1. A competitive inhibitor of an enzyme is usually:

    a. a highly reactive compound.

    b. a metal ion such as Hg2+ or Pb2+.

    c. structurally similar to the substrate.

    d. water insoluble.

    e. a poison.

2. A non-competitive inhibitor of an enzyme catalyzed reaction

    a. binds to the Michaelis complex (ES).

    b. decreases Vmax.

    c. is without effect at saturating substrate concentrations.

    d. can actually increase reaction velocity in rare cases.

    e. The first and second choices are both correct.

3. A competitive inhibitor has the following effect on a Lineweaver-Burke (double reciprocal) Plot.

    a. It moves the entire curve to the right.

    b. It moves the entire curve to the left.

    c. It changes the y-intercept.

    d. It changes the x-intercept.

    e. It has no effect on the slope.

4. The degree of inhibition α by a competitive inhibitor is obtained from

    a. measurement of Vmax.

    b. measurement of the y-intercept on a Limeweaver-Burke Plot.

    c. measurement of KM.

    d. crystallographic studies.

    e. is unrelated to the binding affinity of the inhibitor to the enzyme.

5. An allosteric inhibitor of an enzyme usually

    a. binds to the active site.

    b. participates in feedback regulation.

    c. denatures the enzyme.

    d. causes the enzyme to work faster.

    e. is a hydrophobic compound.

6. Which of the following statements about the reversible inhibition is incorrect?

a. Non-competitive inhibition occurs when the binding site for the enzyme and the substrate is same.

b. Competitive inhibition occurs when a substrate and an inhibitor compete for the same active site on the enzyme.

c. Non-competitive inhibition of an enzyme cannot be overcome by adding large amounts of substrate.

d. Competitive inhibitors are often similar in chemical structure to the substrate of the inhibited enzyme.

7. The following data are obtained from an enzyme-catalyzed reaction in the presence and absence of inhibitor A.

[S] mM	V mM/ml/min In absence of A	V mM/ml/min In presence of A
0.2	5.0	3.0
0.4	7.5	5.0
0.8	10.0	7.5
1.0	10.7	8.3
2.0	12.5	10.7
4.0	13.6	12.5

1. Using double-reciprocal plot determine the type of inhibition.

2. Does inhibitor A combine with E, or ES or both? Explain.

Short Questions with answers

How are enzymes classified?

A. They are classified into five major classes.

Q. What are those classes?

A. Oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.

Q. What is the function of oxidoreductases?

A. Transfer of hydrogen.

Q. Give an example of oxidoreducatase.

A. Alcohol dehydrogenase.

Q. What is the function of transferases?

A. Transfer of groups other than hydrogen.

Q. Give an example of transferase.

A. Hexokinase.

Q. What is the function of hydrolases?

A. Cleave bond after adding water.

Give an example of a hydrolase.

A. Acetyl choline esterase.

Q. Peptidases are classified under which class of enzyme?

A. Hydrolases.

Q. What is the function of lyases?

A. Cleave bond without adding water.

Q. Which enzyme will add water to a double bond, without breaking the bond?

A. Hydratase.

Q. Give an example of lyase.

A. Aldolase.

Q. Give an example of isomerase.

A. Triose phosphate isomerase.

Q. What is the function of ligases?

A. ATP dependent condensation of two molecules.

What is the difference between synthase and synthetase?

A. Synthetases are ATP-dependent enzymes catalysing biosynthetic reactions; they belong to Ligases. Synthases are enzymes catalysing biosynthetic reactions; but they do not require ATP directly; they belong to classes other than Ligases.

Q. Give examples of synthetases.

A. Carbamoyl phosphate synthetase, arginino succinate synthetase, PRPP synthetase, glutamine synthetase.

Q. Give examples of synthases.

A. Glycogen synthase, ALA synthase, IMP synthase.

Q. What are co-enzymes?

A. Enzyme may contain a non-protein part, the co-enzyme. The co-enzyme is essential for the biological activity of the enzyme. A co-enzyme is alow molecular weight organic substance, withoutwhich the enzyme cannot exhibit any reaction. Co-enzyme accepts one of the products of the reac-tion; and so act as a co-substrate.

Q. What is holo-enzyme?

A. When apo-enzyme and co-enzymes are added, holo-enzyme is produced. Fully active enzyme is called Holo-enzyme.

Q. How are co-enzymes classified?

A. (a) Those taking part in reactions catalysed by oxidoreductases by donating or accepting hydrogen atoms or electrons. (b) Those co-enzymes taking part in reactions transferring groups other than hydrogen.

Give some examples of co-enzymes involved in oxidoreductases.

A. NAD, NADP, FAD.

Q. What is the full form of NAD?

A. Nicotinamide adenine dinucleotide.

Q. What is FAD?

A. Flavin adenine dinucleotide.

Give some examples of co-enzymes involved inreactions other than hydrogen transfer

A. Thiamine pyrophosphate, pyridoxal phosphate, biotin, co-enzyme A, ATP.

Q. What is the full form of ATP?

A. Adenosine triphosphate.

Q. What is the function of ATP?

A. It is the energy currency in the body. During the oxidation of food stuffs, energy is released, a part of which is stored as chemical energy in the form of ATP. Other reaction requiring energy are coupled with ATP.

Q. Name the enzymes containing copper.

A. Superoxide dismutase, tyrosinase, cytochromeoxidase.

Q. Which metal is required for the action of Kinases?

A. Magnesium.

Q. Chloride ions activate which enzyme?

Give some examples of co-enzymes involved in reactions other than hydrogen transfer (Page 32)

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A. Amylase.

Q. Which enzyme contains molybdenum?

A. Xanthine oxidase.

Q. Name some iron containing enzymes.

A. Cytochrome oxidase, catalase, peroxidase, xan-thine oxidase.

Q. What is Michaelis-Menten Theory ?

A. It is otherwise called enzyme-substrate complex theory. The enzyme combines with the substrate,to form an enzyme-substrate complex, which immediately breaks down to the enzyme and the product.

Q. What is Fischer's theory?

A. It states that the three dimensional structure of the active site of the enzyme is complementary to the substrate. Thus, enzyme and substrate fit each other like a key and its lock.

Q. What is Koshland's induced fit theory?

A. The substrate induces conformational changes in the enzyme, such that precise orientation of catalytic groups is effected.

Q. What is active site of an enzyme?

A. That area of the enzyme where catalysis occurs is referred to as active site or active center.

Q. What is meant by serine proteases?

A. Proteases (proteolytic enzymes) having a serine residue at its active center.

Q. Give an example of a serine protease.

A. Trypsin, chymotrypsin, thrombin.

Q. Thermodynamically, how reactions are classified?

A. Exothermic, isothermic and endothermic reactions.

What is exothermic reaction?

A. Here energy is released from the reaction, and therefore reaction essentially goes to completion, e.g. urease enzyme, converting urea to ammonia+ CO2 + energy.

Q. What is endergonic reaction?

A. Energy is consumed and external energy is to be supplied for these reactions. In the body this is usually accomplished by coupling the endergonic reaction with an exergonic reaction, e.g. Hexokinase reaction, Glucose + ATP = Glucose-6-Phos-phate + ADP.

Q. What are the salient features of enzyme kinetics?

A. Enzymes lower activation energy. They increase the chemical reaction, but do not alter equilibrium of the reaction.

What are the factors influencing enzyme reaction?

A. Enzyme concentration, substrate concentration, product concentration, temperature, pH and presence of activators or inhibitors.

Q. What is Km value?

A. Substrate concentration (expressed in moles/L)at half-maximal velocity is the Km value.

Q. What does it indicate?

A. It denotes that 50% of enzyme molecules are bound with substrate molecules at that particu-lar substrate concentration.

What is its significance?

A. Km is independent of enzyme concentration. Km value is thus constant for an enzyme. It is the char-acteristic feature of a particular enzyme for a specific substrate. Km denotes the affinity of enzyme to substrate. Thus, the lesser the numerical value of Km, the affinity of the enzyme for the substrate is more.

Q. What is the use of assessing the Km value of an enzyme? What is the application?

A. Determination of Km value is also useful to understand the natural substrate of an enzyme. Study of Km value will also differentiate the competitive and non-competitive inhibitions.

Q. What is the effect of temperature on enzyme velocity?

A. The velocity of reaction increases when temperature is increased, reaches a maximum and then falls (Bell-shaped curve)

Why it falls?

A. when temperature is more than 50ºC, heat denaturation and consequent loss of tertiary structure of protein occurs.

Q. What is the effect of pH on the activity of an en-zyme?

A. Each enzyme has an optimum pH, on both sides of which the velocity will be drastically reduced. The graph will show a bell-shaped curve.

What is the explanation for the effect of pH?

A. The pH decides the charge on the amino acid residues at the active site. The net charge on the en-zyme protein would influence substrate binding and catalytic activity.

Q. What is the optimum pH of usual enzymes?

A. Usually enzymes have the optimum pH between6 and 8.

Q. Are there any important exceptions for this general rule?

A. Pepsin (optimum pH 1-2), alkaline phosphatase(optimum pH 9-10) and Acid phosphatase (4-5).

Q. What is zymogen?

A. It is otherwise called pro-enzyme. Inactive zymogen is activated by removal of a piece of the pro-enzyme.

Give an example of zymogen is activated?

A. By splitting a single peptide bond, and removal of a small polypeptide from trypsinogen, the ac-tive trypsin is formed. This results in unmasking of the active centre.

What is the significance of zymogen activation?

A. Gastro-intestinal enzymes are synthesised in the form of pro-enzymes, and only after secretion into the alimentary canal, they are activated. This pre-vents autolysis of cellular structural proteins. Coagulation factors are seen in blood as zymogen form, their activation takes place only when necessity arises. This prevents intravascular coagulation.

Q. What are the different types of inhibitions of enzyme activity?

A. Competitive inhibition, non-competitive inhibition, suicide inhibition, and allosteric regulation.

Q. What are salient features of competitive inhibition?

A. Competitive inhibitor is a structural analogue. 2.It is reversible. 3. Km is increased. 4. Vmax is not changed.

Q. Give examples of competitive inhibition

A. Malonate inhibits succinate dehydrogenase.

Q. Give examples of clinical application of competitive inhibition.

A. Sulfonamide inhibits PABA incorporation in bac-teria, and so acts as an antibacterial agent. Meth-otrexate inhibits folate reductase system, dicoumarol inhibits vitamin K.

What is the immediate treatment for methanol poisoning?

A. Methanol is oxidised by alcohol dehydrogenase to formaldehyde which causes the acute toxicity. Antidote to methanol poisoning is ethanol which is the natural substrate for alcohol dehydrogenase. So ethanol is preferentially utilised.

Q. What are the salient features of non-competitive inhibition?

A. Non-competitive inhibitor has no structural similarity with the substrate. 2. It is generally not re-versible 3. Km is not changed. 4. Vmax is reduced.

Q. Give examples of non-competitive inhibition

A. Di-isopropyl fluoro phosphate inhibits trypsin, fluoride inhibits and enolase.

Q. Iodo-acetate inhibits enzyme by reacting withwhich group at the active site of the enzyme?

A. Sulfhydryl group.

What is the mechanism of inhibitory action of Di-isopropyl fluoro phosphate?

A. It inhibits enzymes with serine in their active cen-tres, e.g. acetylcholine esterase.

Q. What is suicide inhibition?

A. In suicide inhibition, the structural analogue is converted to a more effective inhibitor with the help of the enzyme to be inhibited. The inhibitor makes use of the enzyme's own reaction mechanism to inactivate it.

What is the other term for suicide inhibition?

A. Mechanism based inactivation.

Q. Give examples for suicide inhibition

A. Ornithine decarboxylase (ODC) is inhibited bydifluro methyl ornithine (DFMO). Another ex-ample is Allopurinol which is oxidised by xan-thine oxidase to alloxanthine that is a strong in-hibitor of xanthine oxidase.

Q. What is allosteric inhibition?

A. Allosteric enzyme has one catalytic site where the substrate binds and another separate allosteric site where the modifier binds.

Q. What are the salient features of allosteric inhibition?

A. (1) The inhibitor is not a substrate analogue. (2) Itis partially reversible when excess substrate is added. (3) Km is usually increased. (4) Vmax is reduced. (5) Most allosteric enzymes possess quaternary structure. They are made up of subunits.

Give examples for allosteric inhibition.

A. ALA synthase, aspartyl trans-carbamoylase, HMG CoA reductase

Q. What is covalent modification?

A. It means, either addition of a group to the enzyme protein by a covalent bond; or removal of a group by cleaving a covalent bond

Give some examples of covalent modification

A. Glycogen synthase is inactive, in the phosphorylated state, whereas glycogen phosphorylase is active when phosphorylated.

Give examples of multi-enzyme complexes.

A. Fatty acid synthase, pyruvate dehydrogenase, and alpha keto glutarate dehydrogenase.

Q. What are the types of specificity shown by enzymes?

A. Absolute specificity, group specificity and stereospecificity.

Q. Give an example for absolute specificity

A. Urea is the only substrate for urease.

Q. Give an example for group specificity

A. trypsin can hydrolyse peptide bonds formed by carboxyl groups of arginine or lysine residues.

What are iso-enzymes?

A. They are physically distinct forms of the same enzyme activity. They have identical catalytic properties, but differ in structure.

Q. How to differentiate iso-enzymes.

A. Electrophoresis, heat stability, km value, inhibi-tor specificity, and tissue localization.

Lactate dehydrogenase has how many polypep-tide subunits?

A. Four. It is a tetramer.

Q. Lactate dehydrogenase has how many iso-en-zymes?

A. Five

Q. What are they?

A. H4, H3M, H2M2, M3H and M4 varieties, forming five iso-enzymes. All these five forms are seen in all persons.