Advanced Biochemistry and Enzymology

3 credit (2+1+0)

Dr. Amit Vij (Guest Instructor)

Stereochemistry and Physical:

Stereochemistry of Carbon compounds: Optical isomerism, chirality, symmetry elements, enantiomers, diastereomers, DL and RS notations, racemization, stereoisomerism and geometrical isomerism, cis – trans and E – Z conventions.

Chemical equilibrium: Basic concepts of ionic equilibria in solution, hydrolysis of salts, pH, buffer, Henderson Hasselbalch equation and their applications. Equilibrium constants (Kc, Kp and Kx) for homogeneous reactions.

Thermodynamics: First law of thermodynamics, basic concepts of entropy and second law of thermodynamics, free energy changes, standard free energy change and its relation to equilibrium constant. Oxidation – reduction reactions in biological systems. Feasibility of reactions. ATP-Structure, properties and energy currency of the cell, Importance of Coupled reactions, High energy compounds.

Biomolecules structure and function

Amino acids and Proteins: Nomenclature, classification and buffering properties, zwitterionic structure, reaction of amino acids, unusual amino acids, non protein amino acids; the peptide bond and its properties. Primary structure and strategies for its determination, Secondary structure of protein and its prediction methods (Ramachandran Plot).Tertiary and quaternary structures. CD as a sensitive indicator in chain conformation of proteins. Overview of purification of proteins and criteria of purity. Protein folding with special emphasis on molecular chaperones.

Nucleic Acids: Structures of purine and pyrimidine bases, nucleosides, nucleotides, RNA, & DNA (differences), base pairing schemes, keto -enol tautomerism of bases and its consequences. Structure and function: Types of DNA- A, B and Z. Supercoiling of the DNA molecule topoisomers and superhelixes. RNA Structure-types of RNA, structure of mRNA, tRNA, rRNA, siRNA, micro RNA with emphasis on importance of structure to its function; Denaturation of DNA , Spectroscopic properties of DNA, hyperchromic and hypochromic shift. Sequencing of DNA and RNA. Structure determination of Nucleic acids by enzymatic, chemical and diffraction methods. Structure and Dynamics by NMR.

Techniques to study macromolecular structure.

Spectroscopic techniques: Principles of colorimeter, spectrophotometer, fluorimeter. Beer-Lambert's Law and its limitations. Extinction coefficient, fluorescent probes and their applications

Protein analysis by mass spectrometry: Mass spectrometry (general and technical), Sample preparation, Interpretation of mass spectra, Mass analysis of intact proteins and peptides.

Basic X ray crystallography and Cryo-electron microscopy

Carbohydrate metabolism and Plant Biochemistry.

Introduction to Metabolism– Basic concepts, Metabolism-Compartmentalization.

Carbohydrate Metabolism: Glycolysis, Gluconeogenesis and its regulation. TCA cycle - Regulation, Glyoxylate cycle, amphibolic & anaplerotic reactions. Pentose phosphate pathway (HMP shunt) & its regulation, Glycogen metabolism and regulation. Oxidative phosphorylation.

Plant Biochemistry – i) Photosynthesis - Light harvesting complexes; mechanisms of electron transport; ii) Photoprotective mechanisms; CO_2 fixation-C3, C4 and CAM pathways. **'light' and 'dark' reactions**: photorespiration iii) Secondary metabolites - Biosynthesis of terpenes, phenols and nitrogenous compounds and their roles.

Enzymology:

Mechanisms of enzyme action: Enzyme definition and characteristics, mechanism of enzyme action, activation energy, collision & transition state theories, lock and key model, induced fit hypothesis, Proximity orientation effect, strain and distortion theory.

Enzyme kinetics: Derivation of Kinetics of single-substrate enzyme-catalysed reactions (Michelis Menten and Briggs-Haldane equation). Enzyme activity, specific activity, turnover number. Kinetics of bi-substrate reactions (elementary idea only).

Types of catalysis Acid-base catalysis, covalent catalysis, Techniques for studying the mechanism of enzyme action; chemical modification, site directed mutagenesis and substrate analogues.

Typical enzyme mechanisms and events at the active site: Mechanism of reaction catalyzed by serine proteases-trypsin and chymotrypsin, carboxypeptidase, lysozyme, triose phosphate isomerase, ribonuclease and rotational catalysis-ATPase.

Enzyme regulation: General mechanisms of enzyme regulation. Allosteric enzymes (ATCase). Cooperativity phenomenon. Hill plot for hemoglobin and myoglobin. Sigmoidal kinetics and their physiological significance. Reversible and irreversible covalent modification, feedback inhibition, control of enzyme by products, substrates and adenylate energy charge, monocyclic and multicyclic cascade systems.

Suggested readings:

1. Wilson and Walker. A biologist's guide to principles and techniques of practical biochemistry. 5th ed. Cambridge University Press 2000.
2. Friefelder and Friefelder. Physical Biochemistry – Applications to Biochemistry and Molecular Biology. WH Freeman &Co. 1994
3. Upadhyay, Upadhyay and Nath. Biophysical Chemistry Principles and Techniques. Himalaya Publ. 1997.
4. Lehninger's Principles of Biochemistry - D. L. Nelson and M. M. Cox
5. Biochemistry – L. Stryer
6. Biochemistry, 2004, Donald Voet and Judith Voet, John Wiley and sons. ISBN – 047119350
7. David Sheehan (2009). Physical Biochemistry: Principles and Applicatons, John Wiley & Sons Ltd, Chichester, England
8. Biochemistry and Molecular Biology of Plants. Buchanan, Greussem and Jones, AAPS (2000)
9. Plant Biochemistry; Hans-Walter Heldt and Birgit Piechulla, Academic Press (2004)
10. Enzymes; Trevor Palmer, East – West Press Pvt. Ltd., Delhi (2004)
11. Streochemistry of organic compounds by D Nasipuri.
12. **Molecular Cell Biology 8th EditionBy** Harvey Lodish, Arnold Berk, Chris A. Kaiser, Monty Krieger, Anthony Bretscher, Hidde Ploegh, Angelika Amon and Kelsey C. Martin.
13. **Nucleic acid structure, properties and function by Victor A Bloomfield, Donald M Crothers,Ignacio Tinoco Jr.**