Subject Code : P8BC3

CORE COURSE III – ENZYMES AND ENZYME TECHNOLOGY

Unit 1

Enzymes- Active site. Definition. Investigation of active site structure. Identification of intermediates– trapping, chemical inference, isotope exchange and rapid quenching methods. Photo oxidation. Enzyme modification using chemicals. Modification using proteases. Affinity labeling using active site directed reagents– TPCK, TLCK. Effect of changing pH, multienzyme complexes and multifunctional enzymes. A brief account of nonprotein enzymes- ribozymes and DNA enzymes.

Unit 2

Enzyme kinetics- pre-steady state and steady state kinetics. Fast kinetics to elucidate the intermediates and rate limiting steps (flow and relaxation methods) effect of pH, temperature, enzyme and substrate concentration. Michaelis-Menten plot, linear transformation Lineweaver- Burk plot, Eadie-Hofstee plot, and Hanes-Woolf equations. Significance of Km and Vmax. King-Altman procedure. Kinetics of allosteric enzymes- MWC and KNF models. Hill's equation and co-efficient. Sequential and non sequential bisubstrate reactions.

Unit 3

Enzyme inhibition- irreversible and reversible competitive, noncompetitive, uncompetitive, mixed, inhibitions. Kinetic differentiation and graphical methods. Examples. Determination of inhibitor constant, therapeutic, diagnostic and industrial applications of enzyme inhibitors. Mechanism of enzyme action- acid base catalysis, covalent catalysis, strain, proximity and orientation effects. Mechanism of action of lysozyme, chymotrypsin, DNA polymerases, RNase.

Unit 4

Coenzymes structure and function- mechanism of pyridine nucleotides, flavin nucleotides, coenzyme A pyridoxal phosphate, thiamine pyrophosphate, tetrahydrofolate and B_{12} coenzymes, multienzyme complexes. Metal dependent and metalloenzymes. Isoenzymes.

Enzyme regulation: General mechanism of enzyme regulation, feedback inhibition and feedforward stimulation. Enzyme repression, induction and degradation, control of enzymic activity by products and substrates. Zymogens. Immobilization of enzymes and their applications. Enzyme engineering. Creation of artificial enzymes.

Unit 5

Enzyme electrodes, enzyme biosensors and their applications, ELISA, EMIT.

Enzymes of industrial and clinical significance, sources of industrial enzymes, thermophilic nzymes, amylases, glucose isomerases, cellulose degrading

enzymes, pectic enzymes, lipases, proteolytic enzymes in meat and leather industry, detergents and cheese production. Enzymes as thrombolytic agents, anti-inflammatory agents, debriding agents, digestive aids, therapeutic use of enzymes.

Books recommended

1. T.Palmer. Understanding enzymes. Prentice Hall.
2. Principles of Biochemistry – Zubay 4th ed. 1998, William C.Brown Publ.
3. Ratledge and Kristiansen. Basic Biotechnology. 2nd ed. Cambridge Univ. Press, 2001.
4. Dixon and Webb. Enzymes 3rd ed. Longmans, 1979.
5. Stryer. Biochemistry 5th ed. Freeman, 2002.
6. Whitehurst RJ. Enzymes in Food Technology. CRC Press, 2001.
7. Uhlig H. Industrial enzymes and their applications. John Wiley, 1998.
8. Marangoni AG. Enzyme Kinetics. A modern approach. John Wiley & Sons, 2002.
9. Balasubramanian et al. Concepts in Biotechnology Universities Press (India) Ltd., 1998.