Biochemistry

Unit 1 : Techniques

Chromatographic techniques: Ion exchange, gel filtration, affinity , GLC, chromatofocusing, HPLC and FPLC.

Electrophoretic techniques: Polyacrylamide gel electrophoresis, SDS-PAGE, 2D-electrophoresis, agarose gel electrophoresis, isoelectric focusing, pulsed field electrophoresis, Separation of proteins, lipoproteins and nucleic acids. Visualizing separated components; staining, fluorescence, PAS staining, zymogram and reverse zymogram.

Spectroscopic techniques: Fluorimeter, Beer-Lambert's Law and its limitations. Extinction coefficient, fluorescent probes and their applications.

Physical methods of determining size, shape and structure of molecules:

NMR and IR: Principles and applications. CD and its applications to proteins.

Isotopic tracers: Heavy isotopes and radio isotopes, Mass spectrometer. Ionization, fragmentation, m/e, time of flight, MALDI and ESI.

Radioisotopes in Biology: Concept of half-life, decay constant, detection and quantitation - GM counter, solid and liquid scintillation counter. Autoradiography and their applications.

Microscopic techniques: Scanning and transmission electron microscopes, freeze-etch and freeze-fracture methods for EM, Confocal microscopy.

Unit 2: Biomolecules:

Carbohydrate: Structure elucidation: degradation, graded acid hydrolysis, periodate oxidation, degradation of oxopolysaccharides, methylation, acetylation, GC-MS.

Glycobiology: Glycoproteins; N- and O-glycosylation, lectins, carbohydrates in tissue engineering. Proteoglycans. Structural polysaccharides; hyaluronan, chitin, chondroitin and pectin. Determination of site of glycosylation and type of linkage (o-glycosyl and n-glycosyl).

Proteins: Determination of primary structure: Sequencing strategies; N-terminal and C-terminal, sequencing methods. Automated sequanators. Determiantion of s-s-bond position. Secondary structure of protein; α, β sheet, β bend, β turn and super secondary structures. Secondary structure prediction methods; Ramachandran plot. Tertiary and quaternary structures.

Lipids: Oils, fats, and waxes. Cholesterol, phosopholipids, glycolipids, sphingolipids, cerebrosides and gangliosides.

Nucleic Acids: Physiochemical properties of nucleic acids, melting of DNA, Tm; factors affecting Tm, Cot curve, classification of DNA based on cot curve. Chemical reactions of DNA and RNA.

Sequencing of DNA: Dideoxy method. Chargaff's rule, secondary structure of DNA. Watson and Crick model; B and Z DNA, other models of DNA structure. Secondary structure of tRNA and clover leaf model. Other secondary structural features in DNA, stem loop structure, palindromic sequences, cruciforms. DNA protein interaction; zinc finger leucinc zipper, helix-turn-helix, other motifs.

Unit 3: Enzymology:

General aspects: Nomenclature and IUB classification of enzymes. Enzyme specificity, specific activity, assay methods; coupled enzyme assays, continuous, end point and kinetic assay. Units of enzyme activity, IU and Katal.

Enzyme kinetics: Michaelis-Menten equation, steady state approach. Vmax, Km and their significance. Linear transformation of Michaelis-Menten equation; Lineweaver-Burk plot, Eadie-Hofstee, Haynes-Wolf and Cornish-Bowden.

Inhibition: Reversible and irreversible inhibition; competitive, non competitive, uncompetitive product inhibition and suicide inhibition. Determination of Ki and Kd.

Cooperativity: Binding of ligands to macromolecules; Scatchard plot, cooperativity, positive and negetive cooperativity. Oxygen binding to hemoglobin. Homotropic and heterotropic effectors, aspartyltranscarbamylase as an allosteric enzyme.

Mechanisms of action of specific enzyme: Factors influencing enzyme catalysis Chymotrypsin; zymogen activation, acid-base catalysis, charge relay net work. Lysozyme. RNA as an enzyme, coenzymic action of NAD^+, FAD, TPP, PLP, Biotin, CoA, folic acid and lipoic acid.

Metabolic regulation of enzyme activity: Feed back regulation, fine control of enzyme activity.

Fast reactions - Stopped flow, temperature jump method.

Unit 4: Metabolism:

Carbohydrate metabolism: Gluconeogenesis, Cori cycle, citric acid cycle and their regulation, energetics, anaplerosis, glyoxylate cycle. HMP shunt pathway, interconversion of hexoses. Utilization of non glucose sugars.

Metabolic disorders: Disorders of carbohydrate metabolism; diabetes mellitus, classification.

Lipids: Degradation of triacylglycerols, phospholipids, sphingolipids and regulations; lipase, hormone sensitive lipase, phospholipases and sphingomyelinase. Energetics of fatty acid degradation. Chain elongation and desaturation. Cholesterol metabolism and its regulations: Metabolism of circulating lipids; chylomicrons, HDL, LDL and VLDL. Reverse cholesterol transport by HDL. Foam cell formation. Regulation of blood cholesterol, triglycerides, LDL and HDL. Obesity. Phospholipids: Inter conversion of phospholipids. Biosynthesis of prostaglandins, thromboxanes, leukotrienes and ether lipids.

Integration of metabolic pathways: Integration of carbohydrate and lipid metabolism, and their regulation and manipulation. Hormonal regulation of glucose metabolism**:** Effect of insulin and glucogon, catecholamines, growth hormones and carticosteroids on carbohydrate and lipid metabolism in different tissues.

Proteins: General mechanisms of degradation in cells; ubiquitin-proteosome pathway, lysosomal pathway.

Non ribosomal peptide synthesis: glutathione and gramicidine. Biosynthesis of creatin and polyamines.

General mechanisms of amino acid metabolism and regulations: Deamination, transamination, decarboxylation, desulphuration, Ketogenic and glucogenic amino acids. Regulation of amino acid biosynthesis; transglutaminase cycle, urea cycle. In born errors of amino acid metabolism; Phenyl Ketonuria, alkaptonuria, maple syrup urine.

Purines and pyrimidines: Uric acid formation. Salvage pathways. Gout and Lysch-Nyhan syndrome. Conversion of nucleotides to deoxynuclotides. Mchanisms of action of methotrexate, 5-fluorouridine, azathymidine.

Biosynthesis of cofactors: NAD^+, FAD and coenzyme A, polyamine biosynthesis and their metabolic role.

Unit 5: Molecular Biology:

Introduction: Central dogma of molecular biology.

DNA-antiparellel nature: Nearest neighbor base frequency analysis. Replication of DNA, semi conservative nature; Messelson and Stahl experiment. Replication of double stranded DNA, direction of replication, discontinuous replication, Okazaki fragements. DNA polymerase I, II, and III, DNA ligase, DNA topoisomerases. Fidelity of replication, replication in viruses, rolling circle model, Trombone model, translesion synthesis (DNA pol IV and V).

Transcription: Colinerity of genes and proteins, RNA polymerase I, II and III. RNA biosyntehsis in prokaryotes and eukaryotes; initiation, elongation and termination. RNA dependent RNA synthesis, RNA replicase of Qβ virus. Processing of eukaryotic RNA, cap addition, poly A tail addition, RNA editing. Processing of tRNA and mRNA transcripts.

Translation: Universality features of the genetic code and degeneracy of codons. 3D structure of prokaryotic and eukaryotic ribosomes, ribosomal protein synthesis; initiation elongation and termination. Aminoacyl tRNA synthetases and their role in translation accuracy.

Regulation of gene expression in prokaryotes: Operon model; lac operon, structure and regulation. Galactose operon; role of two promoters. Arabinose operon; positive control. Tryptophan operon; attenuation control.

Eukaryotic gene regulation: DNA methylation. Chromatin structure, chromatin remodeling, Swi/Snf, remodeling assay, ChIP.

Regulation at the level of translation: Secondary structure in the 5' and 3' untranslated region; regulation of ferretin and transferring, mRNA. Role of upstream AUG codons. (GCN 4 gene regulation), protein splicing inteins.

Post translation modification of proteins, signal cleavage, disulphide bond formation, O and N-glycosylation, folding of nascent protein, role of chaperones, attachment of glycosyl anchor, and other modifications.

Enzymes in DNA and RNA degradation: Nucleases, ribonucleases, classification and role.

Unit 6: Immunology:

Introduction: Definitions; antigenicity, immunogenicity, innate and acquired immunity. Primary and secondary lymphoid organs, self and non-self discrimination. Antigens and antibodies; haptens, epitopes and paratopes. Valency of antigen and epitope analysis.

Structure of immunoglobulins, hyper variable region, isotypic, allotypic and idiotypic variations.

Cellular Basis of Immunity: Primary and secondary immune response. Reticuloendothelial system, T, B and accessory cells. Development of T and B cells. Sub sets of T and B cells. T and B cell receptors, antigen processing and presentation. T and B cell interaction. Cytokines and co-stimulatory molecules; lymphokines, interleukins, structure and function of IL-lβ, IL-2, and TNFα. Suppression of immune response, immunoglobulin genes, generation of immunoglobulin diversity, gene rearrangement, clonal selection theory of Burnet.

MHC: Role of MHC in immune response.

In vitro antigen-antibody reaction: Precipitation, agglutination, complement fixation, immuno diffusion, immunoelectrophoresis, immunofluorescence, RIA and ELISA.

Defense system in plants: Host parasite interaction in plants.

Unit 7: Genetic engineering and biotechnology:

Genetic Engineering: Restriction-modification systems, restriction enzymes; type I, II and III, specificity, sticky ends and blunt ends, isoschizomers. Gene cloning; genomic cloning, shot gun cloning and cDNA cloning.

Vectors: Plasmids, phage, cosmids and phagemid. Yeast cloning vectors, plant vectors, bacterial artificial chromosome, SV40, shuttle vectors, construction of expression vectors.

Ligation: Blunt end and sticky end ligation, use of linkers and adopters, homo polymer tailing, colony hybridization, plaque hybridization.

Transformation: Micro injection, electroporation, lipofection, calcium phosphate method, protoplast fusion/somatic cell hybridization and biolistic methods.

Transgenic plants and animals, gene knock out.

Techniques: PCR, nested PCR and their applications in cloning, agriculture and medicine. RT-PCR and Real time PCR techniques and their applications.

Identifying the right clones: Direct screening; insertional inactivation of marker gene, visual screening, plaque phenotype. Indirect screening; immunological techniques, hybrid arrest, translation and hybrid select translation. Screening using probes; construction of gene probes, hybridization and labeling.

Blotting techniques: Dot blot, Southern, Northern, Western blot, DNA foot print assay, DNA finger print assay, gel retardation assay, nuclease protection assay. RFLP, RAPD,

Applications: Gene therapy, applications in agriculture medicine, industry. GM foods, terminator gene, negative impact of genetic engineering.

Unit : 8 Hormones and cell signaling:

Endocrine System: Chemistry of hormones, hormones produced by hypothalamus, pituitary, thyroid, parathyroid, pancreas, adrenals, gonads and intestine.

Regulation of hormone production and release: hypothalamus-pituitary-target organ axis and regulation by feed back mechanism.

Cell signaling: Autocrine, paracrine, juxtacrine, and endocrine signaling.

Peptide hormones: General mechanisms of cell signaling by hydrophilic factors, transmembrane receptors, G protein coupled receptors, receptor tyrosine kinase, eicosanoid receptors.

Second messengers: IP_3, DAG, cAMP, protein kinases. Nitric oxide signaling; generation and action.

Steroid hormones: General mechanism of action. Receptor down regulation, desensitization and up regulation.

Cell cycle and its regulation: CDKs, apoptosis.

Insect hormones: Moulting hormone, ecdysone and juvenile hormones. Pheromones-Mechanism of perception, action and use in pest control.

Unit 9: Membrane Biology

Biomembranes: Physicochemical properties of biological membranes; compositions, supra molecular organization-Singer and Nicholson's model.

Membrane asymmetry; lipids, proteins and carbohydrates and their dynamics. Biogenesis of lipids and proteins, polarized cells, membrane domains; caveolae and rafts. Membrane lipid and protein turnover, intracellular targeting of proteins. Biogenesis of sub cellular organelles.

Methods to study the membrane structure: Lipid transfer proteins, phospholipases, chemical methods, amino-phospholipid translocation, TNBS reagent, freeze fracture and freeze etching. Lipid vesicles; liposome preparations and application, function of sterols in membranes. FRET, FRAP and single particle tracking.

Membrane transport: Laws of diffusion across membranes, simple diffusion, facilitated diffusion and active transport. Glucose transporters, Ca^{2+} ATPase, Na^{+}-K^{+} ATPase, bacterial phosphotransferase system. Endocytosis, receptor mediated endocytosis, exocytosis, ion channels; gated and non-gated, aquaporin channel.

Nerve transmission: Acetylcholine receptor and neurotransmitters, mechanisms of nerve conduction, resting and action potential, ion channels, ionophores, patch clamp technique. Synaptic transmission, GABA, NMDA, structure and function.

Muscle contraction: Mechanisms, role of calcium, calmodulin and phospholamban.

Unit 10: Bioenergetics

Thermodynamics: I, II and III laws of thermodynamics. Enthalpy, entropy, free energy and chemical equilibrium.

High energy compounds: Energy currency, ATP, ADP, creatine phosphate, phosphoenol pyruvate as energy rich compound.

Photosynthesis: Photosynthetic apparatus in plants, photosystems I and II, light harvesting antenna complex. Electron flow and phosphorylation; cyclic and noncyclic, oxygen evolution, Calvin cycle. C3, C4 and CAM cycle. Photorespiration, bacterial photosynthesis.

Respiration: Plant mitochondrial electron transport and ATP synthesis.

Nitrogen metabolism: Importance of nitrogen in biological systems, nitrogen cycle. Nitrogen fixation; symbiotic and non-symbiotic, nitrogenase complex, energetics and regulation.

Mitochondrial electron transport: Entry of reducing equivalents for oxidation; malate-aspartate shuttle, glycerol phosphate shuttle.

Organization of respiratory chain complexes, structure and function of the components; Fe-S proteins, cytochromes, Q cycle, proton transfer, P/O ratio, respiratory control, oxidative phosphorylation, uncouphers and inhibitors.

ATP synthesis, ATP synthase complex, binding change mechanism, proton motive force, Mitchell's hypothesis.

Substrate level phosphorylation, futile cycles and their application.

Biochemical calculations: Specific gravity, percent solution, dilution and dilution factors, ionic strength; molarity, normality, mole concept, Avogadro principles.

Acids and bases, buffers, pH, pKa, Henderson-Hasselbach equation, buffer capacity, buffering capacity of amino acids.

Determination of LD_{50}, ED_{50}, IC_{50}.