

# NPTTEL VIDEO COURSE – PROTEOMICS

## PROF. SANJEEVA SRIVASTAVA

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### HANDOUT

### LECTURE-08

### ENZYME: BASIC CONCEPTS, CATALYTIC AND REGULATORY STRATEGIES

#### Slide 1

Today, we will talk about enzyme: basic concepts, catalytic and regulatory strategies.

#### Slide 2

Lecture outline:

- Basic concepts of enzyme including enzyme kinetics, energetics and enzyme inhibition.
- Different type of catalytic strategies and regulatory strategies.

#### Slide 3

So as you all know, enzymes play very important role in biochemistry All enzymes are proteins therefore it will essential to study about enzymes while studying basic concepts of amino acids and proteins.

Although it may not be directly linked to the proteomics but understanding of enzyme and proteins is very fundamental for the advanced understanding of concepts related to proteomics.

#### Slide 4

So what are enzymes?

- Enzymes are molecular catalysts.
- Almost all enzymes are proteins and as we have discussed in the previous lecture there are twenty amino acids that make up the basic building blocks of all proteins.
- Enzymes can accelerate a given reaction up to million folds. Let's take an example of carbonic anhydrase which catalyzes hydration of carbon di oxide. Now this enzyme can catalyze  $10^6$  molecules per second.
- Enzymes are highly specific and catalyze single or closely related reaction.

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In terms of enzyme specificity, let me give you few examples-

- Trypsin- it is highly specific, it cleaves peptide on carboxyl side of Lys/Arg.
- Another enzyme thrombin, which participates in blood clotting, it is even more specific than trypsin. It catalyses hydrolysis of Arg/Gly bonds in a very specific peptide sequence and therefore offers high specificity.

### Slide 6

The six major classes of enzymes which are grouped according to type of the reaction they catalyze. I will not go in much detail but briefly let me talk what type of reactions these classes of enzymes are offering.

- Oxidoreductase- it catalyzes oxidation and reduction. Example- Pyruvate dehydrogenase
- Transferase- these enzymes can transfer the certain groups so group transfer can be mediated by this group of enzymes. Example- Hexokinase
- Hydrolase- these can catalyze hydrolysis reactions. Example- Chymotrypsin
- Lyase is another group of enzymes which adds groups to double bonds or removes groups from the double bond. Examples include fumarase.
- Isomerase as the term says isomerization so this group of enzyme can transfer groups within the molecule. Example- Phosphogluco-isomerase
- Ligase- it catalyses bond formation by using ATP. Example- Glutamine synthetases

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So let's talk about enzyme activity and how various cofactors govern these enzyme activity.

- Catalytic activity of many enzymes depends on presence of small molecules which are known as cofactors.
- There could be two groups here as shown in the slide metals and small organic molecules.
- The coenzymes are small organic molecule such as those derived from the vitamins, the prosthetic group that is a tightly bound coenzyme.
- The apoenzymes are enzyme without its cofactor and holoenzyme is the combination of the apoenzyme with cofactor to give rise to the complete catalytic enzyme.

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- Most enzymes are made up of a protein part known as the apoenzyme as well as a cofactor which can either be an organic molecule known as a coenzyme or a metal ion.
- These cofactors are essential for the enzyme to be catalytically functional and the complete functional enzyme is referred to as the holoenzyme. Pyruvate dehydrogenase is a complex enzyme which uses
- Thiamine pyrophosphate as its coenzyme while carbonic anhydrase uses zinc ion as its cofactor.
- Enzymes are classified on the basis of the reactions that they catalyze. Most enzymes are named by adding the suffix 'ase' either to their substrate or the type of activity they carry out. However as more enzymes came to be known, it became increasingly difficult to name them in this manner.
- Classification by international organizations has therefore led to six enzyme classes with many subgroups within each class, depending upon the type of reaction being catalyzed.
- Every enzyme has a unique, four-part classification number known as the Enzyme Commission number (E.C. number), in which subclass number gives finer details about that particular enzyme reaction.

### Slide 9

Let's now talk about free energy.

- So remember the concept of entropy- $\Delta G = \Delta H - T\Delta S$
- So  $\Delta G$  must be negative for a spontaneous reaction. So the energy which is required to convert substrates to product that is rate of reaction.
- Enzymes can only affect rate of a reaction so enzymes cannot alter reaction equilibrium. They can accelerate attainment of equilibrium concentration.

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So what is transition state and activation energy?

- When you take a reaction from substrate to product, it goes through intermediate transition state. The activation energy is differences in free energy between transition state and substrate.
- The activation energy is a barrier to reactions. Now if you look at the graph, enzyme decreases activation energy and alter the attainment of reaction equilibrium by facilitating of  $S^*$ .

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### Energetics of enzymatic reactions

- Conversion of substrate to product proceeds through formation of a transition state.
- The free energy of activation of an uncatalyzed reaction is very high. Enzymes form favorable interactions with the substrate and facilitate formation of the transition state by lowering the free energy of activation.
- The transition state then dissociates to give the product and regenerates free enzyme.
- For a reaction to be spontaneous, the  $\Delta G$  must be negative. It must be emphasized that enzymes do not alter the equilibrium of a reaction.

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#### The Enzyme-Substrate complex-

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- The enzyme-substrate complex or ES formation is first step in catalysis.
- Enzymes are very selective in choice of substrate they can bind which ultimately dictates specificity of these catalysts.
- These substrates are bound to active site of enzyme, there have been experimental evidence that enzyme-substrate or ES complex do form and various strategies which have shown that ES complex forms include maximal velocity, X-ray crystallography and spectroscopy.

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#### So what is active site?

- The active site is a region of enzyme that binds of substrate.
- It provides a 3-D cleft. It is formed by the amino acids from different parts of the sequence.
- So it's not necessary that amino acids present only in that pocket are going to form active site but even it can be from different regions of given protein which could form active site.
- Active site amino acids form only a small portion of enzyme, while other amino acids, they form the scaffold.

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To understand enzyme-substrate binding different models have been proposed.

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- The two popular models – one is lock and key model which was proposed by Emil Fisher in 1890. This model explains that the complementary interaction can determine the specificity.
- So if you have an enzyme and a substrate is present. Now the active site present on the enzyme should be complementary in shape with the substrate. Something similar to a key is very specific to a given lock, and then only it will open.

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- Other model is induced fit which was proposed by Daniel Koshland in 1958.
- So the active site, they form a shape which is complementary to the substrate and only after substrate has been bound.
- So enzyme can change the shape of substrate binding. In this case the substrate structure does not have to be specific to the active site of enzyme.
- It can be dictated by enzyme and shape can be changed which will be complementary for the substrate binding.

### Models for enzyme-substrate binding

- Fischer's hypothesis is aptly defined as the 'lock-and-key' hypothesis.
- Any lock, which is analogous to an enzyme, can have only one suitable key of appropriate shape and size to open it.
- The various available keys, which are analogous to the thousands of substrates available, can attempt to open the lock but only one will be the perfect fit that is capable of opening the lock.
- Similarly only one particular substrate will fit into the active site of the enzyme and the enzymatic reaction can occur.
- According to the Fischer's hypothesis, enzymes and their substrates possess specific complementary geometric shapes that fit exactly into each other. This model accounts for the specificity of enzymes but fails to account for stabilization of the transition state.
- Koshland modified this hypothesis and suggested that the active site of an enzyme gets continually reformed based on the interactions that it establishes with the substrate molecule.
- This accounts for both the enzyme specificity and the stabilization of the transition state since the enzyme is not considered to be a rigid molecule.

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Let's talk about enzyme kinetics.

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In 1913, scientist Michaelis and Menten, they derived a model to explain the concept of enzyme-substrate complex which is fundamental to understand enzyme reactions.

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The Michaelis-Menten explained kinetic characteristics. The role of enzyme catalysis ( $V_0$ ) that is number of moles of product formed per second. It varied with substrate concentration; it increases linearly as substrate concentration increases levels of a maximal velocity at higher substrate concentration. So an enzyme (E) that catalyzes substrate (S) to give rise to product (P) can be understood in this reaction.



Now E combines with S to form ES complex in presence rate constant  $K_1$  whereas ES complex can be dissociated in to E and S with rate of constant  $K_{-1}$ . ES complex can form product with a rate constant  $K_2$ . ES complex can be reformed by reverse reaction with a rate constant  $K_{-2}$ .

### Slide 18

Michaelis-Menten equation.

- The  $V_0$  rate of formation of product can be derived by this equation where  $K_M$  is Michaelis constant.

$$V_0 = V_{\max} \frac{[S]}{[S] + K_M}$$

$V_{\max}$  – reaction rate when enzyme is fully saturated with S  
 $K_M$  – Michaelis constant  
S – substrate concentration

- Now this equation is fundamental to understand various problems. So let's say we take this equation and there is one situation where substrates concentration is less than  $K_m$  or Michaelis constant. Now what will be the equation? In this case  $V_0$  will become  $V_{\max} / K_m [S]$ . So rate is directly proportional to substrate, the reaction is first order.
- Now, let's take another scenario where  $[S]$  is more than  $K_m$ . Now what will be the equation?
- $V_0 = V_{\max}$  so rate is maximal and it is independent of substrate concentration. In this case reaction is of zero order.

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- Now let's take third situation where substrate concentration is equal to  $K_m$  now in this case equation will become-
- $V_0 = V_{max}/2$ , so substrate concentration at half maximal velocity is known as Michaelis constant or  $K_m$ .

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So  $K_m$  is substrate concentration at half maximal velocity as you can see in the graph. The  $K_m$  is a measure of strength of enzyme substrate complex so higher  $K_m$  indicates weaker bonding and low  $K_m$  indicate stronger binding.

### Slide 20

So another scientist gave Lineweaver-Burk plot or double reciprocal plot which is a plot of  $1/V_0$  versus  $1/s$ . it is reverse of  $V_0$  or  $S$  which was taken in case of Michaelis-menten reaction.

### Slide 21

#### Kinetics of enzymatic reactions

- Enzymes catalyze the formation of product from its substrate via an enzyme-substrate intermediate complex. During the initial stages of the reaction, the equilibrium favors product formation rather than dissociation of the [ES] complex to give back the substrate. The number of moles of product formed per second during these stages determines the reaction velocity for that particular enzyme.  $V_0$  has an almost linear relation with substrate concentration when the substrate concentration is low but becomes independent at higher concentrations.
- The Michaelis-Menten model for enzyme kinetics assumes that the breakdown of [ES] complex to give back free substrate is negligible and also assumes steady-state conditions whereby the rates of formation and breakdown of the [ES] complex are equal.
- The reaction velocity increases linearly with substrate concentration when [S] is low but becomes independent at higher concentrations. The maximal velocity ( $V_{max}$ ) that can be achieved by an enzyme refers to the state in which all its catalytic sites are occupied. The substrate concentration at which the reaction velocity is equal to half its maximal value is known as the Michaelis constant,  $K_m$ .
- The Lineweaver-Burk equation or the double reciprocal plot is a useful tool that can be plotted using simple experimental data from kinetics experiments. This equation is derived from the Michaelis-Menten equation by taking reciprocals on

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both sides and then plotting a graph of  $1/V_o$  Vs  $1/[S]$ . The Y-intercept on this graph can be used to deduce the value of  $V_{max}$  while the X-intercept gives the value of  $K_m$ .

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Enzyme inhibition-

- The enzyme inhibition can provide insights into catalysis.
- Enzyme activity can be inhibited by small molecules and ions. The enzyme inhibition provides major control mechanism in biological system.
- There are two type of inhibition- reversible and irreversible

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- The reversible inhibitors dissociate rapidly and the enzyme-inhibitor complex (EI) is dissociated. There are three different strategies for reversible enzyme inhibition- competitive, non-competitive and uncompetitive or mixed inhibition.

### Slide 24

#### Competitive inhibition

The enzyme binds to substrate or inhibitor and forms enzyme-substrate or enzyme-inhibitor complex but do not enzyme-substrate-inhibitor (ESI) complex. The inhibitor is similar in structure to the substrate which binds the active site of enzyme. It reduces rate of catalysis and also reduce the number of enzyme-substrates which are formed by biding at the same sit as shown in the figure.

### Slide 25

#### Uncompetitive inhibition

- Inhibitor and substrate bind at different sites.
- Enzyme-substrate-inhibitor complex is formed so by increasing the substrate concentrations the inhibition cannot be overcome which was the case in competitive inhibition.
- The reaction rate increases more slowly at lower substrate concentration as compared to the uncompetitive.



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### Noncompetitive (mixed) inhibition

The inhibitor binds only to ES complex and form ESI complex. It lowers the apparent Michaelis constant  $K_m$  value. The uncompetitive inhibition cannot be overcome by addition of excess substrate. One of the examples is glyphosphate, an herbicide.

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- After talking about reversible enzyme inhibition, now let's discuss irreversible enzyme inhibition.
- The inhibitor is very tightly bound to enzyme either covalently or non-covalently in such a way that this inhibition is irreversible.
- Let's take the very classical example penicillin, the first antibiotic which was discovered. It was irreversibly inhibiting a key enzyme transpeptidase in bacterial cell was synthesis and kill the bacteria.
- Now in irreversible enzyme inhibition different classes- group specific, reactive substrates and suicide inhibitors.
- The group specific irreversible inhibitor, these inhibitors can react with specific side chain of amino acid.
- The reactive substrates or affinity labels, they are structurally similar to the substrate and covalently bind to the active site.
- They are more specific for enzyme active site than group specific reagents. The suicide inhibitors, they are highly specific substrates which can modify the active site of an enzyme.
- They are most specific for modification of enzymes active site.
- Enzyme inhibition can either be reversible, where the inhibitor can dissociate quickly from the enzyme, or irreversible, where the inhibitor dissociates very slowly from the enzyme and can covalently modify the enzyme thereby rendering it unsuitable for further catalysis reactions.
- Reversible inhibition can be further classified as competitive, uncompetitive and mixed inhibition.
- In competitive inhibition, the inhibitor molecule is structurally similar to the substrate and therefore binds to the enzyme at the active site.
- Binding of inhibitor prevents substrate from binding, thereby decreasing the reaction rate.
- The  $V_{max}$  in this type of inhibition remains the same and only the  $K_m$  is altered. Competitive inhibition can be overcome by suitably increasing the substrate concentration, which allows the substrate to out-compete the inhibitor for the enzyme's active site.

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In the case of uncompetitive inhibition, the substrate and inhibitor both have different binding sites on the enzyme. However, the inhibitor binds only to the enzyme-substrate complex and not to the enzyme alone. Binding of inhibitor to the ES complex prevents any further reaction and no product formation is observed. Both the  $K_m$  and  $V_{max}$  are found to decrease with this type of inhibition.

A mixed inhibitor or noncompetitive inhibitor also binds to the enzyme at a site distinct from the substrate binding site with the difference being that it can bind either to enzyme or ES complex. Binding of either one brings about conformational changes in the enzyme structure thereby affecting binding of the other. This inhibition can be reduced but not overcome by increasing substrate concentration. Both  $K_m$  and  $V_{max}$  are altered in this type of inhibition.

### Slide 27

Now let's move on to catalytic strategies-

Enzymes are very effective and extremely specific catalyst; the enzymes employ various mechanisms to facilitate catalysis for example covalent catalysis, acid-base catalysis and metal ion catalysis. In the next few slide we will more specifically talk about these strategies.

### Slide 28

So covalent catalysis-

In this active site contains reactive group which covalently attaches to the substrate. Let's take example of chymotrypsin which contains a highly reactive serine (Serine-195). It plays a very central role in catalytic activity of chymotrypsin.

### Enzymes: Catalytic strategies & regulatory mechanisms

Covalent catalysis

- Covalent catalysis involves the formation of a transient covalent bond between the nucleophile present in the enzyme and the substrate molecule. Formation of this bond provides an alternative reaction pathway that has lower activation energy than the uncatalyzed reaction.
- Several amino acid side chains act as effective nucleophiles that facilitate the reaction. The enzyme is regenerated in its unaltered form at the end of the reaction.

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- Chymotrypsin is one such enzyme that carries out catalysis by covalent modification. It possesses a catalytic triad of histidine, aspartic acid and serine at its active site with the serine at position 195 serving as a highly powerful nucleophile. The reaction between the serine hydroxyl group and the unreactive carbonyl group of the substrate helps in bringing about product formation with regeneration of the enzyme after the reaction. Covalent modification of this serine residue led to irreversible inactivation of the enzyme.

### Slide 29

#### Acid-base catalysis-

- A molecule plays a role of proton donor or acceptor; this is other than the water molecule. Let's take an example of carbonic anhydrase where zinc ion is bound to imidazole ring of three histidine residues and water molecule. So histidine facilitates hydrogen ion removal from zinc bound water and generates hydroxyl ion.
- Acid-base catalysis
- Biochemical reactions involving the formation of unstable charged intermediates are often stabilized by transfer of protons to or from the substrate or intermediate. For non-enzymatic reactions, acid-base catalysis may involve only the hydronium or hydroxyl ions of water, referred to as specific acid-base catalysis.
- In many cases, however, water alone does not suffice to catalyze the reaction. In such cases, proton transfer is facilitated by weak organic acids or bases. Organic acids act as proton donors while organic bases can serve as proton acceptors.
- In case of enzyme catalyzed reactions, weak proton donors or acceptors are often present as amino acid side chains at the active site of the enzyme itself. The precise positioning of these groups within the active site mediates proton transfer reactions which can provide rate enhancements of several orders of magnitude.
- Acid-base catalysis is a common mechanism of action employed by many enzymes. It is often used in combination with another mechanism such as covalent catalysis. The ease of stabilization of charged intermediates by the amino acid side chains helps in lowering the activation energy for product formation.
- Chymotrypsin is one such enzyme that employs both covalent catalysis as well as acid-base mechanism. The arrangement of the catalytic triad, consisting of Aspartic acid, Histidine and Serine, at the enzyme's active site is such that the histidine residue serves as a general base catalyst by polarizing the hydroxyl

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group of serine. The alkoxide ion thus generated in the serine residue makes it a more powerful nucleophile.

- Following substrate binding and nucleophilic attack of the serine on the carbonyl group, the geometry of the intermediate becomes tetrahedral and the negative charge developed on the carbonyl oxygen gets stabilized through interactions with other side chains of the proteins, in a site known as the oxyanion hole. An internal proton transfer then causes the tetrahedral intermediate to collapse and generate the acyl-enzyme intermediate after which the amine group is released from the active site.
- Once the amine group leaves the enzyme's active site, it is replaced by a molecule of water which carries out hydrolysis of the ester group of the acyl-enzyme intermediate. Mechanism for hydrolysis again proceeds via formation of a tetrahedral intermediate with histidine acting as a general acid catalyst and the negative charge on oxygen being stabilized by residues in the oxyanion hole.
- The tetrahedral intermediate then breaks down to liberate the second product in the form of a carboxylic acid along with regeneration of the enzyme which is then ready for another round of catalysis. Internal proton transfers between amino acid side chains therefore play a vital role in acid-base catalysis by enzymes.

### Slide 30

The third strategy is metal ion catalysis. Metal ions serve as bridge between enzyme (E) and substrate (S). They increase binding energy and hold substrate in appropriate conformation which is required for catalysis. Let's take example of nucleoside monophosphate (NMP) kinase. So all these enzymes utilize ATP as substrate, the NMP kinase catalyze the inter-conversion of ATP and NMP into nucleoside di-phosphate (NDP) by transferring phosphoryl group.

#### Metal ion catalysis

- Metal ions, either present in solution or bound to the enzyme itself, facilitate catalysis by forming favorable interactions between enzyme and substrate or in the transition state.
- Metal ions in the active site of the enzyme typically react with a water molecule and activate it by facilitating generation of a strong nucleophile in the form of a hydroxide ion.
- The nucleophilic alkoxide ion attacks the unreactive carbonyl group to form a tetrahedral intermediate in which the charges are stabilized by the metal ion.
- These favorable interactions help in orienting the substrate and enzyme in suitable positions for transition state, and subsequently, product formation.

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- Carbonic anhydrase is an enzyme responsible for hydration and dehydration reactions of carbon dioxide and bicarbonate respectively and has been found to have a divalent zinc ion associated with its activity.
- The zinc ion in its active site is bound to the imidazole rings of three histidine residues as well as to a molecule of water.
- This binding to water facilitates formation of the hydroxide nucleophile with concomitant release of a proton.
- The generated hydroxide ion at the active site then attacks the carbon dioxide substrate, converting it into a bicarbonate ion. The negative charge generated on the oxygen atom is stabilized by interactions with the zinc ion.
- Binding of another molecule of water to the zinc ion at the active site of the enzyme leads to release of the bicarbonate ion and regeneration of the enzyme molecule ready for another round of catalysis. Several pH related studies have provided substantial proof for this mechanism.

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After briefly discussing catalytic strategies, now let's discuss regulatory strategies.

- The metabolic pathways are complex, especially, when we are talking at the proteomic scale. So it includes one or more enzymes which exhibit greater effect on rate of overall sequence.
- So in response to a given trigger or signal, these regulatory strategies of enzyme can modulate catalytic activity and allows cells to meet energy requirements and eventually it will dictate the cell growth and survival.

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So first strategy is allosteric regulation-

- The allosteric proteins possess regulatory site and multiple functional sites.
- The activity is modulated by specific regulatory molecules.
- These regulatory molecules bind at different site than the active site and trigger conformational changes which are transmitted to active site. The allosteric proteins possess property of co-operativity.
- So activity at one functional site can affect activity of other sites in cooperative manner.
- Allosteric proteins are information transducers in response to signal molecule their activity is modified.
- Let's take an example of hemoglobin, where oxygen binding curve is sigmoidal that is a cooperative binding behavior.

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- Binding of carbon dioxide and hydrogen ion promotes release of oxygen and there is allosteric linkage between binding of hydrogen ions, carbon dioxide and oxygen.

### Regulatory strategies

- Activity of all enzymes must be regulated to ensure that they function only to the desired extent at the appropriate locations within an organism.
- Common mechanisms of regulation include allosteric or feedback inhibition, control of isozyme forms, reversible covalent modification and proteolytic activation.
- Allosteric enzymes, these possess distinct regulatory and catalytic sites, are often found as the first enzyme of a reaction pathway.
- Regulation of the first enzyme of a pathway by the final product of the pathway is known as feedback inhibition.
- Binding of the regulator molecule to the regulatory site of the enzyme triggers a series of conformational changes that are ultimately transmitted to the active site where substrate binding is then inhibited.
- It has been observed that allosteric enzymes do not obey regular Michaelis-Menten kinetics.
- Aspartate transcarbamoylase, which catalyzes the first step of pyrimidine biosynthesis, is an allosteric enzyme having distinct regulatory and catalytic subunits.
- Binding of substrate to the catalytic subunits induces conformational changes that stabilize the relaxed state or R state of the enzyme, thereby facilitating the enzymatic reaction.
- The inhibitor for this enzyme is CTP which is the final product of the pathway.
- Binding of inhibitor to the regulatory subunits stabilizes the tense state or T state of the enzyme thereby preventing the reaction from taking place.

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### Isozymes

- Isozymes are multiple forms of a enzyme. Isozymes catalyse the same reaction but differ in structural characteristics.
- Isozymes regulate the same reaction differently at at different locations.
- They differ in  $K_m$ ,  $V_{max}$  and different regulatory properties.
- Isozymes are expressed in different organelle or even at different stages of development.

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- So essentially it can allow the very intricate development of metabolism by fine tuning various reactions responsible in metabolism to meet the need of a given tissue or developmental stage.
- Isoenzymes are homologous enzymes within a single organism that differ slightly in their amino acid sequence but catalyze the same reaction.
- These enzymes are mostly expressed in different tissues and have differing kinetic parameters such as substrate affinity ( $K_m$ ) and maximum velocity ( $V_{max}$ ).
- They may also have different regulator molecules that allow them to be differentially expressed and regulated depending on the requirement for that particular tissue.

Lactate dehydrogenase is an enzyme involved in anaerobic glucose metabolism that is present as two isozyme forms in human beings. The tetrameric heart enzyme, which requires an aerobic environment to function, has higher affinity for its substrate than the muscle enzyme. Despite having 75% sequence homology, they also differ in that high levels of pyruvate allosterically inhibit the heart enzyme but not the muscle form.

### Slide 34

Now let's discuss reversible covalent modification-

- This is a very effective process to control enzyme activity.
- You have heard example of phosphorylation. Protein kinase can catalyze protein phosphorylation and ATP serves as the phosphoryl group donor. There is another enzyme protein phosphatase which removes phosphoryl group by hydrolysis process.
- Reversible covalent modification is another commonly employed enzyme regulatory strategy. The most widely observed modification is phosphorylation, which is carried out by various enzyme kinases with the help of ATP as a phosphoryl donor. Some enzymes are more active in their phosphorylated forms while others are less active in this form. Dephosphorylation is carried out by the phosphatase enzyme. Enzymes involved in glycogen metabolism are regulated by reversible phosphorylation.
- Apart from phosphorylation, which most commonly takes place at serine, threonine and tyrosine residues, other reversible covalent modifications include adenylation, uridylation, methylation and ADP-ribosylation which modify different amino acid residues of the proteins.

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- Lastly, we will discuss a regulatory strategy, enzyme activation by proteolytic cleavage, which allows the enzyme to cycle between the active and inactive state.
- The inactive state which is the precursor is known as zymogen or proenzyme. One of the classical enzymes controlled by this mechanism is the enzyme chymotrypsin which is present in digestive tract so trypsin converts chymotrypsin which is a zymogen into the active chymotrypsin by hydrolyzing peptide bond. The blood clotting due to the zymogen activation is another example.
- Several enzymes exist in their inactive forms, known as zymogens, where they do not possess any catalytic activity. In order to become active, they need to be activated by hydrolysis of one of more peptide bonds by various proteases. This removal of certain regulatory residues irreversibly converts the enzyme into its active form. Unlike reversible modification, the enzyme is degraded after completion of catalysis.
- Several digestive enzymes as well as clotting factors are regulated by proteolytic activation. Chymotrypsin, a digestive enzyme that hydrolyzes proteins in the small intestine, exists in its zymogen form within membrane bound granules after synthesis in the acinar cells of the pancreas. The proteolytic enzyme trypsin converts it into its fully active form by cleavage of a peptide bond between arginine at position 15 and isoleucine at position 16. The resulting enzyme known as pi-chymotrypsin is acted upon by other such molecules to yield the completely active and stable alpha-chymotrypsin which consists of three chains linked by inter-chain disulphide bonds.

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So in summary today we discussed various concepts related to enzyme which are highly effective catalyst. There are several catalytic mechanisms which are employed by the enzymes which include acid-base catalysis, covalent catalysis and metal-ion catalysis; we discussed some of these mechanisms in some details. The activities of metabolic pathways in cells are regulated by control of the activities of the certain regulatory enzymes and four regulatory strategies were discussed.

So I hope now you have some basic understanding of enzymes and different catalytic and regulatory strategies.