UNIVERSITY OF TORONTO
FACULTY OF ARTS AND SCIENCE
BIOCHEMISTRY 210HF

Second Midterm Examination: November 4th, 2008
Total Duration: 1.5 hours

Examiners: Drs. R. Reithmeier, R.R. Baker and R. Andreopoulos

Candidates may use simple calculators (supplied by candidates).

This examination is worth 25 % of the final grade for the course. There are 25 multiple choice questions worth 1 mark each for a total of 25 marks. Of the 25 multiple choice questions, 16 are from Dr. Reithmeier (Part A) and 9 are from Dr. Baker/Dr. Andreopoulos (Part B).

For the multiple choice questions you are to choose ONE answer for each question and fill in the appropriate circle on the computer card with a soft lead pencil (**not pen!**). Marks are not deducted for wrong choices but no marks are given if more than one circle is filled in per question.

We cannot help you if you make transcriptional errors. It is thus not a good idea to leave entering your answers on the card to the last few minutes of the exam.

When not entering answers on your card you should cover the answer card with your question paper.

Please note that the examiners make every effort to ensure there is only one suitable answer for each multiple choice question. However, if you are convinced that a particular question has two answers, you should select the answer that seems **MOST** appropriate.

Answer all the multiple choice questions.

Biochemistry 210 Mid-term Examination

Lipids and Biological Membranes

Dr. Reinhart Reithmeier

Instructions: For each of the following 16 questions select ONE of the following choices on the Scantron Card:
A if ALL statements are TRUE
B if ALL statements are FALSE
C if ONE statement is FALSE and THREE are TRUE
D if TWO statements are FALSE and TWO are TRUE
E if THREE statements are FALSE and ONE is TRUE

(1 mark each, 16 marks total)

1. i) Fatty acids occurring in nature typically contain trans, not cis double bonds.
ii) Fatty acids with longer acyl chains have a lower melting temperature.
iii) Fatty acids with double bonds have a higher melting temperature.
iv) Fatty acids containing omega-3 and omega-6 double bonds are not required in the diet.

2. i) Phospholipids consist of a glycine backbone, two fatty acyl chains, phosphate and a polar headgroup.
ii) Phospholipids can be neutral (zwitterionic) like phosphatidylcholine or anionic like phosphatidylserine.
iii) Phospholipids, when sonicated in water, can form liposomes consisting of a lipid bilayer enclosing an aqueous compartment.
iv) Phospholipids like phosphatidylinositol can be phosphorylated by kinases and are involved in cell signaling events.

3. i) Cholesterol is an important component of biological membranes.
ii) Cholesterol is a precursor for steroid hormones, bile salts and prostaglandins.
iii) Cholesterol is a rigid, planar molecule containing a fused 4-ring system and a hydroxyl group at C-3
iv) Cholesterol comes exclusively from our diet.

4. i) Biological membranes are perfectly symmetrical, with the same phospholipids on both sides of the bilayer.
ii) Biological membranes can be described by the fluid mosaic model, which proposed that globular proteins are embedded in a fluid lipid bilayer.
iii) Biological membranes display oligosaccharides attached to lipids and proteins to the cell interior.
iv) Biological membranes contain extrinsic membrane proteins that typically span the lipid bilayer and intrinsic membrane proteins that are associated with the surface of the membrane.

Cont'd

5. i) Membrane proteins that span the lipid bilayer are more hydrophilic than soluble proteins.
ii) Membrane proteins can be visualized by freeze-fracture electron microscopy.
iii) Membrane proteins containing beta-barrels are found in the outer membrane of some bacteria.
iv) Membrane proteins are too big to diffuse in the plane of the membrane.

6. i) Transmembrane segments have a high content of hydrophobic amino acids such as leucine, isoleucine, and valine.
ii) Transmembrane segments rarely contain charged residues like lysine and glutamate, even though they are good helix formers in soluble proteins.
iii) Transmembrane segments can contain amino acids that are poor helix formers like isoleucine and valine.
iv) Transmembrane segments can contain glycine residues, that mediate specific helix-helix interactions.

7. i) Transmembrane segments are typically alpha-helical in conformation.
ii) Transmembrane segments typically contain ~20 hydrophobic amino acid residues allowing them to span the 30Å thick hydrophobic phase of the membrane.
iii) Transmembrane segments can pack together in a specific manner in multi-span membrane proteins.
iv) Transmembrane segments can rapidly change their conformation from an alpha-helix to a beta-strand.

8. i) Membrane proteins are often present in low amounts in native tissues.
ii) Membrane proteins need to be solubilized using detergents, which preserves lipid-protein interactions.
iii) Membrane proteins are easy to crystallize accounting for the high number of structures in the protein data base (PDB).
iv) Membrane proteins in detergent micelles are too small for NMR analysis.

9. i) SDS (sodium dodecyl sulfate) is an ionic detergent that can efficiently solubilize membranes.
ii) SDS gel electrophoresis can be used to determine the subunit molecular weight of proteins.
iii) SDS gel electrophoresis can be used to separate a complex mixture of proteins.
iv) SDS is commonly used to purify membrane proteins in their native state.

10. i) A mutation in glycine residues in the transmembrane segment of glycophorin A could affect its dimer structure.
ii) A mutation in asparagine residues in the extra-cellular domain of glycophorin A could affect the attachment of sugars.
iii) A mutation that introduces proline residue into the transmembrane segment of glycophorin A could disrupt the alpha helix.
iv) A mutation that introduces a stop codon on the amino-terminal side of the transmembrane segment of glycophorin A could result in production of an extracellular soluble protein.

Cont'd

11. i) Bacteriorhodopsin uses the energy of light to produce a proton gradient across the membrane of the bacterium that can be used to generate ATP.
ii) Bacteriorhodopsin contains 12 transmembrane segments that were first visualized by electron microscopy.
iii) Bacteriorhodopsin contains a covalently attached retinal group that links the protein to the membrane.
iv) Circular dichroism spectroscopy suggests that bacteriorhodopsin has a very high tryptophan content.

12. i) Oligosaccharide chains on membrane proteins come in two types, N-linked attached to the side-chain of lysine and O-linked attached to the side chains of serine or threonine.
ii) Oligosaccharide chains are also linked to certain lipids, creating glycolipids.
iii) Oligosaccharides are involved in cell-cell interactions important for cell recognition.
iv) Oligosaccharides have a high information content due to the variety of monosaccharides that can be linked together in different ways.

13. i) Aquaporin transports water that normally moves slowly across biological membranes.
ii) Aquaporin forms a beta-barrel that spans the red cell blood cell membrane.
iii) Aquaporin contains a central hydrophilic pore.
iv) Aquaporin is very selective for water and but can also transport ions.

14. i) Alpha-hemolysin is mushroom-shaped membrane protein that lyses cells.
ii) Alpha-hemolysin contains a beta-barrel that spans the lipid bilayer.
iii) Alpha-hemolysin consists of 7 identical subunits, each contributing 2 beta-strands to the pore.
iv) Alpha-hemolysin has a pore large enough to allow leakage of molecules like ions, ATP and amino acids from the cell.

15. i) Membrane transport involving channels and pores move substances rapidly up concentration gradients.
ii) Membrane transport involving passive transport requires a protein carrier that moves substances up concentration gradients.
iii) Membrane transport involving primary active transport systems use a direct source of energy (ATP) and move substances down their concentration gradients.
iv) Membrane transport involving secondary active transport uses ion gradients to co-transport substances down their concentration gradients.

16. i) The β2 adrenergic membrane receptor binds the water-soluble adrenalin on the cytosolic side of the membrane.
ii) The β2 adrenergic membrane receptor can cause a GTPase to become active, which transduces a signal to an effector enzyme.
iii) The β2 adrenergic membrane receptor can activate an effector enzyme, adenylyl cyclase that converts ATP to cAMP
iv) The β2 adrenergic membrane receptor's action can be modified by caffeine, which blocks the enzyme, phosphodiesterase that normally degrades cAMP, resulting in an increase in cellular cAMP.

Cont'd

PART B: Dr. Baker's/Dr. Andreopoulos' Section.

INSTRUCTIONS: For each question you are asked to select one INCORRECT or CORRECT option

17. Which ONE of the following concerning hemoglobin or myoglobin is INCORRECT?

a) Fe^{3+} in methemoglobin does not bind oxygen, instead water is found at the oxidized heme sites.

b) CO has a high affinity for hemoglobin, so that low levels of CO can be very toxic.

c) If a genetic mutation changed His F-8 (His-93) to Ala F-8 (Ala-93) in the myoglobin amino acid sequence, likely this mutant myoglobin would have a greatly diminished colour when compared with normal (non-mutant) myoglobin.

d) It is important that the imidazole rings of both His E-7 (His-64) and His F-8 (His-93) be in the protonated (charged) form to maintain efficient heme function in myoglobin.

e) At $pO_2 > 80$ torr, hemoglobin will be found principally in the R_4 conformational state.

18. Which ONE of the following is INCORRECT?

a) Red blood cells arriving at the lungs will carry deoxyHb in the T_4 conformational state.

b) At pO_2 values seen at the tissues (20-40 torr), a smaller proportion of hemoglobin subunits will be oxygenated at pH 7.2 compared with pH 7.6.

c) The binding of 2,3-bisphosphoglycerate to deoxyhemoglobin should reduce the net positive charge found among amino acid residues lining the cavity of the deoxyhemoglobin tetramer.

d) Deoxyhemoglobin can deliver as many molecules of CO_2 to the lungs as oxyhemoglobin can deliver molecules of oxygen to the tissues.

e) At very high elevations, where there is less than 100% saturation of hemoglobin with oxygen at the lungs, the body responds by elevating hemoglobin's affinity for oxygen.

19. If 1 mL of arterial blood has 150 mg of hemoglobin, how many moles of oxygen can be carried by 1 mL of arterial blood that is fully saturated with oxygen? (Average molecular weight of a Hb subunit = 16,000)

a) 2.3×10^{-9} moles
b) 2.3×10^{-6} moles
c) 9.4×10^{-6} moles
d) 13.2×10^{-9} moles
e) 9.4×10^{-9} moles

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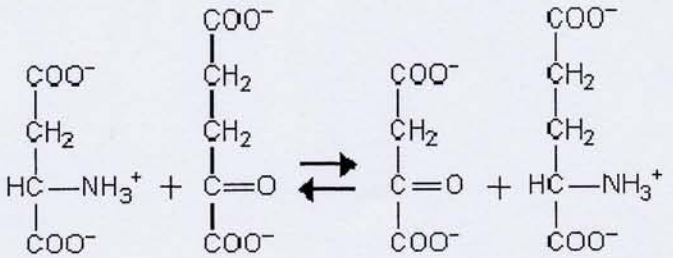
20. Select ONE of the following classes for the enzyme that catalyzes the reaction below:



a) Isomerase
b) Ligase
c) Transferase
d) Hydrolase
e) None of the above

21.

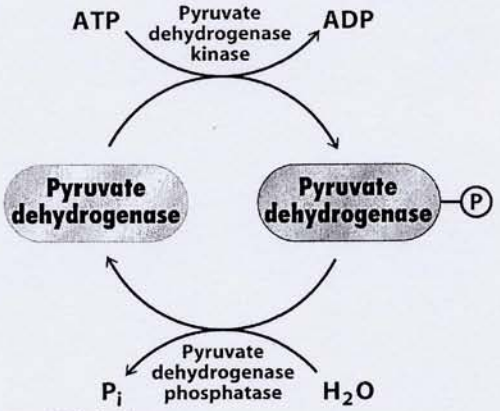


Figure 5-24 Principles of Biochemistry, 4/e
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In the above diagram, regulation of mammalian pyruvate dehydrogenase is shown.
Which ONE of the following statements is INCORRECT?

a) The enzymes pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase have higher degrees of substrate specificity when compared with hexokinase.
b) Pyruvate dehydrogenase can be classified an oxido-reductase, which is typically dependent on cofactors such as Mg^{2+} or Fe^{2+}.
c) The reaction catalyzed by the enzyme pyruvate dehydrogenase kinase is similar to that catalyzed by hexokinase.
d) None of the above three enzymes in the diagram can be classified as ligases.
e) Pyruvate dehydrogenase phosphatase can be classified as a hydrolase.

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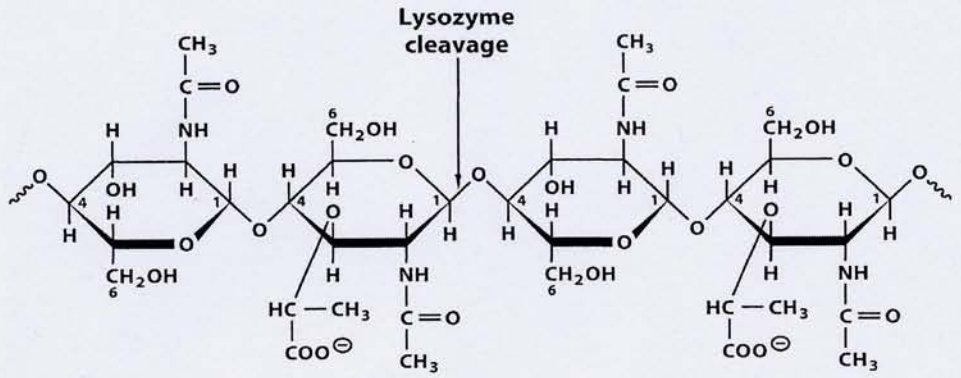
22. Which ONE of the following statements concerning lysozyme's active site is INCORRECT?



a) The substrate binding site of lysozyme has 6 saccharide sites designated A through F.
b) The mechanism of action by lysozyme involves ionic residues Glu-35 and Asp-52 which are located near C-1 of the distorted sugar in the D binding site.
c) The mechanism of lysozyme involves Glu-35 donating a proton to the oxygen involved in the glycosidic bond between residues D and E.
d) The active site of lysozyme constitutes a moderate percentage of the total enzyme volume.
e) Due to its specificity for bacterial cell walls, lysozyme serves as an antibiotic.

23. Which ONE of the following statements concerning enzyme regulation is CORRECT?

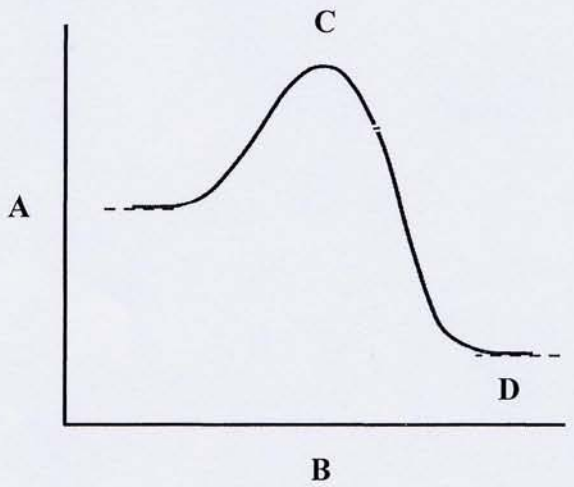
a) The activity of COX can be limited by the availability of the substrates it uses (i.e. arachidonic acid and eicosanoids).
b) The phosphorolysis of pyruvate dehydrogenase in the reaction shown in question (21) is typically characterized as a covalent modification occurring either on a serine, threonine or tyrosine residue.
c) An allosteric modulator of an enzyme does not change its shape and activity.
d) Allosteric regulation of an enzyme is a reversible covalent modification
e) Phophorylase-b can be activated by phosphorylase kinase and the activated phosphorylase will cleave glucose-1-phosphate residues from glycogen that can subsequently be used for energy.

Cont'd

24. Which ONE of the following statements is INCORRECT?

a) During the enzymatic conversion of Thr to Ile, a decrease in Ile formation can reverse the allosteric activation Ile has on E_1 in the reaction.
b) ADP is an allosteric activator of PFK-1.
c) Activation of trypsin occurs in the duodenum by either trypsin or enteropeptidase which cleave a hexapeptide (consisting of mainly polar residues) from the zymogen.
d) Proteins with quaternary structure may have regulatory subunits that can be influenced by allosteric modulators leading to modulation of their catalytic subunits.
e) Zymogens are inactive enzyme precursors

25. For the energy diagram below representing a singe-step non-enzymatic reaction which ONE of the following statements is INCORRECT?



a) A represents the free energy of the reaction.
b) C represents the transition state of the reaction intermediate.
c) An enzyme catalyzed reaction would lower the height of C
d) The lower the height of C the more often the reaction will proceed to D.
e) B represents the activation energy of the reaction.

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