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UNIVERSITY OF SWAZILAND

FINAL EXAMINATION PAPER: MAY 2014

TITLE OF PAPER: BIOCHEMISTRY & CELL BIOLOGY

COURSE CODE: B203

TIME ALLOWED: THREE HOURS

INSTRUCTIONS:
1. ANSWER QUESTION 1 (COMPULSORY) AND ANY THREE OTHER QUESTIONS.
2. ANSWER A TOTAL OF 4 (FOUR) QUESTIONS
2. EACH QUESTION CARRIES TWENTY FIVE (25) MARKS
3. ILLUSTRATE YOUR ANSWERS WITH LARGE AND CLEARLY LABELLED DIAGRAMS WHERE APPROPRIATE

SPECIAL REQUIREMENTS:

1. CANDIDATES MAY USE CALCULATORS

THIS PAPER SHOULD NOT BE OPENED UNTIL PERMISSION HAS BEEN GRANTED BY THE INVIGILATORS

[PLEASE TURN OVER]

Section A (Compulsory)
Answer all questions in this section

Question 1

(a) Explain the difference between apoptosis and cell necrosis. (2 marks)

(b) Calculate the pH of a buffer composed of 0.15 M pyruvic acid and 0.25 M sodium pyruvate, given that K_a of pyruvic acid is 3.2×10^{-3}. (3 marks)

(c) Explain how chaperones assist in the correct folding of polypeptides. (5 marks)

(d) For each of the following methods of separating proteins, describe the principle of the method and the property of proteins that allows their separation.
(i) salting out, (2 marks)
(ii) differential centrifugation, (2 marks)
(iii) isoelectric focusing. (2 marks)

(e) In one or two sentences, describe the usefulness of each of the following in the analysis of protein 1° structure:
(i) Edman reagent (phenylisothiocyanate), (1 mark)
(ii) protease (e.g. trypsin or chymotrypsin), (1 mark)
(iii) reducing agent (e.g. dithiothreitol or β-mercaptoethanol). (1 mark)

(f) Consider the following peptide: ***Gly-Ile-Glu-Trp-Thr-Tyr-Gln-Phe-Arg-Lys***. Predict the products of treatment of the above peptide with each of the following:
(i) Carboxypeptidase, (3 marks)
(ii) Dinitrofluorobenzene (DNFB). (3 marks)

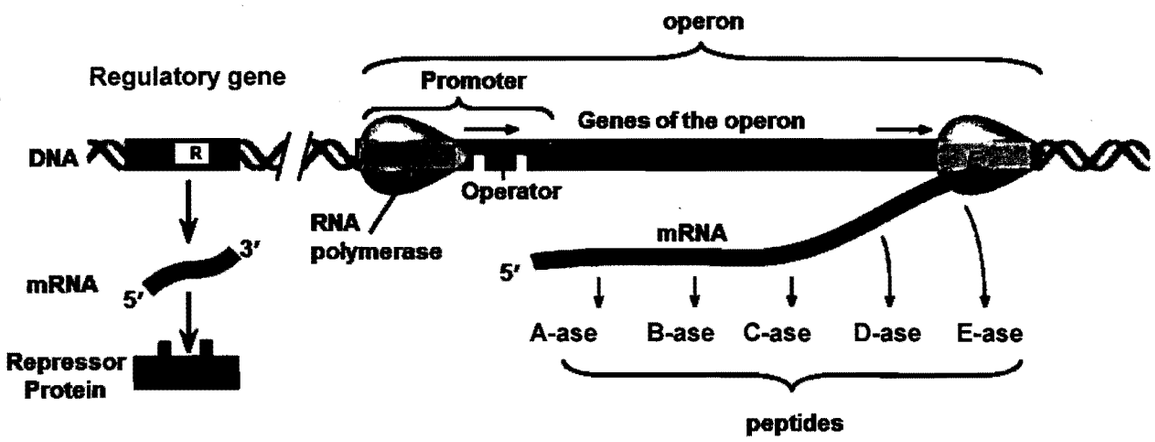
[Total Marks = 25]

Section B

Answer **any three** questions from this Section.

Question 2

(a) The diagram below represents a hypothetical operon in the bacterium *E. coli.* The operon consists of five structural genes (A to E), which code for the enzymes A-ase and B-ase, etc. and also includes the regulatory gene (R), the promoter and the operator regions as shown below.



When a certain compound **X** is added to the growth medium of *E. coli,* the structural genes are expressed at a 50-fold higher rate than in the absence of **X**. Give answers to the following, making as much reference to the above diagram as possible.

(i) Describe an operon and its relationship to polycistronic mRNA. (3 marks)
(ii) Explain the roles of the regulatory gene and compound **X** in the expression of the above operon. (4 marks)
(iii) Is regulation of expression of the above operon positive or negative? Justify your answer, giving the differences between these two controls of prokaryotic gene expression. (4 marks)
(iv) Explain whether or not the above operon is constitutive, repressible or inducible, stating the distinguishing features of each mode of expression. (4 marks)

(b) Describe briefly the relationship between chromatin structure and transcription in eukaryotes. (5 marks)

(c) With reference to the roles of proto-oncogenes and tumor-suppressor genes in cell cycle, explain why cancer is regarded as a genetic disease. (5 marks)

[Total Marks = 25]

Question 3

(a) For a reaction that can take place with or without catalysis by an enzyme, predict the effect of the enzyme on the following:

(i) standard free energy change of the reaction, (1 mark)
(ii) activation energy of the reaction, (1 mark)
(iii) initial velocity of the reaction. (1 mark)

(b) Explain any three modes by which inhibitors can impede enzyme-catalyzed reactions. Sketch annotated graphs to illustrate how these modes can be experimentally distinguished from each other. (14 marks)

(c) Bukelwa measured the initial rate of an enzyme-catalyzed reaction as a function of substrate concentration in the presence and absence of an unspecified inhibitor. Results are shown in the table below.

[S]/mM	V_0 (mmol product/second)	
	Inhibitor absent	**Inhibitor present**
0.0001	33	17
0.0002	50	29
0.0005	71	50
0.001	83	67
0.002	91	80
0.005	96	91
0.01	98	95
0.02	99	98
0.05	100	99
0.1	100	100
0.2	100	100

Without drawing any graph, use the data above to elucidate the V_{max} and K_m in the absence and presence of inhibitor. Hence, deduce the kind of inhibitor that is involved. (8 marks)

[Total Marks = 25]

Question 4

(a) Rearrange by temporal order and briefly describe the biochemical roles of the following enzymes in prokaryotic DNA replication.

(i) DNA helicase, (1 mark)
(ii) primase, (1 mark)
(iii) DNA polymerase I, (1 mark)
(iv) DNA ligase, (1 mark)
(v) topoisomerases, (1 mark)
(vi) DNA Polymerase III. (1 mark)

(c) A given mRNA sequence might be translated in any of three reading frames. Describe how prokaryotic and eukaryotic ribosomes determine the correct reading frame, prior to translation. (4 marks)

(c) The template strand of a segment of double-stranded DNA contains the sequence:

5'-CTT TGA TAA GGA TAG CCC TTC-3'

(i) Write down the sequence of the mRNA that can be transcribed from this strand (indicate its 5' and 3' ends). (2 marks)

(ii) Write down the amino acid sequence that could be coded by the mRNA sequence above, using only the first reading frame of the mRNA (indicate the N-terminus and the C-terminus). (4 marks)

(iii) Suppose the other (complementary) strand is used as a template for transcription. What is the amino acid sequence of the resulting peptide, using only the first reading frame? (4 marks)

(NB: You may refer the Genetic Code below)

(d) Explain the process by which tRNAs are charged with their cognate amino acids, the fidelity of this charging and the role of aminoacyl-tRNA synthetases. (5 marks)

[Total Marks = 25]

The Genetic code

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

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Question 5

(a) Briefly describe the two possible metabolic fates of pyruvate produced by glycolysis in humans, and explain the circumstances that favor each. (5 marks)

(b) The hydrolysis of ATP is highly exergonic:

$ATP + H_2O \rightarrow ADP + P_i$ $\Delta G^\circ = -30.5$ kJ/mol

However, the conversion of glucose into glucose 6-phosphate, which must occur in the breakdown of glucose, is thermodynamically unfavorable (endergonic):

Glucose + P_i $\rightarrow$glucose 6-phosphate + H_2O $\Delta G^\circ = +13.8$ kJ/mol

Explain how cells overcome this problem and hence, use the above equations to calculate the standard free-energy change (ΔG°) for the phosphorylation of glucose. (5 marks)

(c) If a 0.08 M solution of glucose 1-phosphate is incubated at 25°C with a catalytic amount of phospho-glucomutase, the glucose 1-phosphate is transformed to glucose 6-phosphate until equilibrium is reached. At equilibrium, the concentration of glucose 1-phosphate is 4.5×10^{-3} M and that of glucose 6-phosphate is 8.6×10^{-2} M. Write the expressions for the calculation of K'_{eq} and ΔG° for this reaction, hence calculate K'_{eq} and ΔG°. (R = 8.315 J/mol·K) (5 marks)

(d) Show the three reactions in the citric acid cycle in which NADH is produced. None of these reactions involves molecular oxygen (O_2), but all three reactions are strongly inhibited by anaerobic conditions. Explain this phenomenon. (5 marks)

(e) When the acetyl-CoA produced during β-oxidation in the liver exceeds the capacity of the citric acid cycle, the excess acetyl-CoA forms ketone bodies such as acetone, acetoacetate, and D-β-hydroxybutyrate. Explain why this occurs in severe, uncontrolled diabetes. (5 marks)

[Total Marks = 25]

Question 6

(a) During starvation, more urea production occurs. Explain this observation. (5 marks)

(b) Degradation of amino acids yields compounds that are common intermediates in the major metabolic pathways. Explain the distinction between glucogenic and ketogenic amino acids in terms of their metabolic fates. (5 marks)

(c) Explain, giving examples, why vitamins of the B complex are so important in anabolic and catabolic processes. (10 marks)

(d) Explain the formation of NADPH and ATP during photosynthesis, indicating how these two molecules can be used during carbon dioxide fixation. (5 marks)

[Total Marks = 25]

END OF QUESTION PAPER