives us an indirect idea indirect, but very good reflection of what is the amount of insulin that is secreted produced in our system ok.

So, again remember zymogen is proinsulin activation via covalent modification. Now, another type of covalent modification is phosphorylation. Phosphorylation what is phosphorylation in which a phosphate group is added to an enzyme. So, an enzyme can become phosphorylated and now becomes a phosphorylated enzyme ok. So, when it is not phosphorylated we call it dephosphorylated and when it is active or phosphorylated we call it phosphorylated form.

So, an enzyme can be activated by addition of phosphate group, it can be deactivated by removal of phosphate group, it can be activated by removal of phosphate group, it can be deactivated by removal of phosphate group mind it see. So, phosphorylated enzyme this chart here is a few here are few example for example, acetyl CoA carboxyl as fatty acid synthesis glycogen synthesis these are the enzymes who are inactive in phosphorylated state. Whereas, we see these are the few examples that are few example of enzyme that are activated in phosphorylated state. If you try to remember or memorize the whole thing this by making a chart it is possible, but believe me it is very unscientific and it will drive you mad.

So, concept clearing should be done. So, I am here to clear your concept remembering this golden rule ok. Glucagon activates all catabolic enzymes by phosphorylation, insulin activates all anabolic enzymes by dephosphorylation. This fundamental concept if you can memorize believe me you will be able to explain or you will be able to correlate with any reaction if you can assess whether the pathway is anabolic or catabolic. To understand whether the pathways anabolic or catabolic is very easy, if a simpler form of nutrient is getting converted into storage form it is an anabolic pathway, when a some bigger storage form is being broken to form active energy it is catabolic pathway. Now, if we remember this and if we look back in our previous example we can see for example, glycogen phosphorylase is a pathway where glycogen is being broken down to produce glucose it is catabolic.

Therefore, since it is catabolic so glucagon is activating all catabolic enzymes by phosphorylation. So, this enzyme is activated in phosphorylated state alright again

anabolic enzyme. For example, glycogen synthase, glycogen synthesis is a anabolic pathway glucose is being converted to glycogen we are building something anabolic. So, insulin will help it and this enzyme is activated in dephosphorylated form ok. There are two intermediates you should remember just for multiple choice question purpose insulin does the dephosphorylation with the help of protein phosphatase and glucagon.

So, dephosphorylation is happening in the is being done with the help of phosphatase and activation by glucagon that is phosphorylation is being done by kinase specifically cyclic AMP dependent protein kinase. So, whenever you see kinase mind it glucagon it is activating catabolic enzyme it will activate mind it the reverse phenomena is also true it means what all the catabolic enzymes are inhibited by insulin and they are inactive in dephosphorylated form. And all the anabolic enzymes are inhibited by glucagon and they are inactive in phosphorylated form ok. Just remember one and you can easily deduce all the other three ok. So, just if this was overwhelming I request you to pause the video just go 5 minutes back see the chart watch it twice it will be very clear right.

So, again now if you look at the chart you can easily even if you do not know all the metabolic pathway in detail you will be easily able to answer what enzyme is active in what form all right. So, the to conclude the lecture session has covered the role of allosteric enzymes how allosteric enzymes are regulated we understood the feedback inhibition the end product inhibition we understood how covalent modification helps in regulation of enzyme activity and we have also understood how hormones play a crucial role in covalent modification. These are the few reference for this slide. Thank you for your attention.