BIO303 – BIOCHEMISTRY II

Midterm Questions

Q-1 What are types of RNA?

Small nuclear RNA (snRNA)

• Small nuclear RNA (snRNA) are large number of small stable RNA species found in eukaryotic cells.
• Most of them are complexed with proteins to form ribonucleoproteins.
• They are distributed in the nucleus, in the cytoplasm or in both.
• They are significantly involved in rRNA and mRNA processing and gene regulation.

Large & Small Noncoding Regulatory RNAs

• One of the most exciting discoveries in the last decade of eukaryotic regulatory biology has been the identification and characterization of regulatory non-protein coding RNAs (ncRNAs).
• NcRNAs exist in two general size classes,
• Small consisting of microRNA (miRNAs) and silencing (siRNAs)
• Large consisting of long noncoding RNAs (lncRNAs)
• The small ncRNAs termed microRNA (miRNAs) and silencing (siRNAs) typically inhibit gene expression at the level of specific protein production by targeting mRNAs through one of several distinct mechanisms.
• Both siRNAs and miRNAs typically hybridize, via the formation of RNA–RNA hybridization to their targeted mRNAs.

Long non-coding RNAs (lncRNAs).

• LncRNAs, which as their name implies, do not code for protein (ie, the mRNA encoding genes).
• ncRNAs make up a significant portion of eukaryotic transcription.
• ncRNAs play many roles ranging from contributing to structural aspects of chromatin to regulation of mRNA gene transcription by RNA polymerase II.

Q-2 Define snRNA.

Small nuclear RNA (snRNA)

• Small nuclear RNA (snRNA) are large number of small stable RNA species found in eukaryotic cells.
• Most of them are complexed with proteins to form ribonucleoproteins.
• They are distributed in the nucleus, in the cytoplasm or in both.
• They are significantly involved in rRNA and mRNA processing and gene regulation.

Q-3 Electrons move from electrophile to nucleophile. Name four methods.

Q-4 Write importance of Gluconeogenesis.

- To maintain the blood glucose concentration during prolonged fasting or starvation when sufficient carbohydrate is not available from the diet or glycogen reserves.
- It maintains the level of intermediates of the TCA cycle even when fatty acids are the main source of acetyl coA in the tissues.
- It clears the lactate produced by muscles and erythrocytes and glycerol produced by adipose tissue.

BIO303 – BIOCHEMISTRY II

Midterm Questions

Q-5 Define upstream processing.

• Upstream processing includes formulation of the fermentation medium, sterilization of air, fermentation medium and the fermenter, inoculum preparation and inoculation of the medium. The fermentation medium should contain an energy source, a carbon source, a nitrogen source and micronutrients required for the growth of the microorganism along with water and oxygen, if necessary.

A medium which is used for a large scale fermentation, in order to ensure the sustainability of the operation, should have the following characteristics;

1. It should be cheap and easily available
2. It should maximize the growth of the microorganism, productivity and the rate of formation of the desired product
3. It should minimize the formation of undesired products

Q-6 Write down three steps of enzyme catalyzed reaction.

• The distinguishing feature of an enzyme catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called the active site.
• The surface of the active site is lined with amino acid residues with side chains that complement and bind the substrate and catalyze its chemical transformation.
• Often, the active site encloses a substrate, sequestering it completely from solution.
• The enzyme substrate complex, whose existence is central to the action of enzymes.
• Enzyme-catalyzed reactions have three basic steps:
• Binding of substrate: E+S↔ES
• Conversion of bound substrate to bound product: ES ↔ EP
• Release of product: EP ↔ E+P

Q-7 Write down the components of fermenter.

The heart of the fermentation process is the fermenter.
• A biorector is a device in which the organisms are cultivated and motivated to form a desired product
• Closed vessel or containment designed to give a right environment for optimal growth and metabolic activity of the organism.
• Fermenter: for microbes/ Bioreactor: for eukaryotic cells.
• Size variable ranging from 20-250 million litres or Large scale production (..To1000-million L capacity)

Q-8 Write the steps of synthesis of phosphoenoalpyruvate into pyruvate.

• The first of the bypass reactions in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvate (PEP).
• This reaction cannot occur by simple reversal of the pyruvate kinase reaction
• Instead, the phosphorylation of pyruvate is achieved by a sequence of reactions that in eukaryotes requires 2 enzymes in both the cytosol and mitochondria.
• Pyruvate is first transported from the cytosol into mitochondria or is generated from alanine within mitochondria by transamination, in which the alpha -amino group is transferred from alanine (leaving pyruvate) to an alpha-keto carboxylic acid.
• In mitochondria, pyruvate is converted to oxaloacetate in a biotin (coenzyme)-requiring reaction catalyzed by pyruvate carboxylase, a mitochondrial enzyme.

BIO303 – BIOCHEMISTRY II

Midterm Questions

Q-9 What are reducing equivalents and its examples?

In biochemistry, the term reducing equivalent refers to any of a number of chemical species which transfer the equivalent of one electron in redox reactions. Examples of reducing equivalents are: A lone electron (for example in reactions involving metal ions) A hydrogen atom (consisting of a proton and an electron)

Examples of reducing equivalents are:

- A lone electron (for example in reactions involving metal ions)
- A hydrogen atom (consisting of a proton and an electron)
- A hydride ion ($:H^-$) which carries two electrons (for example in reactions involving NAD)

Q-10 Differentiate between lyases and ligases.

In biochemistry, a lyase is an enzyme that catalyzes the breaking of various chemical bonds by means other than hydrolysis and oxidation, often forming a new double bond or a new ring structure. The reverse reaction is also possible.

Ligase, an enzyme that uses ATP to form bonds, is used in recombinant DNA cloning to join restriction endonuclease fragments that have annealed. The ligase commonly used is T4 DNA ligase, which was first isolated from E. coli that were infected with the lytic bacteriophage T4.

Q-11 What are Ligands?

Ligand

• In biochemistry and pharmacology, a ligand (from the Latin ligandum, binding) is a substance (usually a small molecule), that forms a complex with a biomolecule to serve a biological purpose.
• In a narrower sense, it is a signal triggering molecule, binding to a site on a target protein.
• A molecule bound reversibly by a protein is called a ligand.
• Ligands include substrates, inhibitors, activators, and neurotransmitters.
• A ligand may be any kind of molecule, including another protein.
• A ligand binds at a site on the protein called the binding site, binding site is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character.
• Furthermore, the interaction is specific: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few.
• The binding of a protein and ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding called induced fit.
• A given protein may have separate binding sites for several different ligands.
• In a multi-subunit protein, a conformational change in one subunit often affects the conformation of other subunits.
• Interactions between ligands and proteins may be regulated, through interactions with additional ligands.
• These other ligands may cause conformational changes in the protein that affect the binding of the first ligand.

BIO303 – BIOCHEMISTRY II

Midterm Questions

Q-12 Write role of cryptochromes in plant growth and mammalian circadian rhythms.

• Family of flavoproteins,
• Found in both bacteria and eukaryotes
• mediate the effects of blue light on plant development and the effects of light on mammalian circadian rhythms (oscillations in physiology and biochemistry, with a 24-hour period)
• Homologs of Photolyases
• **Photolyases:** Use energy of light to repair defects in DNA

Q-13 What is submersion process?

Submersion processes

• In the submersion processes, the microorganisms grow in a liquid medium provided with the substrate
• Except in traditional beer and wine fermentation, the medium is held in fermenters and stirred to obtain a homogeneous distribution of cells and medium.
• Most processes are aerobic, and for these the medium must be vigorously aerated.
• Used for pickling vegetables and producing soy sauce.
• All important industrial processes (production of biomass and protein, antibiotics, enzymes are carried out by submersion processes.

Q-14 Differentiate between DNA and RNA.

Differences between DNA and RNA

• Although sharing many features with DNA, RNA possesses several specific differences.
• Size: They are considerably smaller than DNA
• Sugar: In RNA, the sugar moiety to which the phosphates and purine and pyrimidine bases are attached is
ribose rather than the 2'-deoxyribose of DNA.
• Pyrimidine: The pyrimidine components of RNA differ from those of DNA.
• Instead of thymine, RNA contains the ribonucleotide of uracil.
• Thymine is present in the rare case of tRNA
• Single Strand:
• RNA typically exists as a single strand whereas DNA exists as a double-stranded helical molecule.

Q-15 What is NcRNA?

Long non-coding RNAs (lncRNAs).

• LncRNAs, which as their name implies, do not code for protein (ie, the mRNA encoding genes).
• ncRNAs make up a significant portion of eukaryotic transcription.
• ncRNAs play many roles ranging from contributing to structural aspects of chromatin to regulation of mRNA gene transcription by RNA polymerase II.

Q-16 What is cytochrome?

Cytochromes are redox-active proteins containing a heme, with a central Fe atom at its core, as a cofactor. They are involved in electron transport chain and redox catalysis. They are classified according to the type of heme and its mode of binding.

Compiled by:
Sir Mughal

BIO303 – BIOCHEMISTRY II

Midterm Questions

Q-17 What diseases are caused from ion defect?

Ion channel diseases are associated with defects in proteins called ion channels typically are marked by muscular weakness, absent muscle tone, or episodic muscle paralysis.

- Andersen-Tawil syndrome
- Hyperkalemic periodic paralysis
- Hypokalemic periodic paralysis
- Myotonia congenita
- Paramyotonia congenita
- Potassium-aggravated myotonia

Q-18 What is oxidation and reduction?

Oxidation can be defined as the reaction in which a substance loses electron or gains oxygen or loses hydrogen. Similarly reduction can be expressed as a reaction in which a substance gains electrons or gain hydrogen or loses oxygen. Both reactions are called redox reactions collectively.

Q-19 What is ping pong mechanism?

Ping-pong mechanism of nucleoside diphosphate kinase: The enzyme binds its first substrate (ATP in our example), and a phosphoryl group is transferred to the side chain of a His residue. ADP departs, and another nucleoside (or deoxynucleoside) diphosphate replaces it, and this is converted to the corresponding triphosphate by transfer of the phosphoryl group from the phosphohistidine residue.

• This reaction is fully reversible, so after the intense demand for ATP ends, the enzyme can recycle AMP by converting it to ADP which can then be phosphorylated to ATP in mitochondria.

• A similar enzyme guanylate kinase, converts GMP to GDP at the expense of ATP.

• By these pathways energy conserved in the catabolic production of ATP is used to supply the cell with all required NTPs and dNTPs

• Phosphocreatine (PCr) serves as a ready source of phosphoryl groups for the quick synthesis of ATP from ADP. The phosphocreatine concentration in skeletal muscle is considerably higher than those in the other tissues. The enzyme creatine kinase catalyzes the reversible reaction.

Q-20 Write advantages of fermentation.

- Preserves and enriches the food, improves digestibility, and enhance the taste and flavor of the food.
- Potential of enhancing the food safety by controlling the growth and multiplication of a number of pathogens in foods.
- Important contribution to human nutrition, particularly in developing countries, where economic problems pose a major barrier to ensuring food safety.

Q-21 Where does gluconeogenesis occurs?

• Gluconeogenesis occurs mutually in the cytosol and mitochondria.

• Gluconeogenesis occurs in all animals, plants, fungi, and microorganisms.

• The reactions are essentially the same in all tissues and all species.

Q-22 What are elimination reactions?

An elimination reaction is a type of chemical reaction where several atoms either in pairs or groups are removed from a molecule. The removal usually takes place due to the action of acids and bases or the action of metals. It can also happen through the process of heating at high temperatures. The removal

of a hydrogen atom and a halogen atom, for example, is known as dehydrohalogenation; when both leaving atoms are halogens, the reaction is known as dehalogenation.

Q-23 What is DNA denaturation?

DNA Denaturation

• Separation of the two strands of the double helix when hydrogen bonds between the paired bases are disrupted. Disruption can occur in the laboratory if the pH or the salt concentration of the DNA solution is altered & if the solution is heated above 80°C .
• Disruption of the hydrogen bonds between paired bases and of base stacking causes unwinding of the double helix to form two single strands complete separation of DNA stands along the entire length.
• No covalent bonds in the DNA are broken.

Q-24 Write first step of glycolysis.

• In the first step of glycolysis, glucose is activated for subsequent reactions by its phosphorylation at C-6 to yield glucose 6-phosphate, with ATP as the phosphoryl donor:
• This reaction is irreversible under intracellular conditions, and catalyzed by hexokinase.
• The acceptor in the case of hexokinase is a hexose, normally D-glucose, hexokinase also catalyzes the phosphorylation of other common hexoses, such as Dfructose and D-mannose, in some tissues.
• Hexokinase requires Mg+2 for its activity, because the true substrate of the enzyme is not ATP-4 but the MgATP-2 complex.
• Hexokinase and other nine enzymes of are soluble cytosolic proteins.

Q-25 What is oxidative phase?

1. The Oxidative Phase Produces Pentose Phosphates and NADPH
2. The first reaction of the pentose phosphate pathway is the oxidation of glucose 6-phosphate by glucose 6- phosphate dehydrogenase (G6PD) to form 6- phosphoglucono-delta-lactone, an intramolecular ester.
3. NADP+ is the electron acceptor, and overall equilibrium is in the direction of NADPH formation.
4. The lactone is hydrolyzed to the free acid 6- phosphogluconate by a specific enzyme lactonase, and then 6-phosphogluconate undergoes oxidation and decarboxylation by 6-phosphogluconate dehydrogenase.

Pentose Phosphate Pathway NADPH formed in the oxidative phase is used to reduce glutathione to GSSG (Glutathione disulfide) and to support reductive biosynthesis. The other product of the oxidative phase is ribose 5-phosphate, which serves as a precursor for nucleotides, coenzymes, and nucleic acids. In cells that are not using ribose 5-phosphate for biosynthesis, the non-oxidative phase recycles six molecules of the pentose into five molecules of the hexose glucose 6- phosphate, allowing continued production of NADPH and converting glucose 6- phosphate to CO2.
• The reaction generates a second molecule of NADPH.
• Phosphopentose isomerase converts ribulose 5- phosphate to its aldose isomer, ribose 5-phosphate.
• In some tissues, the pentose phosphate pathway ends at this point.

BIO303 – BIOCHEMISTRY II

Midterm Questions

Q-26 Explain Laws of Thermodynamics.

1. First law

The first law is the principle of the conservation of energy: it states that:
"for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or **destroyed."**

2. Second law

The second law of thermodynamics states that "the universe always tends towards increasing disorder: in all natural processes, the entropy of the universe increases.

Q-27 What are industrial products of fermentation?

Ethanol, Glycerol, Lactic Acid, Acetone, Butanol, a-amylase.

Biomass Production

Baker's Yeast, SCP (Single Cell Protein), Probiotics, Bio fertilizers, Vaccines, Bioweapons

Q-28 What is reduction potential?

Reduction potential (redox potential, oxidation/reduction potential), is a measure of the tendency of a chemical species to acquire electrons and thereby get reduced. Units: Volts (V), or millivolts (mV).. denoted by *E*.

Q-29 What is use of fructokinase?

The liver enzyme fructokinase catalyzes the phosphorylation of fructose at C-1 rather than C-6.

Q-30 What are components of Ribosomes?

There is a wide variety of RNAs,

- messenger RNAs (mRNAs)- transfer genetic information from DNA to the protein-synthesizing machinery.
- ribosomal RNAs (rRNAs)- contribute to the formation and function of ribosomes
- transfer RNAs (tRNAs)- adapter molecules that carry specific amino acids for protein synthesis.

Q-31 What are components of Eukaryotic ribosomes?

The mammalian ribosome contains two major nucleoprotein subunits:

- a larger one with 60S
- a smaller one with 40S.

The **60S** subunit contains

- a 5S rRNA
- a 5.8S rRNA
- a 28S rRNA
- More than 50 specific polypeptides.
- The **40S** subunit is smaller and contains a single 18S rRNA.
- Approx. 30 distinct polypeptide chains.