

BIO506 – BIOCHEMISTRY II

FINAL TERM MOST IMPORTANT QUESTIONS

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1. Define pH. Write equation to calculate pH with reference to pH of water.

The pH of a solution is defined as the logarithm to the base 10 of the reciprocal of the $[H^+]$, i.e., the negative logarithm of the $[H^+]$.

$$pH = -\log [H^+]$$

The pH of water at 25°C, in which H^+ and OH^- ions are present in equal numbers, is 7.0

$$pH = -\log[1 \times 10^{-7}] = 7$$

2. Explain competitive and non-competitive inhibition.

COMPETITIVE INHIBITION:

This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy i.e. active site and, therefore, competes with the substrate for that site. Reversible inhibitors bind to enzymes through non covalent bonds. Dilution of the enzyme-inhibitor complex results in dissociation of the reversibly bound inhibitor, and recovery of enzyme activity.

NON-COMPETITIVE INHIBITION:

Inhibitors bind enzymes at sites distinct from the substrate-binding site and generally bear little or no structural resemblance to the substrate. Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate.

3. Differentiate between oxidation and reduction.

Oxidation is a process of the addition of oxygen or removal of hydrogen. Reduction is a process of removal of oxygen or the addition of hydrogen. According to the electronic concept, the loss of electrons is called an oxidation reaction and addition of electron is called reduction reaction.

4. What are types of enzyme inhibitors?

Enzyme inhibitors are molecular agents that interfere with catalysis, slowing or halting enzymatic reactions. Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor. Enzymes catalyze virtually all cellular processes, so it should not be surprising that enzyme inhibitors are among the most important pharmaceutical agents known. Inhibitors can be classified on the basis of their site of action on the enzyme, on whether they chemically modify the enzyme, or on the kinetic parameters they influence. Two broad classes of enzyme inhibitors:

1. Reversible
2. Irreversible.

5. What are bulges and loops in RNA?

Loops: a short series of unpaired bases in a longer paired helix.

Bulges: regions in which one strand of a helix has "extra" inserted bases with no counterparts in the opposite strand.

6. What is transaldolase?

Transaldolase is an enzyme of the non-oxidative phase of the pentose phosphate pathway. In humans, transaldolase is encoded by the TALDO1 gene.

7. Write five types of reactions in cells.

The reactions going on in biological systems can be categorized into 5 types:

1. Reactions that make or break carbon-carbon bonds
2. Internal rearrangements, isomerization, and eliminations
3. Free-radical reactions
4. Group transfer
5. Oxidation-reduction reactions

8. What are advantages for using triacylglycerols for energy storage?

Advantages of using triacylglycerols for energy storage

1. Fats are highly reduced hydrocarbons with a large energy of oxidation.
2. Fats are insoluble molecules that aggregate into droplets. They are unsolvated and no storage mass is water.
3. Fats are chemically inert. They can be stored without fear of unfavorable reactions.

9. Which ions are formed by the ionization of water and what happens to Hydrogen ions?

Ionization of Water

Although many of the solvent properties of water can be explained in terms of the uncharged H₂O molecule, the small degree of ionization of water into hydrogen ions (H⁺) and hydroxide ions (OH⁻) must also be taken into account. Pure Water is slightly ionized. Water molecules have a slight tendency to undergo reversible ionization to yield a hydrogen ion (a proton) and a hydroxide ion, giving the equilibrium reaction:



Hydrogen ions formed in water are immediately hydrated to hydronium ions (H₃O⁺). The ionization of water can be measured by its electrical conductivity. Pure water carries electrical current as H₃O⁺ migrates toward the cathode and OH⁻ toward the anode.

10. What is continuous fermentation?

A Continuous fermentation is a process in which fresh medium (substrate) is continuously added in the bioreactor, and biomass or products containing left over nutrients and microorganisms are continuously removed at the same rate to keep the culture volume constant. Under these conditions the cells remain in the logarithmic phase of growth.

11. Define fermentation in terms of biotechnology. Describe its types with enzymes and reaction.

In Industrial Biotechnology, fermentation means a process in which microorganisms that are cultured on a large-scale under aerobic or anaerobic conditions, convert a substrate into a product which is useful to man.

Types of fermentation:

Batch fermentation

Batch fermentation refers to “A technique used to grow microorganisms or cells.

- A limited supply of Sterile nutrients (substrate) for growth is inoculated (starter culture) when these are used up, or some other factor becomes limiting, the culture declines.
- The time for which fermentation occurs is called ‘fermentation time’ or ‘batch time’
- The only material added and removed during the course of a batch fermentation is oxygen (in the form of air, if the process is aerobic) and pH control solutions.
- The culture is allowed to grow until no more of the product is being made.

Continuous Fermentation

A Continuous fermentation is a process in which fresh medium (substrate) is continuously added in the bioreactor, and biomass or products containing left over nutrients and microorganisms are continuously removed at the same rate to keep the culture volume constant.

Fed-batch Fermentation

The fed-batch technique was originally devised by yeast producers in the early 1900s to regulate the growth in batch culture of *Saccharomyces*. Yeast producers observed that in the presence of high concentrations of malt, a by-product - ethanol - was produced, while in low concentrations of malt, the yeast growth was restricted. The problem was then solved by a controlled feeding regime, so that yeast growth remained substrate limited.

Few of the common types of enzymes include the following.

- Proteases: Breaks down protein.
- Lipases: Breaks down lipids into glycerol and fatty acids.
- Amylases: Breaks down starch in simple sugars.
- Cellulases: Breaks down cellulose.

12. Explain 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.

13. Write role of snRNA.

Small nuclear RNA (snRNA)- play pivotal roles in RNA processing, particularly mRNA processing.

14. How to find equilibrium constant if A & B are reactant and D & C are product?

The equilibrium constant is given by following equation where [A], [B], [C], and [D] are the molar concentrations of the reaction components at the point of equilibrium, equilibrium constant can be written as:

$$K_{eq} = \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

15. Differentiate between RNA and DNA.

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.

16. What is the effect of inhibition on v_{max} ?

Effect on V_{max}

The apparent V_{max} changes, because the inhibitor is capable of preventing catalysis regardless of whether the substrate is bound to the enzyme.

- Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate.
- Thus, noncompetitive inhibitors decrease the V_{max} of the reaction.
- The noncompetitive inhibitor, in effect, lowers the concentration of the active enzyme and therefore decreases the V_{max} of the enzyme.

17. What is elimination reaction?

An elimination reaction is a type of organic reaction in which two substituents are removed from a molecule in either a one- or two-step mechanism. The one-step mechanism is known as the E2 reaction, and the two-step mechanism is known as the E1 reaction. The loss of water from an alcohol, resulting in the introduction of a C=C bond b/w 2 adjacent carbon atoms. An elimination reaction with no changes in oxidation state.

18. What do you mean by energy hill and transition state?

In biochemistry and pharmacology, the Hill equation refers to two closely related equations that reflect the binding of ligands to macromolecules, as a function of the ligand concentration. A ligand is "a substance that forms a complex with a biomolecule to serve a biological purpose" (ligand definition), and a macromolecule is a very large molecule, such as a protein, with a complex structure of components (macromolecule definition). Protein-ligand binding typically changes the structure of the target protein, thereby changing its function in a cell.

The starting point for either the forward or the reverse reaction is called the ground state.

- The equilibrium between S and P reflects the difference in the free energies of their ground states.
- The free energy of the ground state of P is lower than that of S
- So G'^0 for the reaction is negative and the equilibrium favors P.
- The position and *direction* of equilibrium are *not affected* by any catalyst.
- But there is an energy barrier between S and P: The energy required for alignment of reacting groups formation of transient unstable charges bond rearrangements and other transformations
- This is illustrated by the energy “hill”
- To undergo reaction, the molecules must overcome this barrier and therefore must be raised to a higher energy level.
- This is called the transition state.

19. How non-competitive inhibitor is different from competitive inhibitor?

The competitive inhibitor binds to the active site and prevents the substrate from binding there. The noncompetitive inhibitor binds to a different site on the enzyme; it doesn't block substrate binding, but it causes other changes in the enzyme so that it can no longer catalyze the reaction efficiently.

20. Explain primary structure of tRNA.

A tRNA molecule has an "L" structure held together by hydrogen bonds between bases in different parts of the tRNA sequence. One end of the tRNA binds to a specific amino acid (amino acid attachment site) and the other end has an anticodon that will bind to an mRNA codon.

21. Explain coordination of citric acid and glyoxylate.

The citric acid cycle is a major catabolic pathway producing a considerable amount of energy for cells, whereas the glyoxylate cycle's main function is anabolic - to allow production of glucose from fatty acids in plants and bacteria.

22. What is glycolysis?

Glycolysis is a biological process in which glucose is broken down into pyruvate thus generating two molecules of ATP (Adenosine triphosphate) per molecule of glucose

23. Write chemical functions of cell.

The molecule that powers chemical work within cells is ATP, or adenosine triphosphate. ATP is the energy currency of the cell. Cells produce ATP from cellular respiration. During cellular respiration, glucose is stripped of electrons, which enter the electron transport chain to make large amounts of ATP.

24. Explain reversible and irreversible inhibitors.

Reversible inhibitors typically bind to enzymes through non-covalent bonds. The two most commonly encountered types of reversible inhibition are;

Competitive and Non-competitive.

A substance that permanently blocks the action of an enzyme. In cancer treatment, irreversible enzyme inhibitors may block certain enzymes that cancer cells need to grow and may kill cancer cells. They are being studied in the treatment of some types of cancer. Irreversible inhibitors bind to enzymes through covalent bonds.

25. Write first step of glycolysis. Which enzyme is used in this step?

- 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 D-fructose 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.

26. Explain ping pong mechanism of nucleosides.

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.

27. Name five diseases resulting from ion channel effect.

Neuronal disorders, e.g., epilepsy, episodic ataxia, familial hemiplegic migraine, Lambert-Eaton myasthenic syndrome, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia may result from dysfunction of voltage-gated sodium, potassium and calcium channels, or acetylcholine- and glycine-gated channels.

28. Define pH; write its equation and what is the pH of water at 25°C?

- The degree of ionization of water at equilibrium is small
- At 25°C only about two out of every 10⁹ molecules in pure water are ionized at any instant
- The equilibrium constant for the reversible ionization of water is $K=[H^{+}][OH^{-}]/[H_2O]$
- Since 1 mole (mol) of water weighs 18 g,
- 1 liter (L) (1000 g) of water contains $1000 \div 18 = 55.56$ mol
- Pure water thus is 55.56 molar (M)

- Accordingly, we can substitute 55.5 M in the equilibrium constant expression to yield
- $K_{eq} = [H^+][OH^-]/55.5$
- $K_{eq} = [H^+][OH^-]/55.5$
- On rearranging, this becomes $(K_{eq})(55.5) = [H^+][OH^-] = K_w$
- Where K_w designates the product $(55.5M)(K_{eq})$, the ion product of water at 250C
- The value for K_{eq} , determined by electrical conductivity measurements of pure water, is $1.8 \times 10^{-16} M$ at 250°C
- $K_w = (K_{eq})(55.5) = [H^+][OH^-]$
- $K_w = (1.8 \times 10^{-16}M)(55.5M) = [H^+][OH^-]$
- $K_w = 1.0 \times 10^{-14} M^2 = [H^+][OH^-]$
- $K_w = [H^+][OH^-] = [H^+]^2 = [OH^-]^2$
- Solving for $[H^+]$ gives:
 $[H^+] = \sqrt{K_w} = \sqrt{10^{-14} M^2}$
 $[H^+] = 10^{-7} M$
- Thus the product $[H^+][OH^-]$ in aqueous solutions at 250°C always equals $1 \times 10^{-14} M^2$
- When there are exactly equal concentrations of H^+ and OH^- , as in pure water, the solution is said to be at neutral pH.
- As the ion product of water is constant, whenever $[H^+]$ is greater than $1 \times 10^{-7} M$, $[OH^-]$ must be less than $1 \times 10^{-7} M$, and vice versa.

29. How ΔG and G° are related to each other?

- The actual free-energy change, ΔG
- The standard free-energy change, ΔG°
- Each chemical reaction has a characteristic standard free-energy change, which may be positive, negative, or zero, depending on the equilibrium constant of the reaction.
- Thus ΔG° is a constant: it has a characteristic, unchanging value for a given reaction
- But the *actual* free-energy change, ΔG , is a function of reactant and product *concentrations and of the temperature prevailing during the reaction*, none of which will necessarily match the standard conditions as defined above.
- The criteria for spontaneity of a reaction is the value of ΔG not ΔG° .

30. How malate synthase is used in glyoxalate cycle?

1. Acetyl-CoA condenses with oxaloacetate to form citrate
2. Citrate is converted to isocitrate, exactly as in the citric acid cycle.
3. Then the cleavage of isocitrate by isocitrate lyase, forming succinate and glyoxylate (not the breakdown of isocitrate by isocitrate dehydrogenase)
4. The glyoxylate then condenses with a second molecule of acetyl-CoA to yield malate, in a reaction catalyzed by malate synthase.
5. The malate is subsequently oxidized to oxaloacetate, which can condense with another molecule of acetyl-CoA to start another turn of the cycle.

31. What is transaldose?

- Transaldolase catalyzes a reaction similar to the aldolase reaction of glycolysis, that is, a three-carbon fragment is removed from sedoheptulose 7-phosphate and condensed with glyceraldehyde 3-phosphate, forming fructose 6-phosphate and the tetrose erythrose 4-phosphate.
- Now transketolase acts again, forming fructose 6-phosphate and glyceraldehyde 3-phosphate from erythrose 4-phosphate and xylulose 5-phosphate. Two molecules of glyceraldehyde 3-phosphate formed by two repetition of the process can be converted to a molecule of fructose 1,6-bisphosphate as in gluconeogenesis
- Finally FBPase-1 and phosphohexose isomerase convert fructose 1,6-bisphosphate to glucose 6-phosphate.
- Overall, six pentose phosphates have been converted to five hexose phosphates—the cycle is now complete.
- Transketolase requires the cofactor thiamine pyrophosphate (TPP), which stabilizes a two-carbon carbanion in this reaction.