2.18 40139 ENZYMOLOGY

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GENERAL EDUCATIONAL OBJECTIVES:

1. To study about enzymes and its characteristics
2. To study about enzyme catalysis and enzyme assay
3. To study about immobilized enzymes
4. To study about applications of enzymes

UNIT-1 INTRODUCTION TO ENZYMES

1.1 History of enzymology
1.2 Classification and nomenclature of enzyme according to IUB
1.3 Differences between chemical and biological catalyst
1.4 Characteristics of enzymes
1.5 Concept of holo-enzyme, apo-enzyme, coenzyme & cofactor
1.6 Structure and function of co enzyme- reaction involving TPP, PLP, NAD+, FAD, Coenzyme A and Biotin
1.7 A brief account of Isozymes (Ex: LDH), Ribozymes and Abzymes.

UNIT-2 ENZYME ACTIVITY

2.1 Enzyme specificity - Features of Active site
2.2 Activation energy- definition & Principle
2.3 Enzyme- Substrate Interaction- Lock and key, Induced fit theory and Transition state
2.4 mechanism of enzyme substrate interaction - Concept, mechanism of Chymotrypsin & Lysozyme
2.5 Factors affecting the catalysis- substrate & enzyme concentration, pH, temperature,
2.6 Inhibition- competitive, un-competitive and non-competitive
2.7 Michaelis - Menten equation for uni-substrate reaction and its transformation, significance of Km and Vmax
2.8 Regulation of enzyme activity- Zymogens, Allosterism, Co-operatitvity & product inhibition.

UNIT-3 ENZYME ISOLATION, PURIFICATION AND ASSAY

3.1 Isolation of enzymes- Cytosol and membrane bound
3.2 Purification of enzymes
3.2.1 Separation based on Solubility – Change in pH, Change in Ionic strength
3.2.2 fractionation of enzymes by precipitation
3.2.3 Separation based on molecular size- GPC, Centrifugation, ultrafiltration, Dialysis
3.2.4 Separation based on charge- Ion-exchange chromatography, Isoelctric focusing
3.2.5 Separation based on specific interaction with other biomolecules- Bioaffinity chromatography
3.2.6 Separation based on Hydrophobic interaction - Hydrophobic interaction chromatography
3.2.7 HPLC
3.2.8 Molecular mass determination

3.2.9 Assay of enzyme activity- development of SOP (Standard Operating Procedure) with the use of Colorimetric, Spectrophotometric, Fluorimetric, Chemiluminescent, Radiometric assays, and Chromatographic assays

UNIT-4 IMMOBILIZED ENZYMES

4.1 Introduction, advantages of immobilized enzymes

4.2 Types of enzyme Immobilization- Adsorption, Covalent linkage, Cross linking, Copolymerization, Enzyme entrapment & Encapsulation

4.3 Kinetics of immobilized enzyme

4.4 Commercial uses of immobilized enzymes

UNIT-5 APPLICATIONS OF ENZYMES

5.1 Industrial Applications-

5.1.1 Enzymes used in detergents- protease, amylase, lipase & cellulase

5.1.2 Enzymes used in food industry – protease, amylase, lipase, cellulase, Pectinase, glucose oxidase

5.1.3 Enzymes used in dairy – Rennin, catalase, Lactase, Proteinases

5.1.4 Enzymes used in leather & wool industry – protease, amylase

5.2 Medical applications- Isoenzymes, Asparaginase, Streptokinase, Lysozyme, creatine kinase (CK), Glutamic-oxaloacetic transaminase (GOT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alanine aminotransferase (ALT). Therapeutic Proteins- Cytokines, Interleukin, Interferon, Human Factor VIII and IX

5.3 Biotechnological Applications- polymerases, restriction enzymes & ligases Enzyme immunoassay techniques

5.4 Enzymes – as drug targets

5.5 Biosensors: Introduction, Principle Applications of Biosensors

Reference Books:

1. Principles of Biochemistry by Nelson Coks
2. Enzyme Technology by Palmer
3. Enzymology and Enzyme technology by Dr. S.M. Bhatt, S. Chand Publication
4. Enzymes in Industry Production and applications Biochemical Engineering by James M. Lee, Prentice Hall (1992)
5. Enzymes in Food Processing by Gerald Reed, Academic presses
3. Biosensor Technology- Fundamentals and Applications by Robert R. Buck, et. Al, Dekke Publication