Proteomics: Principles and Techniques

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Lecture No. # 07
Enzymes: Basic concepts, Catalytic and regulatory strategies

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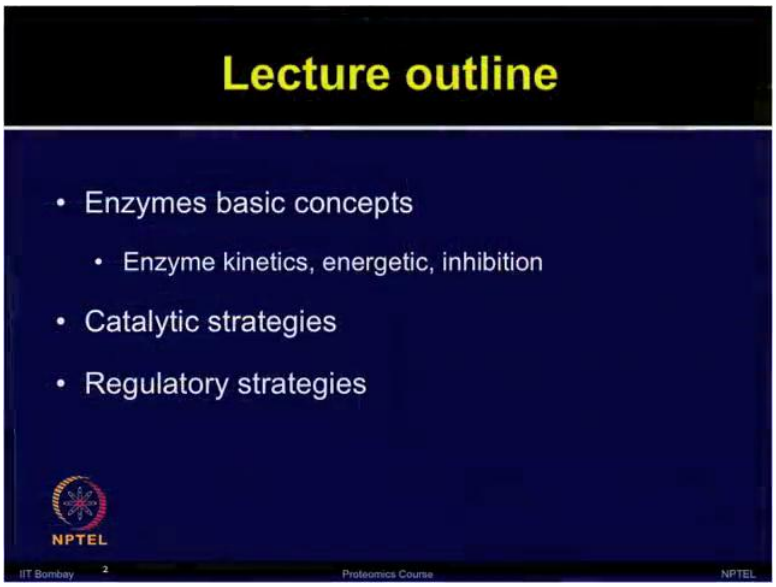

Welcome to proteomics course. Today, we will talk about enzymes; it is basic concept and catalytic and regulatory strategies. So, lecture outline is that first we will talk about different basic concept of enzyme including enzyme kinetics, energetic and enzyme inhibition. Let us then move on to different type of catalytic strategies and regulatory strategies. So, as you all know, enzymes play a very important role in biochemistry, and offer sensitive and a specific method of quantization for various substances. All enzymes are proteins therefore I think it is essential to a study about enzymes while studying the basic concepts of amino acids and proteins. Although it may not be directly linked to the proteomics, but understanding about proteins and enzymes is very fundamental for the advance understanding of concepts related to the proteomics.

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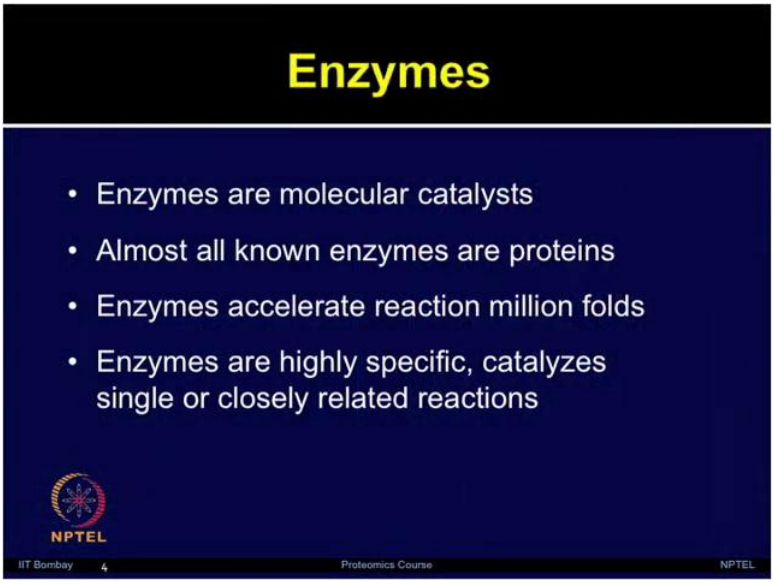

So, what are enzymes? Enzymes are molecular catalyst; almost all the known enzymes are proteins, and as you have discussed in the previous lecture, there are 20 amino acids, which offer array of chemical forces. These enzymes can accelerate the given reaction up to even million folds. For example let us take example of an enzyme carbonic anhydrides, which catalyses hydration of carbon dioxide. Now, this enzyme can catalyse 10 to the power 6 molecules per second. These enzymes are highly specific, and they catalyse single or very closely related reactions. Well, these are just examples, which we will talk during the entire lecture, how enzymes regulate various reactions?

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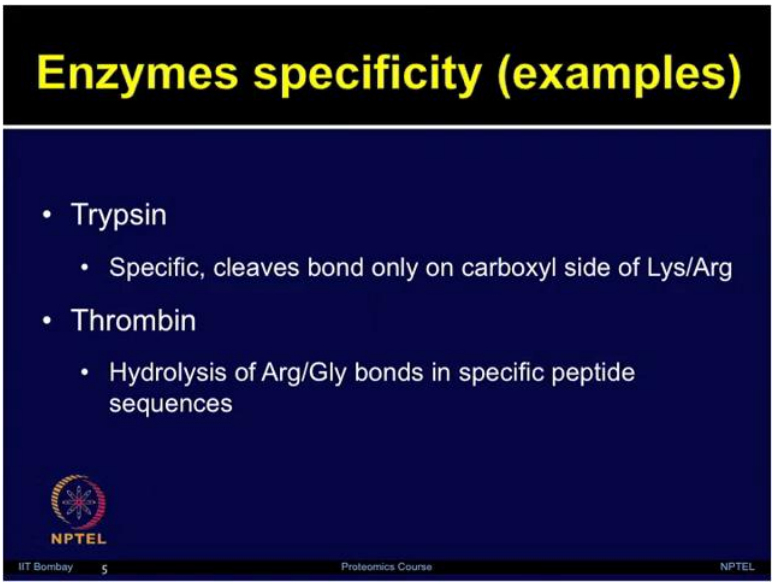

There are certain proteolytic enzymes; for example, which catalyse proteolysis and hydrolysis of peptide bond. In tips of enzyme specificity let me give you few examples, Trypsin it is highly specific it cleaves bond only on the carboxyl side chain on the residues Lysine and Arginine, another enzyme Thrombin which participates in blood clotting, it is even more specific than Trypsin it catalyses hydrolysis of Arginine Glysine bonds in a very specific peptide sequence; and therefore, offers highly specificity.

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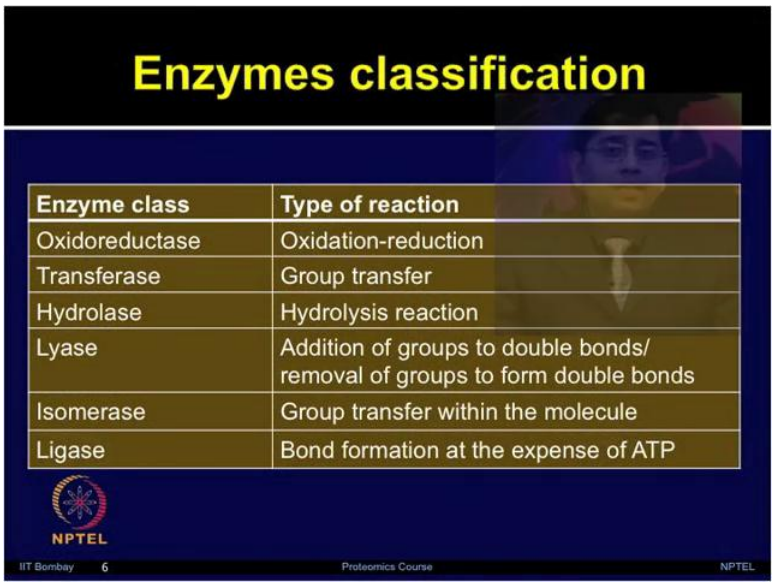

The six major classes of enzymes which are grouped according to the type of reactions they catalyse. I will not go in too much detail, but briefly let me talk what type of reaction they these class of enzymes are offering. Oxidoreductase it catalyses oxidation and reduction; example, asparatate dehydrogenase transfers these enzymes can transfer the certain groups, the group transfer can be mediated by this group of enzymes ; example, Hexokinase. Hydrolase these can catalyse hydrolysis reaction; example, Chymotrypsin Lyase is another group of enzyme which adds group to double bonds or removes groups to form the double bonds; example include, Fumarase, Isomerase as the term says Isomerisation, so this group of enzymes can transfer groups within the molecule; example, Phosphogluco Isomerase. Ligase it catalyses bond formation by using a t p; example, Glutamine Synthesis these are just few examples, but each enzyme class incorporate various enzymes broadly these are the type of reaction which these enzyme can catalyse.

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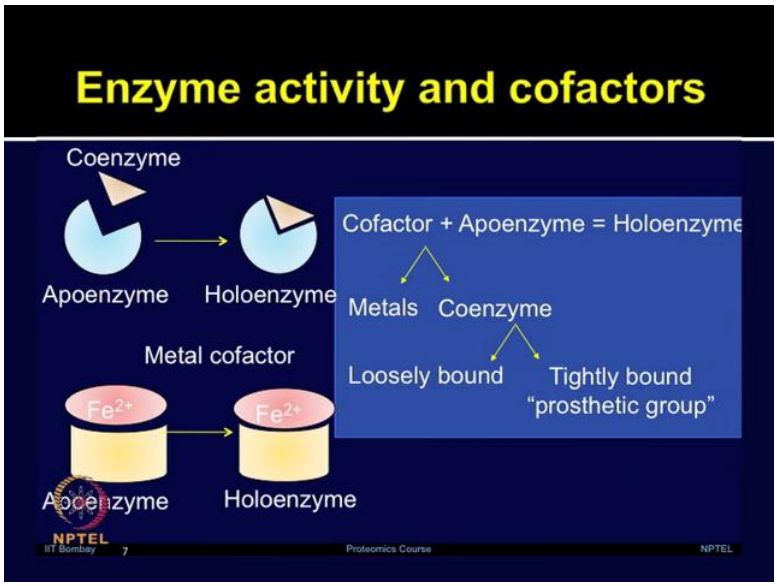

So, let us talk about enzyme activity and how various cofactors govern this enzyme activity? So, many enzymes catalysis activity depends on the presence of small molecules which are known as cofactor. They could be two groups here as shown in the PPT metals and a small organic molecules, the Coenzymes are a small organic molecule such as those derived from the vitamins, the prosthetic group that is a tightly bond coenzyme. The Apoenzymes are enzyme without its cofactor, and Holoenzyme when you combine cofactor and Apoenzyme to give rise to complete catalytic enzyme that is Holoenzyme.

Most enzymes are made up of a protein part known as the Apoenzyme as well as cofactor, which can either be an organic molecule known as coenzyme or a metal ion. These cofactors are essential for the enzyme to be catalytically functional and the complete functional enzyme is referred as Holoenzyme. The Pyruate dehydrogenase is a complex enzyme which uses Thymine Pyrophophatase as it is coenzyme, while another enzyme carbonic anhydrase uses zinc ion as it is cofactor.

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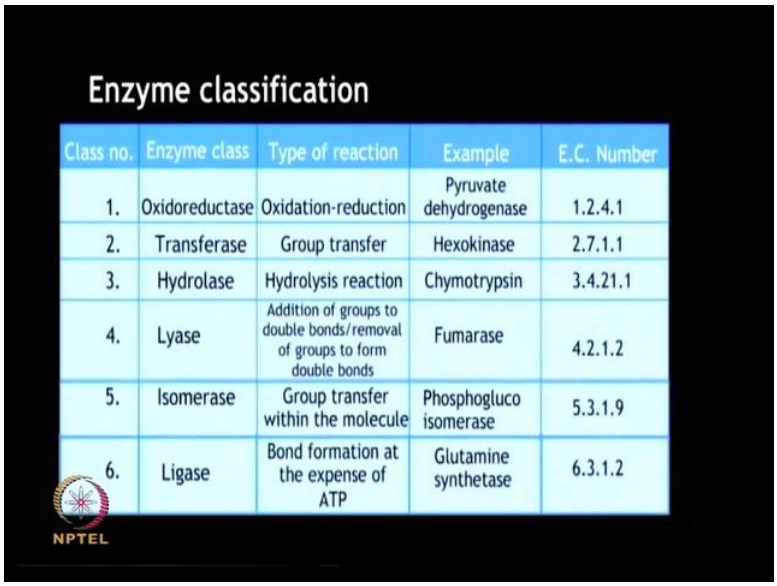

Enzymes are classified on the basis of the reactions that they catalyse, most enzymes are named by adding the suffix ace either to their substrate or the type of activity they carry out; however, Asmore enzymes came to be known it became increasingly difficult to name in this manner, classification by international organisations has therefore, led to six enzyme classes with many sub group within each class depending upon the type of reactions they are catalysing. Every enzyme has a unique four part classification number known as the enzyme commission number or E c number, in which sub class number gives final detail about that particular enzyme reaction. Let us now talk about free energy and enzyme. So, remember the concept of entropy delta G equals to delta h minus t delta s.

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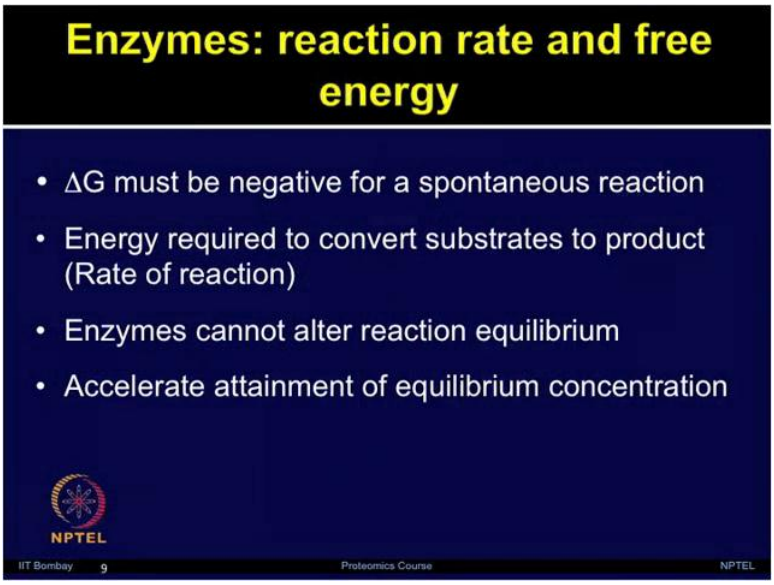

So, delta G must be negative for a spontaneous reaction. So, the energy which is required to convert the substances to product that is rate of reaction, and enzymes can only affect the rate of reaction so, enzymes cannot alter reaction equilibrium they can accelerate attainment of equilibrium concentration. 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.

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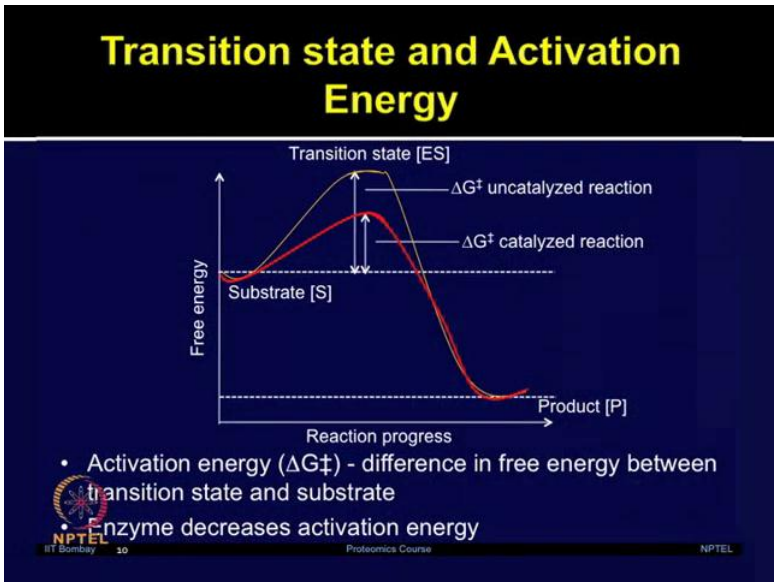

Now, if you look at this graph, the enzyme decreases the activation energy, and alters the rate of attainment of reaction equilibrium by facilitating the formation of S star. Conversion of substrate to product proceeds through formation of a transition state, the free energy of activation of an uncatalized reaction is very high. enzymes form favourable 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 this must be emphasised that enzymes do not alter the equilibrium of a reaction.

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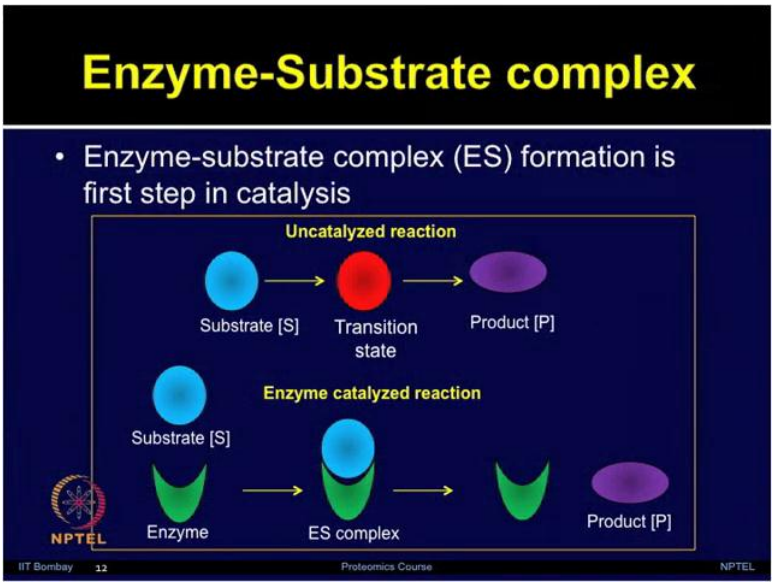

Enzyme substrate complex, the enzyme substrate complex or E S formation is first step in catalysis. enzymes are very selective in choice of substrate they combine which ultimately dictates the specificity of this catalyst, these substrates are bond to active site of an enzyme they have been experimental evidences that enzyme substrate arrhenius complex do form, and various strategies which have shown that E S complex forms include maximal velocity, x ray crystallography, and spectroscopy.

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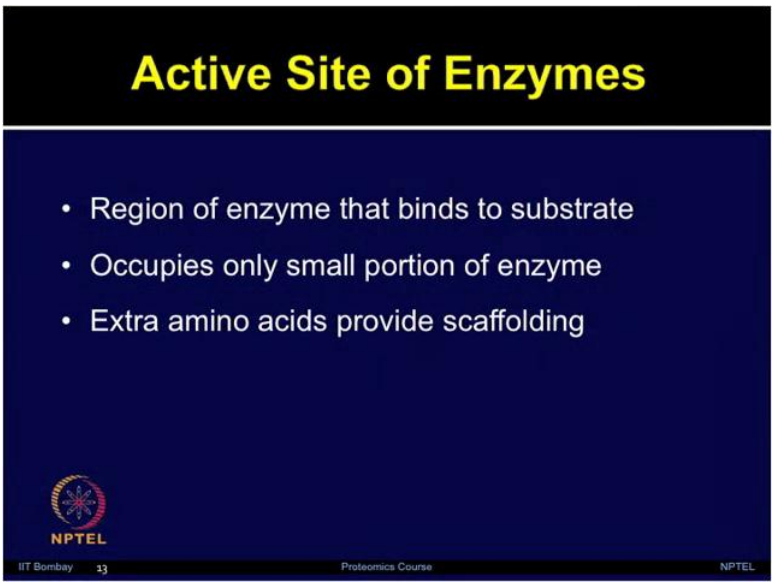

So, what is active site? The active site is a region of enzyme that binds to substrate. It provides a three-dimensional cleft it is formed by amino acid from different parts of the sequence. So, it is not necessarily that the residues in that pocket are only going to form the active site, but even it can be from different regions of given protein which could form the active site, they occupy only small portion of enzymes and some ester amino acids they help in scaffold.

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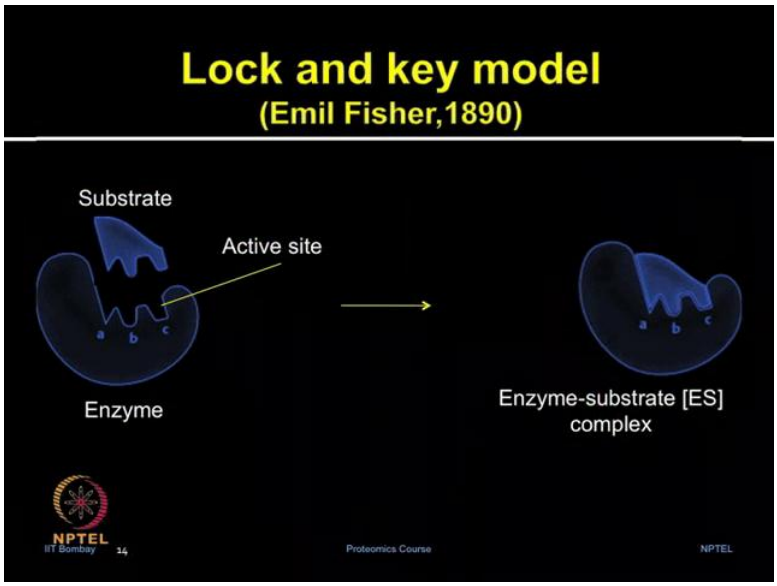

To understand enzyme substrate binding different models have been proposed. The two popular models one is lock and key model, which was proposed by scientist Emil Fischer in 1890, this model explains the complimentary interaction can determine the specificity. So, if you have an enzyme and a substrate is present now the active site of unbound enzyme should be complimentary ensured to the substrate something similar, to the key is very specific for the given lock then only it will open.

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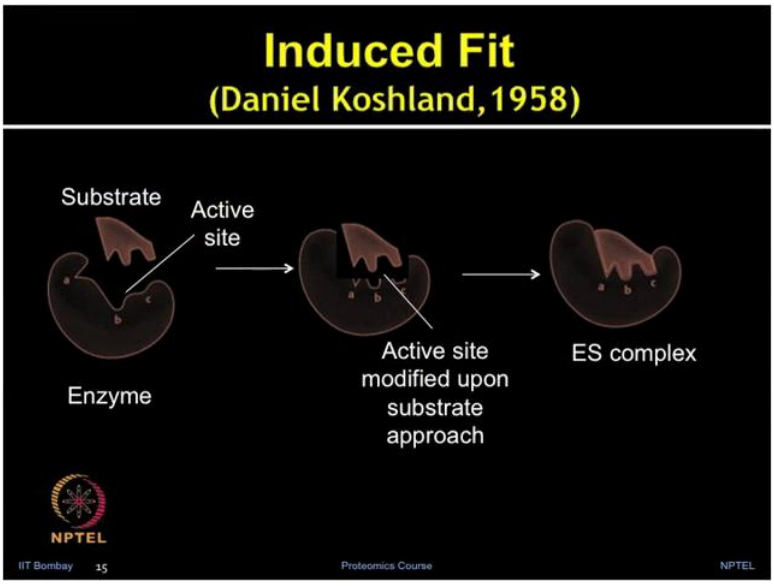

Other model is known as induced fit which was proposed by scientist Daniel Koshland in 1958. So, the active sights they form is shape which is complimentary to the substrate and only after the substrate has been bond. So, enzyme can change the shape of a substrate binding; in this case, the substrate structures do not have to be specific to the active site of enzymes. It can be dictated by enzyme and the shape can be changed which will be complimentary for this binding.

Fischer's hypothesis as 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 can open the lock. Similarly, only one particular substrate will fit into the active site of the enzyme and the enzymatic reactions can occur.

According to the Fischer's hypothesis enzymes and their substrates possess a specific complimentary geometric shapes that fit exactly into each other. This model accounts for the specificity of enzymes that fail to account for a stabilisation of the transition state. Koshland proposed another hypothesis known as Koshland's modified hypothesis suggested that the active site of an enzyme gets continually reformed based on the interaction that it establishes with the substrate molecule, this hypothesis accounts for both the enzyme specificity and the stabilisation of the transition state since enzyme is not considered to be a rigid molecule. Let us talk about enzyme kinetics, in 1913 scientist Michaelis and Menten they derived a model to explain the concept of enzyme substrate complex which is fundamental to understand the enzyme reactions.

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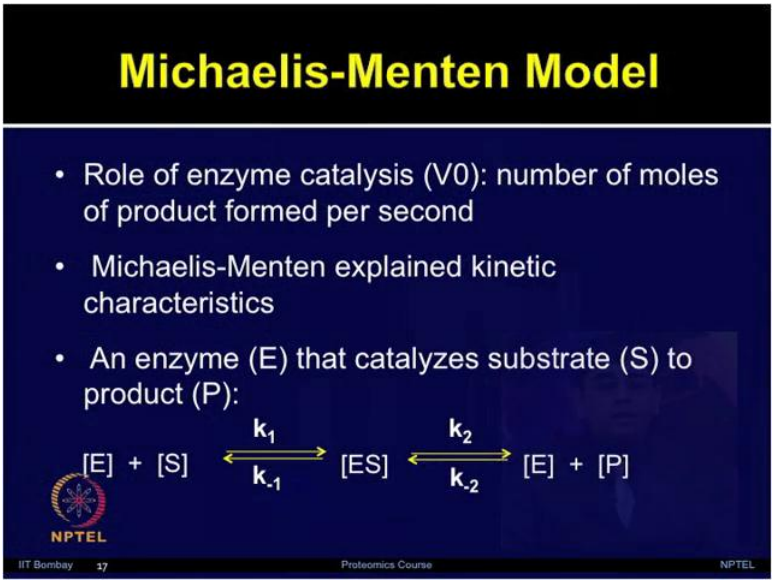

The Michaelis-Menten explained kinetic characteristics, the role of enzyme catalysis or V 0 which is number of moles or product formed per second it varies with substrate concentration S, it increases linearly as the substrate concentration increases levels off at maximal velocity at higher substrate concentration. So, an enzyme E which catalyses a substrate S to give rise to a product P can be understood in this equation, E plus S giving rise to E S, an enzyme substrate complex is broken down to enzyme plus product. Now, E combines with S to form E S complex in presence of rate constant K 1; whereas, the E S complex can be dissociated to form E and S with rate constant K minus 1, E S complex can form product with rate constant K 2, an E S complex can reformed by reverse reaction in rate constant K minus 2.

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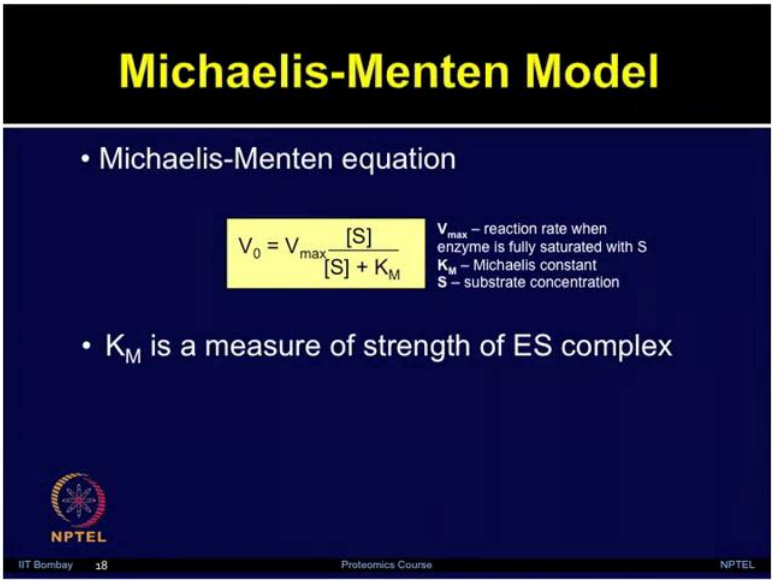

So, here is Michaelis-Menten equation the rate V 0 of formation of product can be derived by this equation, V 0 equals to V max substrate concentration S divided by S plus K M, where K M is Michaelis constant. Now this equation is fundamental to understand various problems. So, let us say we take this equation and there is one situation where substrate concentration is less than K m or Michaelis constant. Now, what will be the equation? In this case V 0 will become V max divided by K m times substrate concentration, so rate it is directly proportional to substrate which is the reaction is first order. Now, let us take another scenario where substrate concentration is greater than K m. Now what will be the equation? V 0 equals to V max, so rate is maximal and it is independent of substrate concentration in this case reaction is 0 orders. Now, let us say third situation where substrate concentration is equal to K M, now in this case the equation will become V 0 equals to V max divided by2, so substrate concentration at half maximal velocity is known as Michaelis constant or K M.

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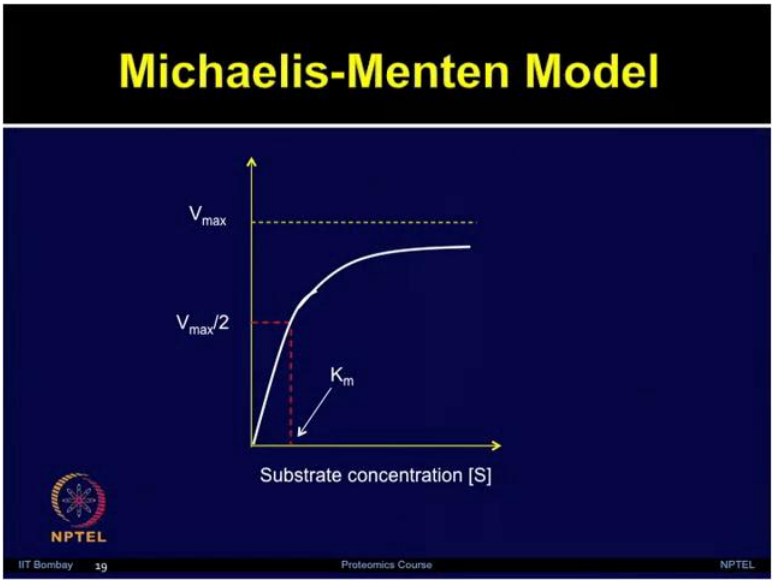

So, K M is substrate concentration at half maximal velocity as you can see in the graph the K M is a measure of a strength of enzyme substrate complex, so higher K M indicates weaker bonding, and low K m is strong binding.

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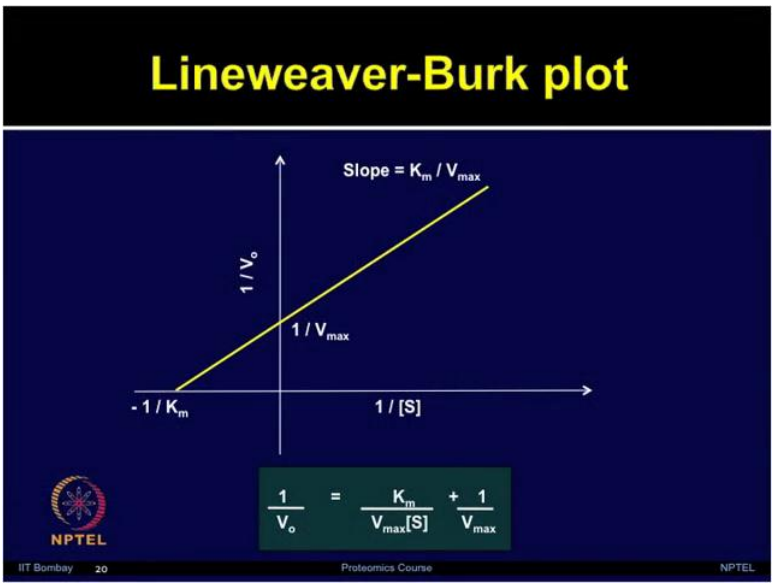

So, another scientist gave Lineweaver-Burk plot or double reciprocal plot, which is a plot of 1 by V 0 verses 1 by S, it is reverse of the V 0 or S which was taken in case of Michaelis reaction. Enzymes catalyze the formation of product from its substrate via an enzyme substrate intermediate complex, during the initial stage of the reaction the equilibrium

favours product formation rather than dissociation of the E S complex to give back this substrate molecule. The number of moles of product formed per second during the restages determines the reaction velocity for that particular enzyme. V 0 has an almost linear relation with substrate concentration, when the substrate concentration is low it becomes independent at higher concentrations. The Michaelis-Menten model for enzyme kinetics assumes that the breakdown of E S complex to give back free substrates negligible, and also assumes a steady state condition, where by the rate of formation and break down of enzyme substrate complex are equal. The reaction velocity increases linearly with substrate concentration when S is low, but becomes independent at higher concentrations. The maximum 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 it is maximum value is known as 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, the this equation is derived from the Michaelis-Menten equation by taking reciprocals on both sides and then plotting a graph of 1 by V 0 verses 1 by substrate. The y intercept on this graph can be used to deduce the value of V max while the x intercept give the value of K M.

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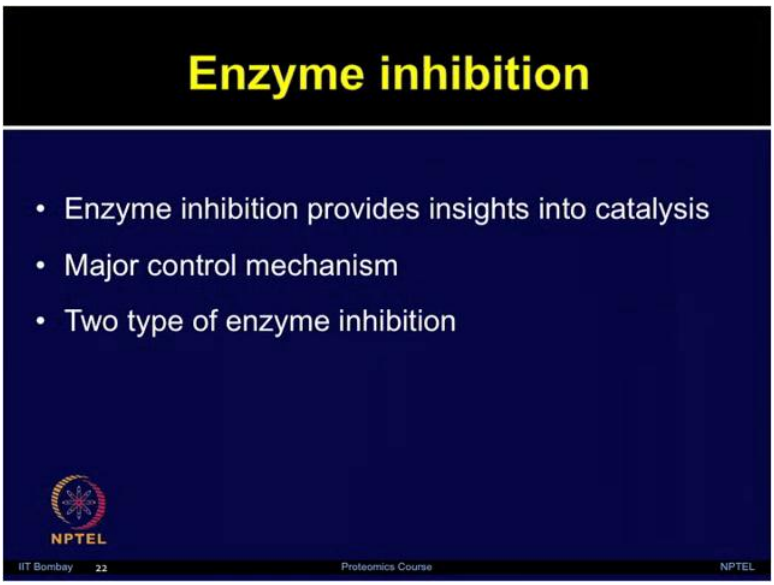

Enzyme inhibition; the enzyme inhibition can provide insights into catalysis, enzyme activity can be inhibited by small molecules and ions, the enzyme inhibition provides major

control mechanism in biological systems. There are two types of enzyme inhibition reversible and irreversible.

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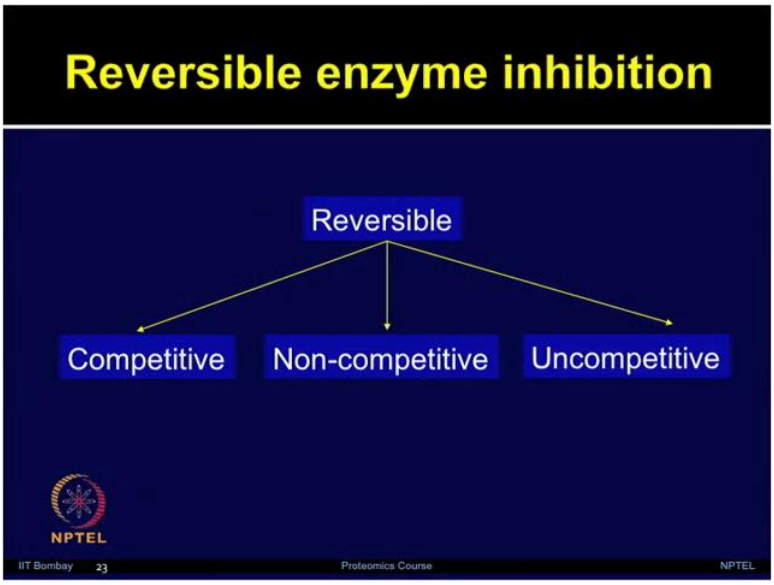

The reversible inhibitors they dissociate rapidly; and the enzyme inhibitor E I complex is dissociated. There are three different strategies for reversible enzyme inhibition; competitive, non competitive, uncompetitive or myth inhibitions.

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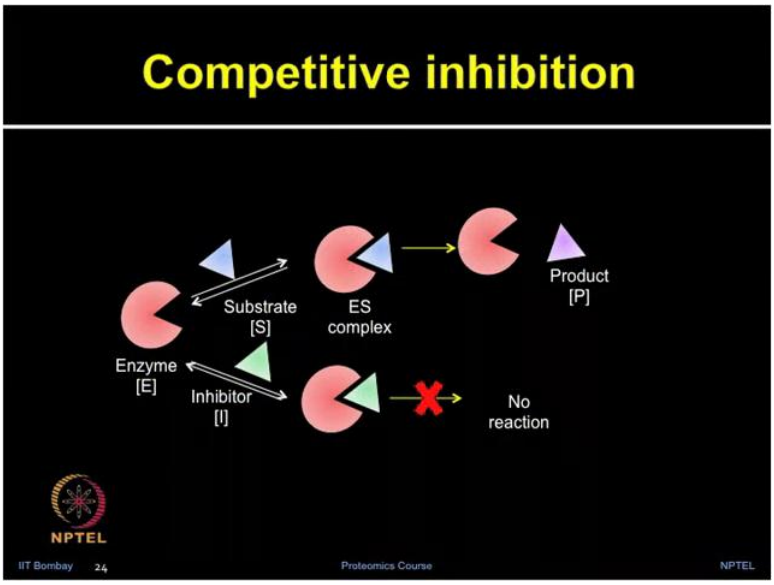

First discuss; first situation competitive inhibition. The enzyme binds to substrate or inhibitor and forms enzyme substrate or enzyme inhibitor complex, but do not form enzyme

substrate inhibitor ESI complex, the inhibitor is similar in structure to the substrate which binds the active site of Enzyme. It can reduce the rate of catalysis and also reduces the number of enzyme substrates which are formed by binding at the same active site as shown in the figure.

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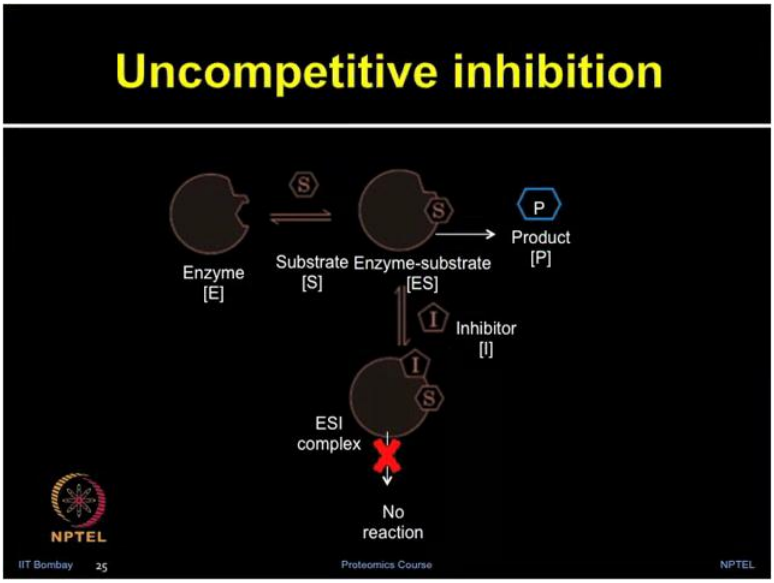

Uncompetitive inhibition; inhibitor and substrate they bind at different sights, enzyme substrate inhibitor complex is formed, so by increasing the substrate concentration inhibition cannot be overcome, which was the case in the previous one of competitive inhibition the reaction rate increases more slowly at lower substrate concentration as compare to the uncompetitive.

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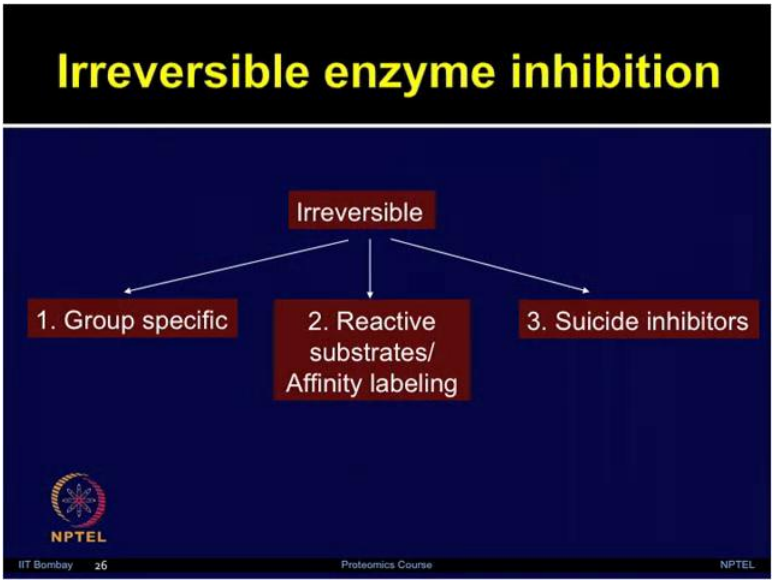

Also talking to reversible enzyme inhibition now let us discusses irreversible enzyme inhibition. Inhibitor is very tightly bound to enzyme either covalently or non-covalently in such a way that this inhibition is irreversible. Let us take the very classical example penicillin the first anti biotech which was discovered, it was irreversibly inactivating a key enzyme Transpeptidase in bacterial cell wall synthesis and killed the bacteria. Now in irreversible enzyme inhibition that different classes; group specific, reactive substrates or suicide inhibitors, the group specific irreversible enzyme this inhibitors can react with the specific sight chain of amino acids, the reactive substrates or affinity labels they are structurally similar to the substrate and covalently bind to the active sight, they are more specific for enzyme active sight then group specific reagents.

The suicide inhibitors they are highly specific substrates which can modify the active sight of an Enzyme, they are most specific for modification of enzymes active sight. 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 catalytic reactions. The 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 sight. 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 K M is altered. The competitive inhibition can be overcome by suitably increasing the substrate concentration which allows the substrate to out compete the inhibitor for the enzymes for the active sight. In case of uncompetitive inhibition this substrate and inhibitor both have different binding sites on the Enzyme.

Binding of inhibitor to the enzyme substrate 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, mixed inhibition or non-competitive also binds to the enzyme at a site distinct from the substrate binding site. However, the difference is that it can bind either to the enzyme or enzyme substrate complex, binding of either one brings about conformational changes in the enzyme structure; thereby, affecting biding 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. Now, for discussing various basic concepts of Enzyme, let us move on to catalytic strategies, enzymes are very effective and extremely specific catalyst. The enzymes employee multiple mechanism to facilitate catalysis; for example, covalent catalysis, a c base catalysis, and metal ion catalysis, in the next few slides we will talk more specifically about these strategies.

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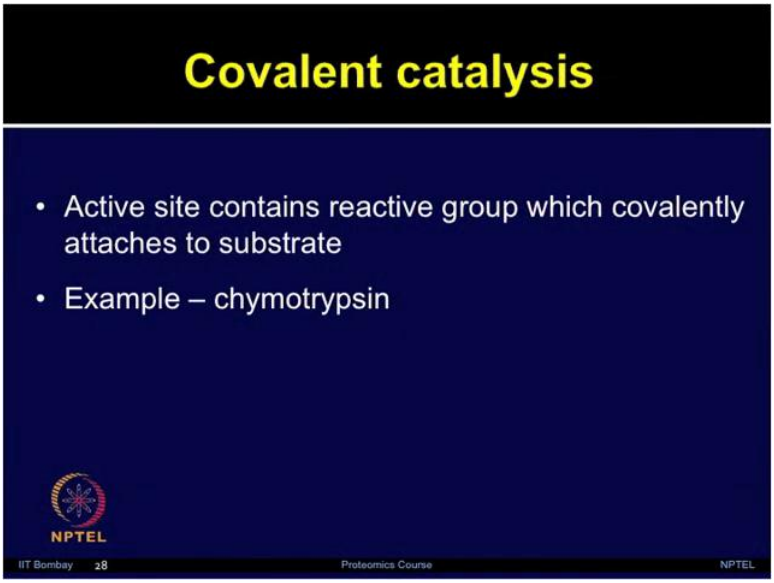

So, covalent catalysis, in this the active site contains reactive group which covalently attaches to the substrate. Let us take example of Chymotrypsin which is contain a highly reactive cyrine at the position cyrine 195, it play a very central role in catalytic activity of Chymotrypsin. Covalent catalysis 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 acids site chain act as effective nucleophile that facilitate this reaction, the enzyme is regenerated in its unaltered form at the end of this reaction.

Chymotrypsin is one such enzyme that carries out catalysis by covalent modification. It poses a catalytic triad of histrine, aspartic acid and cyrine at its active site, with the cyrine at position 195 serving as highly powerful nucleophile, the reaction between the cyrine hydroxyl group and unreactive carbonyl group of the substrate helps in bringing about product formation. Enzyme regeneration occurs after this reaction, covalent modification of the cyrine residue led to the irreversible inactivation of the enzyme. Acid base catalysis, a molecule plays a role of a proton donor or acceptor this is other than the water molecule.

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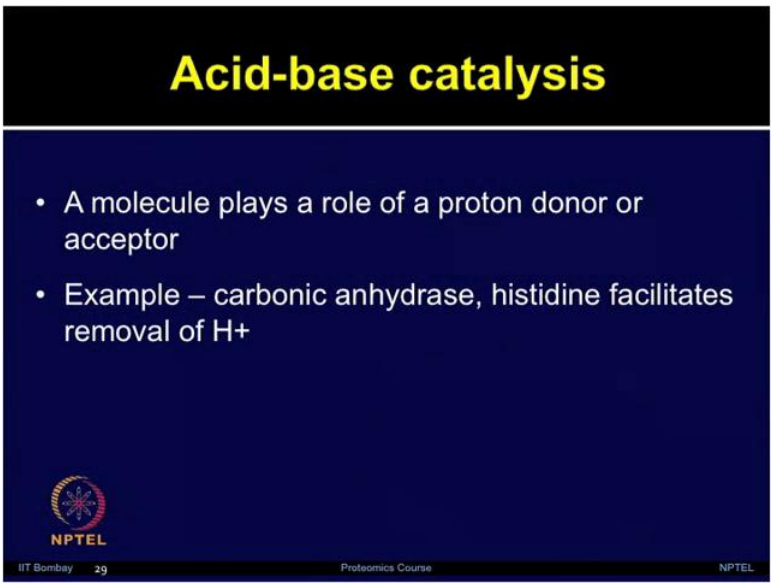

Let us take example of carbonic anhydrase where sic ion is bound to imidazole ring of three histrine residues and water molecule. So, histrine facilitates hydrogen ion removal from zinc bound water and generates hydroxyl ion, biochemical reactions involving the

formation of unstable charge 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. The organic acids act as proton donors while the organic bases can serve as proton acceptors, in case of enzyme catalyze reactions weak proton donors or acceptors are often present as amino acid site chain at the active site of enzyme itself. The precise positioning of these groups within the active site mediate proton transfer reactions which can provide arte enhancement of several order of magnitude, the 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 yield of stabilization of charge intermediates by the amino acid site chain 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, histrine and cyrine at the enzyme active site is such that the histrine residue servers as general base catalysts by polarizing the hydroxyl group of cyrine.

The Alkoxide ion thus generated in the cyrine residue makes it a powerful nucleophile. Following substrate binding and nucleophile attack of the cyrine on the carbonyl group the geometry of intermediate becomes tetrahedral, and the negative charge developed on the carbonyl oxygen gets stabilized through interaction with other site chain of the proteins in a site known as Oxyanion hole. An internal proton transfer then causes the tetrahedral intermediate to collapse and generate acyl enzyme intermediate after which amine group is released from the active site.

Once the amine group leaves the enzymes active site it is replaced by a molecule of water which carries out hydrolysis of ester group of acyl enzyme intermediate, mechanism for hydrolysis proceeds via formation of a tetrahedral intermediate with histrine acting as a general acid catalyst, and the negative charge on oxygen being stabilized by residues in oxyanion hole. The tetrahedral intermediate then breaks down to

liberate the second product in the form of a carboxylic acid along with regeneration of enzyme which is then ready for another round of catalysis. Internal proton transfers between amino acid site chains therefore, play a vital role in acid base catalysis by enzymes.

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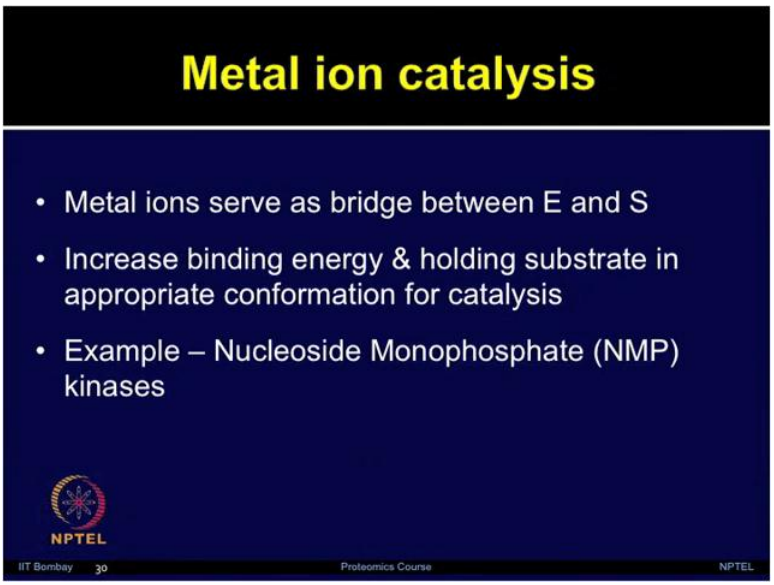

Third strategy is metal ion catalysis, the metal ions serve as bridge between enzyme and substrate, they increase binding energy and hold substrate in appropriate confirmation which is required for catalysis. Let us take example of nucleoside monophosphate kinase NMP, so all these enzymes utilize ATP as substrate. The NMP kinase catalyze inter conversion of ATP and NMP into nucleoside diphosphate NDP by transferring the phosphor group. Metal ions either present in solution or bund to the enzyme itself facilitates catalysis by forming favourable interactions between enzyme and substrates are in the transition state.

Metal ions in the active site of enzyme typically react with a water molecule and activate it by facilitating generation of a strong nucleophile in the form a hydroxide ion. The nucleophilic alkoxide ion attacks the unreactive group to form a tetrahedral intermediate; the tetrahedral intermediate, in which the charges are stabilized by the metal ion; these favourable interactions help in orienting the substrate and enzyme in suitable position for transition state and subsequently product formation. 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 it is active site is bound to the imidazole range of three histidine residues as well as to a molecule of water. This binding to water facilitates formation of 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 interaction with the zinc ions; binding of another molecule of water to the zinc ion, at the active site of enzyme leads to release of the bicarbonate ion and regeneration of the enzyme molecule for another round of catalyses.

Various p h related studies have provided substantial proof for this mechanism. After briefly discussing the catalytic strategies now let us talk about regulatory strategies. The metabolic pathways are very complex especially when we are talking at the proteomic, so it includes one or more enzymes which exhibit greater effect on rate of overall sequence. So, it responds to a given trigger or a signal these regulatory strategies of enzymes can modulate catalytic activity and allow cells to meet the energy requirements and eventually it will dictate the cell growth and survival.

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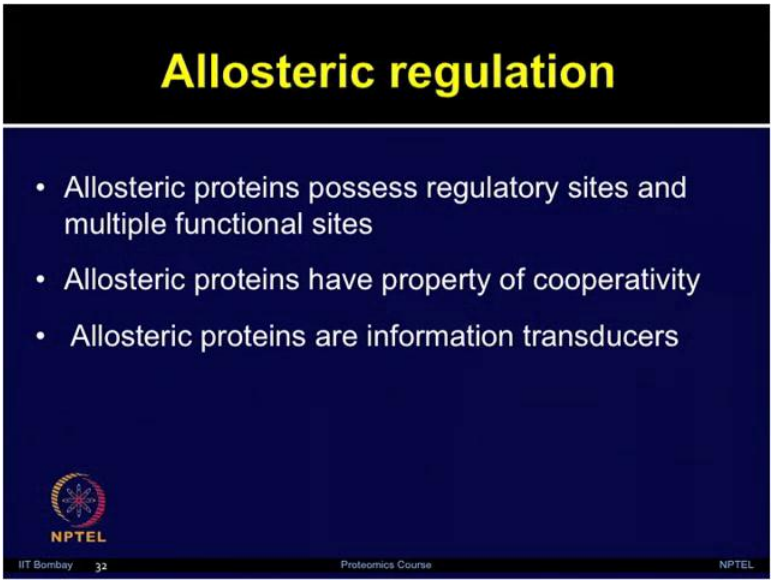

The first strategy is Allosteric regulation. The Allosteric proteins possess regulatory sites and multiple functional sites; the activity is modulated by specific regulatory molecules.

This regulatory molecule binds a different site than the active site and trigger conformational changes which are transmitted to the active site. The Allosteric proteins possess properties of cooperativity, so activity at one functional site can affect the activity of others in cooperative manner. So, the Allosteric enzymes are information transducer in response to the signal molecules there activity is modified. Let us take an example, haemoglobin where oxygen binding curve is sigmoidal that is a cooperative binding behaviour, so binding of hydrogen ion and carbon dioxide promotes release of oxygen, and there is Allosteric linkage between binding of hydrogen ions, carbon dioxide and oxygen. Activity of all enzymes must be regulated to ensure that the function only to the desired extent at the appropriate locations within an organism.

Common mechanism of regulation includes Allosteric or feedback inhibition control of isozyme forms reversible covalent modification and proteolytic activation. Allosteric enzymes these enzymes posses distinct regulatory and catalytic slides and these are often found as the first enzyme of a reaction pathway. Regulation of the first enzyme of a pathway by the final product of pathway is known as feedback inhibition, binding of regulator molecule to the regulatory site of the enzyme triggers a series of conformational changes that are ultimately transmitted to the active side where substrate binding is then inhibited. It has been observed that Allosteric enzymes do not obey regular Michaelis-Menten kinetics, asparate transcarbamoylase which catalyses the first step of pyrimidine biosynthesis is an allosteric enzyme having distinct regulatory and catalytic sub units.

The 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 C T P which is the final product of the pathway, binding of inhibitor to the regulatory subunits stabilizes the tempted state or t state of the Enzyme; thereby, preventing the reaction from taking place. Isozymes or Isoenzymes are multiple forms of enzymes; the isozymes catalyze the same reaction, but differ in structural characteristics.

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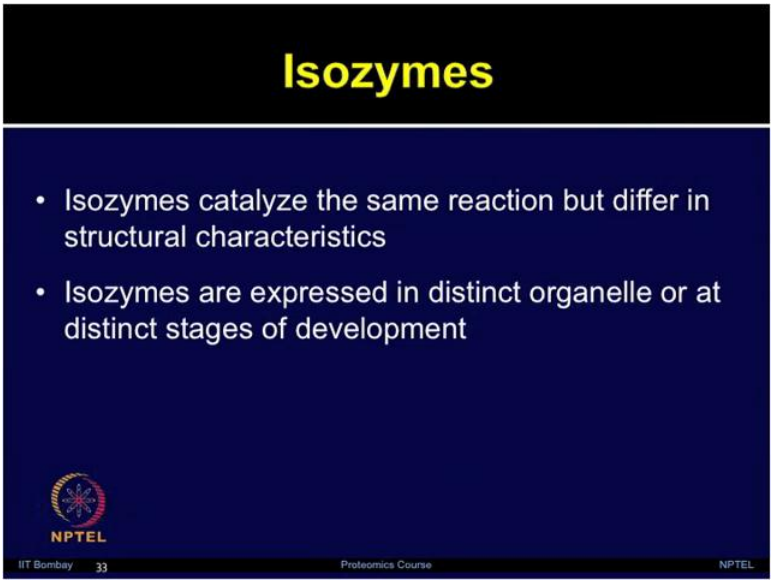

Isozymes varies regulation of same reaction at distinct locations they differ in K M value, V max and different regulatory properties. Isozymes are expressed in different organelles or even at different stages of development. So, essentially it can allow the very intricate development of metabolism by fine tuning various reactions responsible in metabolism to meet the need of given tissue or developmental stage. Isoenzymes are homologous enzymes within a single organism that differ slightly in their amino acid sequence but catalyze a same reaction; these enzymes are mostly expressed in different issue, and have different kinetic parameters such as substrate affinity K M and maximum velocity V max. Lactate dehydrogenase is an enzyme involved in anaerobic glucose metabolism that is present as two isozymes forms an human being, the titrameric heart enzyme which requires an aerobic environment to function has higher affinity for it is substrate than the muscle Enzyme, despite having 75 percent of sequence homology, they also differ in that high levels of hydrate allosterically inhibit the heart Enzyme, but not the muscle form.

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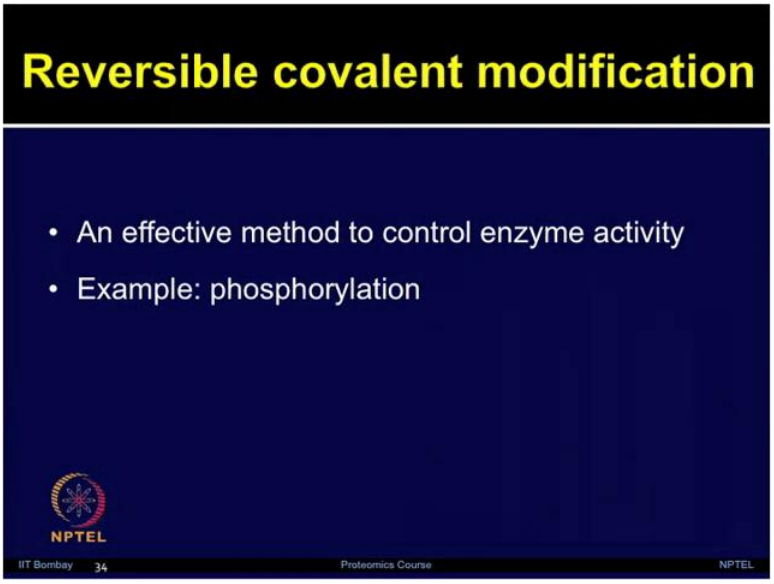

Now, let us talk about reversible covalent modification this is a very effective method to control enzyme activity. You have heard the example of phosphorylation so protein kinesis they can catalyze the phosphorylation reaction, and A T P is served as phosphoral donor. There is another enzyme protein phosphates, which removes the phosphoral group by hydrolysis process, reversible covalent modification is another commonly employed enzyme regulatory strategy. This is most widely observed modification is phosphorylation which is carried out by various enzyme kinesis with the help of a T P as a phosphoral donner, some enzymes are more active if they are phospholated forms while others are less active in this form, d phosphorylation is carried out by the phosphorylation Enzyme, a part from phosphorylation which most commonly takes place at serine threonine and tyrosine residues, other reversible covalent modification include adenylylation uridylyation methylation and ATP ripothilation which modify different amino acid residues of the protein. Now, last regulatory strategy is enzyme activation by proteolytic cleavage.

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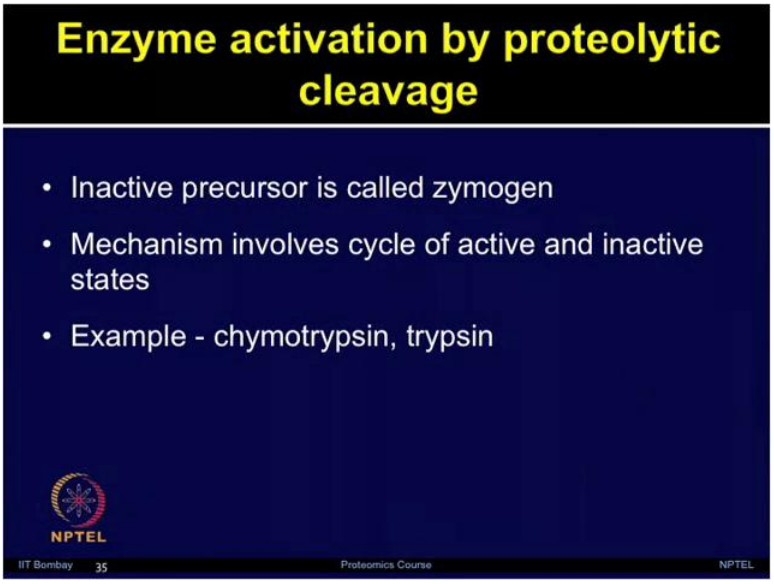

So, enzymes can be controlled by this mechanism which cycles between it is active and inactive states. The in active states which is precursor is known as Zymogen or Proenzyme, one of the classical example is the enzyme chymotrypsin which is present in the digestive tract. So, trypsin converts chymotrypsin which is a Zymogen into the active chymotrypsin by hydrolyzing peptide bond. The blood clotting due to the cascade of 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 or more peptide bonds by various proteases. The 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 enzyme as well as clotting factors are regulated by proteolytic activation. Chymotrypsin a digestive enzyme that hydrolysis protein in the small intestine exist in its zymogen form within membrane bound granos after synthesis in the acinar cells of 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 pichymotrypsin is acted upon by other such molecules to it the completely active and a stable alpha chymotrypsin which consist of three chains linked by inter chain disulphide bonds.

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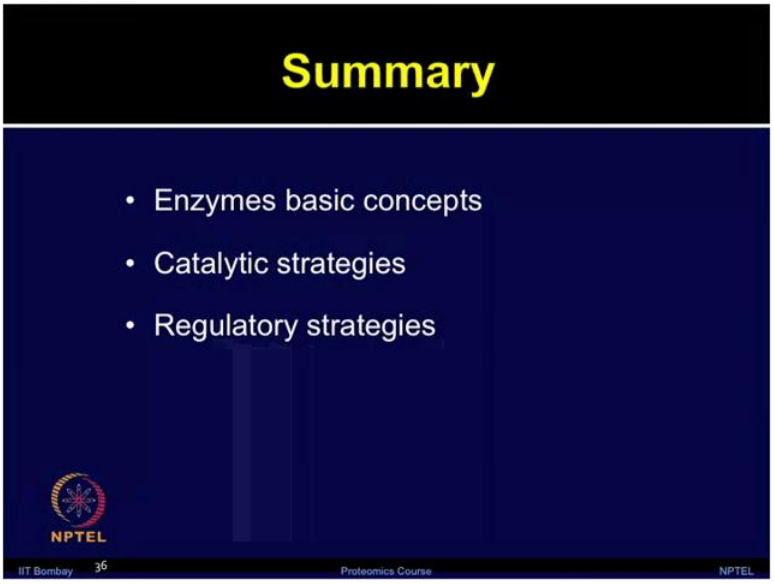

So, in summary today we discussed various concepts related to enzymes which are highly effective catalyst, the several catalytic mechanism which are employed by the enzymes which include acid based catalysis, covalent catalysis and metal ion catalysis. We discussed some of these mechanisms in some detail; the activities of metabolic pathways in cells are regulated by control of the activities of certain regulatory enzymes. And four different regulatory strategies were discussed. So, I hope now you have some basic understanding of enzymes and different catalytic and regulatory strategies. Thank you.