SIBA ADH اسمي

UNIVERSITY OF TORONTO

FACULTY OF ARTS AND SCIENCE

BIOCHEMISTRY 210H/F

December Examinations 2004

Duration: 3 hours

Examiners: Drs. R. Bishop and R. Baker

Candidates may use simple, non-programmable calculators (supplied by candidates).

This examination is worth 50% of the final grade for the course. There are 30 multiple choice questions worth 1 mark each for a total of 30 marks and 6 short answer questions, worth a total of 20 marks.

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.

*****CAREFULLY fill in the appropriate circles on the computer card for your name and student number WITH PENCIL. Please also print your name and number on the card.**

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 and all the short answer questions.
Place your short answers in the examination booklet provided.
Please print your name and student number on your examination booklet.

Multiple Choice Questions

Dr. Baker's section:

(1) Which ONE of the following concerning aspartate transcarbamoylase (ATCase) is INCORRECT?

(a) Both CTP and UTP can inhibit the enzyme activity by allosteric binding at regulatory subunits designated r.

(b) If the sigmoid nature of the enzyme velocity vs [aspartate] plot indicates cooperativity, there appears to be a higher degree of cooperativity when CTP is bound compared with the enzyme that has ATP bound.

(c) The allosteric regulation of ATCase is controlled only by pyrimidine nucleotides.

(d) When the catalytic subunits of ATCase are isolated by themselves the plot of enzyme velocity vs [aspartate] is hyperbolic and it is possible to apply Michaelis-Menten kinetics in order to determine the Km for aspartate for the enzyme.

(2) Which ONE of the following concerning ribonuclease A or ribonuclease S is INCORRECT?

(a) Although RNase S has a break in its primary sequence between amino acids 20 and 21 it is still catalytically active and can hydrolyse substrate RNA on the 3' side of pyrimidine nucleotide components in the RNA chain.

(b) The complete hydrolysis of RNA substrate:

5' GGUAACGAUGCGACUA 3' by RNase A should generate 9 different products containing nucleotide(s).

(c) In the RNase A mechanism the newly generated terminal 3'nucleoside monophosphate will always have either uracil or cytosine as its base.

(d) Water enters the RNase A mechanism by facilitating the formation of the second transition state from the 2', 3'-cyclic nucleoside phosphate intermediate.

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(3) Which ONE of the following concerning chymotrypsin is INCORRECT?

(a) The catalytic triad is composed of Asp 102, His 57 and Ser 195 and the conversion of the side chain of Ser 195 into a strong nucleophile is facilitated by the proximity of His 57 and the stabilization of the protonated His 57 imidazole ring by Asp 102.

(b) In the mechanism of chymotrypsin the two tetrahedral intermediates are stabilized by H bonds at the oxyanion hole.

(c) In the mechanism of chymotrypsin the acyl enzyme intermediate is converted into the amine product first released from the active site.

(d) If iodoacetate reacted with the side chain of His 57, this would block the enzyme's ability to form the first tetrahedral intermediate.

(4) Which ONE of the following is INCORRECT?

(a) The conversion of NAD^+ to NADH happens when a proton is added to the nicotinamide ring of NAD^+ structure.

(b) The only structural difference between NADH and NADPH is the presence of a phosphate ester on one of the ribose ring components in NADPH.

(c) NADH has a nicotinamide component, an adenine component, two ribose components, and two phosphates linked by an anhydride bond.

(d) Two H's (H = proton plus electron) are added to FAD to create the reduced coenzyme $FADH_2$.

$H^+ H^-$ $(H^\cdot) + e^-$

(5) Which ONE of the following concerning carbohydrates is INCORRECT?

(a) The anomeric carbon in α-D-glucopyranose and β-D-galactopyranose is C-1, while the anomeric C in β-D-fructofuranose is C-2.

(b) D-glucose in its straight chain aldohexose form and α-D-glucopyranose both have the general carbohydrate formula $(CH_2O)n$.

(c) The formation of the hemiacetal during the cyclization of D-glucose involves a reaction between the aldehyde C-1 and the –OH at C-5 with a loss of a molecule of water.

(d) While β-maltose and cellobiose are reducing sugars, sucrose and α-D-glucopyranose-1-phosphate are not.

(6) When starch is incubated in a test tube with α-amylase, maltose is formed. Initially this is the alpha anomer of maltose (α-maltose = α-D-glucopyranosyl- (1→4)-α-D-glucopyranose) but with time you find more and more β-maltose in the test tube, until it is the predominant form of maltose. Which ONE of the following BEST explains this finding?

(a) α-amylase produces β-maltose from α-maltose.

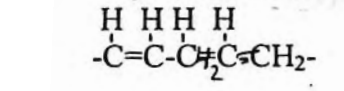
(b) β-maltose is not a reducing sugar.

(c) There is mutarotation of α-maltose, so that the free anomeric carbon in maltose allows the formation of β-maltose.

(d) α-maltose is further hydrolyzed by α-amylase and loses its glycosidic link

(7) Which ONE of the following concerning lipids is INCORRECT?

(a) In mammals fatty acids have long, unbranched chains (8-24 carbons in length) and if they are unsaturated they have the cis double bond structure noted below:



(b) Linoleic acid has 18 carbons and two cis double bonds and is used to form arachidonic acid, an unsaturated fatty acid with 20 carbons and four cis double bonds.

(c) Olive oil is rich in the neutral glyceride triolein, which has three double bonds per mole of this triacylglycerol (TAG).

(d) Trans fatty acids are found in certain snack foods (e.g. donuts and French Fries) as a result of incomplete hydrogenation of vegetable oils (like canola oil or soybean oil) and increased consumption of these trans rich foods is associated with higher risk of cardiovascular disease.

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(8) Which ONE of the following is INCORRECT?

(a) The reaction catalysed by aldolase that produces dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate from β-D-fructose-1,6-bisphosphate has a negative ΔS value.

(b) If the pyruvate kinase (PK) reaction has a $\Delta G^{o'}$ value of –32 KJ/mol, this indicates a K'eq value that is greater than 100,000. (R = 8.315 J/K°.mol)

(c) If the K'eq value for the phosphoglycerate mutase reaction (as it occurs in glycolysis) is 0.16, it means that if the reaction was initiated with a 0.1M concentration of 3-phosphoglycerate, at equilibrium the concentration of 2-phosphoglycerate would be less than 0.020M.

(d) The thiohemiacetal-enzyme intermediate in the reaction mechanism of glyceraldehyde-3-phosphate dehydrogenase is formed by the reaction of the substrate aldehyde group and the –SH of an active site cysteine.

(9) Which ONE of the following is NOT involved in the regulation of PFK-1 in liver cells?

(a) PFK-2 and β-D-fructose-2,6-bisphosphate

(b) The balance between ATP and ADP + AMP in the cytoplasm

(c) β-D-fructose-1,6-bisphosphate

(d) Citrate coming from the Krebs cycle in the mitochondria

(10) Fermentation (anaerobic) in yeast gives no net NADH production for which ONE of the following reasons?

(a) There is no NADH produced by any of the steps in fermentation.

(b) NADH produced by the action of glyceraldehyde-3-phosphate dehydrogenase is consumed in the alcohol dehydrogenase reaction.

(c) Acetaldehyde chemically oxidizes NADH.

(d) NADH generated during fermentation is used to supply carbons in the synthesis of ethanol.

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(11) Which ONE of the following is INCORRECT?

(a) Following the interaction of glucagon with its receptor on the plasma membrane of liver cells, glycogen phosphorylase is activated by a phosphorylation step while glycogen synthase is inactivated by phosphorylation.

(b) Protein kinase A (when activated) carries out the direct phosphorylation of glycogen synthase a, glycogen phosphorylase a, pyruvate kinase and PFK-2 in liver cells.

(c) In liver cells stimulated by glucagon the action of glycogen phosphorylase a is followed by phosphoglucomutase, which allows the isomerization of α-D-glucose-1-phosphate to α-D-glucose-6-phosphate.

(d) Insulin is a hormone released by the pancreas, and insulin interaction with its receptor on insulin re[illegible]sive cells is followed by the activation of protein phosphatase 1 and the action of this phosphatase leads to the inactivation of glycogen phosphorylase.

(12) Which ONE of the following is NOT associated with raised PDH complex activity?

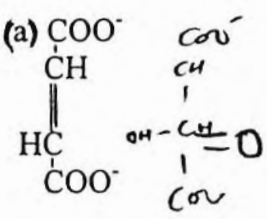
(a) Elevated NADH concentration, Acetyl CoA

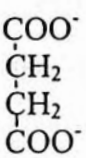
(b) Elevated ADP concentration

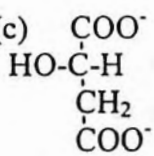
(c) Elevated pyruvate concentration Py

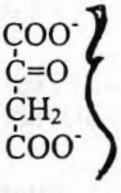
(d) Elevated concentration of Ca^{2+}

(13) Which ONE of the following is a product of malate dehydrogenase during the action of the Krebs cycle? → oxaloacetate

(a) 

(b) 

(c) 

(d) 

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(14) Of the following reactions in the Krebs cycle which option lists all the enzymes that rely on the participation of water for their activity?

i) citrate synthase
ii) aconitase
iii) isocitrate dehydrogenase
iv) α-ketoglutarate dehydrogenase
v) succinyl CoA synthetase
vi) succinate dehydrogenase
vii) fumarase
viii) malate dehydrogenase

(a) i, ii, vii

(b) i, iv, viii

(c) vii, viii

(d) i, iii, v, vi

(15) Which ONE of the following concerning electron transport is INCORRECT?

(a) While $CoQH_2$ can carry both electrons and protons derived from NADH + H^+, the cytochromes carry only electrons, and the electrons donated by $CoQH_2$ would be carried by pairs of cytochromes as the electrons pass down the electron transport chain.

(b) If the electron transport chain is blocked by the action of antimycin A, addition of cytochrome c in its ferrous state to these poisoned mitochondria will allow electron flow and ATP production by oxidative phosphorylation.

(c) The classic P/O ratio for $FADH_2$ is 2 but more recent estimates of this P/O value are closer to 1.5.

(d) The action of CO on electron transport will block mitochondrial electron transport but will not affect the generation of a proton motive force which relies on the pumping of protons from the mitochondrial matrix across the inner mitochondrial membrane.

(16) How many molecules (net) of ATP will be generated if one molecule of D-glyceraldehyde-3-phosphate is added to the cytoplasm of a liver cell? Consider the actions of glycolysis, PDH complex, Krebs cycle, electron transport and oxidative phosphorylation. (Assume classical P/O ratios for reduced coenzymes.)

(a) 15 (b) 19 (c) 29 (d) 36

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(17) Which ONE of the following reactions is NOT found during active gluconeogenesis in liver during prolonged fasting?

(a) The conversion of lactate to pyruvate with NADH production by lactate dehydrogenase.

(b) The action of PEP carboxykinase generating PEP from oxaloacetate with a release of carbon dioxide.

(c) The production of 1,3-bisphosphoglycerate by the action of phosphoglycerate kinase.

(d) An increased production of glucose in the presence of rising concentrations of β-D-fructose-2,6-bisphosphate.

(18) Which ONE of the following does NOT occur during active fat mobilization in fat cells stimulated by adrenaline?

(a) Activation of protein kinase A.

(b) Activation of hormone sensitive lipase by phosphorylation catalyzed by activated protein kinase A.

(c) The requirement of two molecules of water for the generation of three molecules of palmitic acid from the complete degradation of a molecule of tripalmitin in a fat cell.

(d) The release of three fatty acids and glycerol from a molecule of triacylglycerol by the actions of activated hormone sensitive lipase and monoacylglycerol lipase.

(19) Which ONE of the following is NOT a product of one turn of fatty acid β-oxidation using 16:0 CoA as substrate?

(a) $NADH + H^+$

(b) $CH_3\text{-}CH_2\text{-}C(=O)\text{-}S\text{-}CoA$

(c) $FADH_2$

(d) 14:0 CoA

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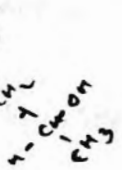
(20) Which ONE of the following is NOT associated with Type I (Juvenile) diabetes (that is NOT under control by insulin injection) ?

(a) Increased synthesis of CH_3-CH(OH)-CH_2-COO^-



(b) Increased fat synthesis

(c) Increased fat mobilization and increased rates of fatty acid β-oxidation.

(d) Cellular dehydration, loss of cellular potassium, polyuria and hypotension.

Dr. Bishop's section

(21) Which ONE of the following is INCORRECT?

(a) Gram-positive bacterial cell walls possess a lipid membrane surrounded by a peptidoglycan layer interspersed with lipopolysaccharide.

(b) Gram-negative bacterial cell walls possess a lipid membrane surrounded by a peptidoglycan layer and an outer membrane.

(c) EDTA can increase the susceptibility of Gram-negative bacteria to lysozyme.

(d) Vancomycin is an antibiotic of last resort for treatment of Gram positive infections.

(22) The relationship between beta-lactamases of class B and C can be said to be:

(a) Homologous

(b) Orthologous

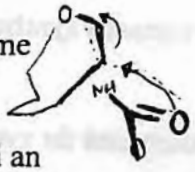
(c) Paralogous

(d) Analogous

(23) Which ONE of the following statements BEST describes the catalytic mechanism of muramidases?

(a) Cleavage by a single Sn2 displacement mechanism involving general acid/base catalysis.

(b) Cleavage by a double Sn2 displacement mechanism via a glycosyl-enzyme intermediate.



(c) Cleavage involving either electrostatic catalysis or covalent catalysis and an oxocarbenium ion transition state.

(d) Cleavage by a single or double Sn2 displacement mechanism involving a glycosyl-enzyme intermediate.

(24) Which ONE of the following statements BEST describes how the RTEM beta-lactamase lowers the activation energy (E_A) in the hydrolysis of penicillin to penicilloic acid?

(a) Lys73 is located in a hydrophobic microenvironment that suppresses its pKa by several orders of magnitude.

(b) Ser70 has nucleophilic character and attacks the carbonyl carbon of the penicillin molecule.

(c) Negative charge forming on the tetrahedral intermediate is stabilized by the oxyanion hole.

(d) Ser130 delivers a proton to the leaving group nitrogen of the beta-lactam amide bond.

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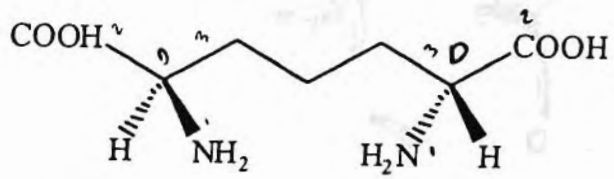
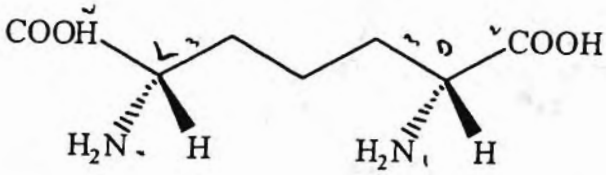
(25) Which ONE of the following is *meso*-diaminopimelic acid?

(a)



(b)



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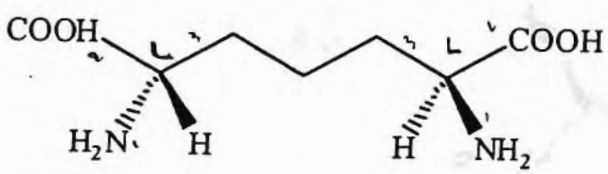
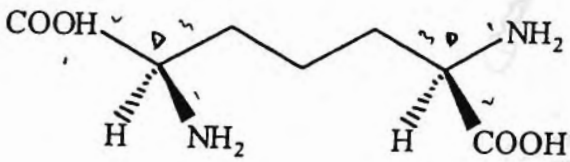


(d)



(26) Which ONE of the following BEST describes the mechanism of the DD-transpeptidase?

(a) The acylation step is like the DD-carboxypeptidase acylation step, and the deacylation step is like the DD-endopeptidase deacylation step.

(b) The acylation step is like the DD-endopeptidase acylation step, and the deacylation step is like the DD-carboxypeptidase deacylation step.

(c) The acylation step is like the reverse of the DD-endopeptidase deacylation step, and the deacylation step is like the reverse of the DD-carboxypeptidase acylation step.

(d) The acylation step is like the DD-carboxypeptidase acylation step, and the deacylation step is like the reverse of the DD-endopeptidase acylation step.

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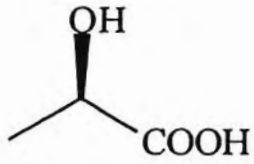
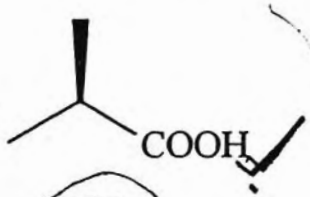
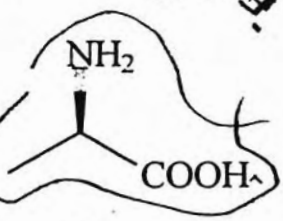
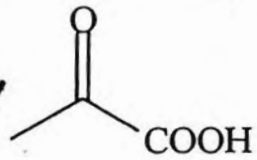
(27) Which ONE of the following metabolites is NOT utilized for the biosynthesis of peptidoglycan?

(a) 

(b) 

(c) 

(d) 

(28) The energy needed to form a peptide bond as catalyzed by the DD-transpeptidase is derived from which ONE of the following:

(a) Alanine racemase

(b) The D-Ala-D-Ala adding enzyme

(c) ATP

(d) D-Alanine

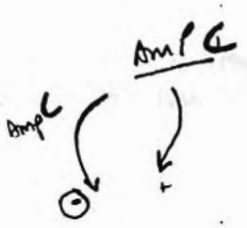
(29) A graduate student has isolated a mutant Gram-negative bacterium that is devoid of GlcNAcase activity. The parent bacterium possesses an inducible *ampC* gene. She reveals that the GlcNAcase mutant has an AmpC phenotype that is consistent with existing dogma of muropeptide recycling. Which ONE of the following phenotypes did the graduate student observe?

(a) Constitutive AmpC overproduction.

(b) Inducible AmpC overproduction.

(c) Inducible AmpC underproduction.

(d) Non-inducible AmpC production.



(30) Which ONE of the following statements BEST describes the mechanism of transglycosylation during peptidoglycan biosynthesis?

(a) A single Sn2 displacement with inversion of the configuration at the anomeric carbon.

(b) A single Sn2 displacement with retention of the configuration at the anomeric carbon.

(c) A double Sn2 displacement with inversion of the configuration at the anomeric carbon.

(d) A double Sn2 displacement with retention of the configuration at the anomeric carbon.

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SHORT ANSWER QUESTIONS

Total Value: 20 marks. Answer all the questions and place your answers in the examination booklets provided.

Dr. Baker's Section (12 marks)

31. (**3 marks**) Describe the acyl CoA synthetase reaction (ACS). Name the substrates and products and name two additional enzymes that assist in promoting the production of the acyl bearing product of ACS.

32. (**3 marks**) When you have not eaten for several hours you must produce glucose to maintain blood glucose levels. If you are relying on glycogen to supply this sugar, indicate the tissue/organ that uses its glycogen for the production of glucose and indicate the important enzyme found principally in this tissue (and not in muscle) that produces glucose for the blood. Give the reaction catalyzed by this enzyme and the names and structures of its substrate and products.

33. (**3 marks**) Explain why mitochondria with leaky inner mitochondrial membranes cannot make ATP, even though they have a fully functional electron transport chain and functional Krebs cycle.

34. (**3 marks**) Explain how the covalent modification of PFK-2 by glucagon in a liver cell shuts down glycolysis in that cell.

Dr. Bishop's section (8 marks)

35 (**4 marks**) A graduate student using site-specific mutagenesis has constructed an Asp52Ala mutant of hen egg white lysozyme and discovered that the mutant enzyme retains approximately 4% of the wild-type catalytic activity in reactions with bacterial cell walls. How do you think this mutant enzyme can still function as a catalyst? According to your proposed mechanism, show how the transition state for the reaction can be stabilized.

36 (**4 marks**) Compare (i.e. indicate what is similar) and contrast (i.e. indicate what is different) the structures of penicillin and acyl-D-alanyl-D-alanine