LECTURE-07

ENZYME: BASIC CONCEPTS, CATALYTIC AND REGULATORY STRATEGIES

TRANSCRIPT

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

So lecture outline is that first we will talk about basic concepts of enzyme including enzyme kinetics, energetics and enzyme inhibition. We will then move on to different type of catalytic strategies and regulatory strategies.

So as you all know enzymes play very important role in biochemistry and offer sensitive and specific method of quantitation for various substances. All enzymes are proteins therefore, it is 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.

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 which offer array of chemical forces. These enzymes can accelerate a given reaction up to million folds. Let's take an example of carbonic anhydrase which catalyses hydration of carbon dioxide. Now this enzyme can catalyze 10^6 molecules per second. Enzymes are highly specific and catalyze single or closely related reaction. The various examples, which we will talk, how enzymes regulate various reactions. There are certain proteolytic enzymes, which catalyze proteolysis and hydrolysis of peptide bond.

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 that trypsin. It catalyses hydrolysis of Arg/Gly bonds in a very specific peptide sequence and therefore offers high specificity.

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

These are few enzymes but each class of enzymes incorporates various enzymes broadly these are the type of reaction which these enzymes can catalyze.

So let's talk about enzyme activity and how various cofactors govern these enzyme acitivity. So many enzymes catalytic activity 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 apoenzyme are enzyme without its cofactor and holoenzyme when you combine apoenzyme with cofactor 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 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.

Let's now talk about free energy and enzyme.

So remember the concept of entropy-

$\Delta G = \Delta H - T\Delta S$

So ΔG 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.

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*.

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 ΔG must be negative. It must be emphasized that enzymes do not alter the equilibrium of a reaction.

The Enzyme-Substrate complex:

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.

So what is active site?

The active site is a region of enzyme that binds to 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 region of given protein which could form active site. They occupy only small portion of enzyme and some extra amino acids, they help in scaffold.

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

Other model is known as 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 not 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 has been 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 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.

Let's 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 enzyme reactions.

The Michaelis-Menten explained kinetic characteristics. The role of enzyme catalysis (V0) 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 equation.

Now E combines with S to form ES complex in presence of rate constant K_1 whereas ES complex can be dissociated to form 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}.

So here is Michaelis-Menten equation. The Vo rate of formation of product can be derived by this equation.

Where K_M is Michaelis constant. 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.

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

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

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.

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. Vo 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 both sides and then plotting a graph of 1/Vo Vs 1/[S]. The Y-intercept on this graph can be used to deduce the value of Vmax while the X-intercept gives the value of Km.

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

The reversible inhibitors, they 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.

So let's talk about the 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 reduces rate of catalysis and also reduces the number of enzyme-substrates which are formed by biding at the same site as shown in the figure.

Uncompetitive inhibition-

Inhibitor and substrate they bind at different site. 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.

After talking about reversible enzyme inhibition, now let's discuss 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'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 killed 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 catalytic 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 Vmax in this type of inhibition remains the same and only the Km 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.

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 Km and Vmax 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 Km and Vmax are altered in this type of inhibition.

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.

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.

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.

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 anyhydrase 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 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.

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.

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. Various pH related studies have provided substantial proof for this mechanism

After briefly discussing catalytic strategies, now let's talk about 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 a trigger or a 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.

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 the active site. The allosteric proteins possess property of cooperativity. 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. 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.

Isozymes are multiple forms of enzyme. Isozymes catalyze the same reaction but differ in structural characteristics. Isozymes varies regulation of same reaction at distinct

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. 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 differet kinetic parameters such as substrate affinity (Km) and maximum velocity (Vmax).

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.

Now let's discuss reversible covalent modification

This is a very effective process to control enzyme activity. You have heard example of phosphorylation. So protein kinases, they can catalyze protein phosphorylation and ATP is served as phosphoryl donor. There is another enzyme protein phosphatase, which removes phophoryl 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 adenylylation, uridylylation, methylation and ADP-ribosylation, which modify different amino acid residues of the proteins.

Now last regulatory strategy, enzyme activation by proteolytic cleavage so enzymes can be controlled by this mechanism, which cycles between its active and inactive state. The inactive state which is precursor is known as zymogen or proenzyme. One of the classical example 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 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 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.

So in summary today we discussed various concepts related to enzymes, 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 detail. 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. Thank you.