

FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY (APK)

MODULE: BIC2A01: BIOCHEMICAL TECHNIQUES AND ENZYMOLOGY

JULY SUPPLEMENTARY EXAMINATION (SSA)

DATE: 15 July 2019

TIME: 15:00-18:00

EXAMINER 1 (Section A)
EXAMINER 2 (Section B)
INTERNAL MODERATORS

Dr L Sitole
Dr M Choene

Dr JT James

TIME 3 HOURS

MARKS 117

NUMBER OF PAGES: 7 PAGES

INSTRUCTIONS: ANSWER ALL THE QUESTIONS.
DO NOT USE RED INK.
PLEASE HAND IN YOUR QUESTION PAPER WITH YOUR EXAM BOOK.

REQUIREMENTS: ANSWER ALL THE QUESTIONS IN YOUR EXAM BOOKS
PROVIDED
ANSWER SECTION A (TECHNIQUES) AND SECTION B
(ENZYMOLOGY) IN TWO SEPARATE EXAM BOOKS

Additional Information:

pKa Values

Carboxyl group : 2.2

Amino group : 9.4

Side Chains : Tyr (10.46); Cys (8.37); Lys (10.54); Arg (12.48); His (6.04); Asp (3.90); Glu (4.07)

SECTION B [57]**Question 1****[27]**

1. Briefly (1-3 sentences, phrases, words, etc.) define the following terms:

[12]

- a) Hybridomas
- b) Protein denaturation
- c) List two methods that separate proteins according to their surface hydrophobicity
- d) Identify two methods to localize a protein within a cell using antibodies
- e) Monoclonal antibody
- f) B-cell epitope

2. Below are seven amino acids. Indicate all characteristics that apply to each amino acid by matching each amino acid to the relevant characteristic (s).

[7]

Amino acid**Characteristics**

Glycine _____
Threonine _____
Proline _____
Phenylalanine _____
Cysteine _____
Glutamic acid _____
Lysine _____

- a) has a charged residue at pH=7
- b) non-polar/hydrophobic
- c) imino acid
- d) has a ring
- e) absorbs light at wavelength 280nm
- f) contains sulphur
- g) polar/hydrophilic

3. Draw the peptide asp-val-glu-gly at pH=7 and circle the peptide bonds.

[8]

QUESTION 2**[11]**

4. Ayanda the Biochemist was purifying some β -galactosidase using the practical guide and she ran into the following problems. Explain why Ayanda's experiments didn't work and recommend how she can fix each of her problems.

- a) Problem 1: There's some β -gal in the flow-through of the column used for purification. [3]
- b) Problem 2: When loading a polyacrylamide gel, the sample diffused out of the well into the running buffer. [2]
- c) An ammonium sulfate cut was made on a newly discovered protein called "Namedoesntmatter-ase". After Ayanda re-suspended the pellet, she analysed it and found no activity. Dr. James then redid the ammonium sulfate step exactly like Ayanda and used her exact reagents. Dr. James found active protein. What happened? [3]
- d) Briefly explain the use of SDS and β -mercaptoethanol in protein denaturation. [3]

QUESTION 3**[19]**

5. Consider the following peptide:

VLCALQNTKAEY GKPMVEAHAGQQWYDAIE

Separate the resulting fragments (after treatment with a mixture of trypsin, V8 protease and CNBr) using anionic exchange chromatography at pH 8.5. (Show the order of elution) [11]

- 6. Give two approaches of eluting a target protein during ion exchange chromatography. [2]
- 7. What is the process of removing a nucleus from a cell during cloning? [1]
- 8. What are two advantages of using eukaryotic recombinant hosts? [2]
- 9. What is selective displacement? [3]

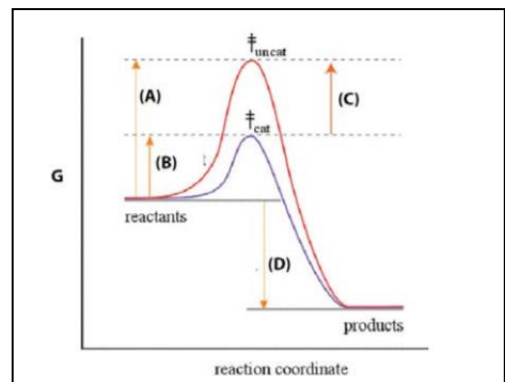
SECTION B [60]**Question 1****[10]**

1. For serine proteases, the first step of the mechanism in which the serine side chain attacks the scissile peptide bond is best described as an example of

- a) general acid catalysis
- b) electrophilic catalysis
- c) electrostatic catalysis
- d) covalent catalysis
- e) transition state binding

2. On the diagram shown comparing the same reaction in the presence and absence of a catalyst, which letter is drawn next to the arrow representing the difference in activation energy between the catalyzed and noncatalyzed reaction?

- a) A
- b) B
- c) C
- d) D
- e) none of the above



3. Induced fit for the formation of enzyme-substrate complex was proposed by:

- a) Fischer
- b) Buchner
- c) Koshland
- d) Leibeg

4. Enzymes that differ in amino acid sequence but catalyze the same reaction are_____

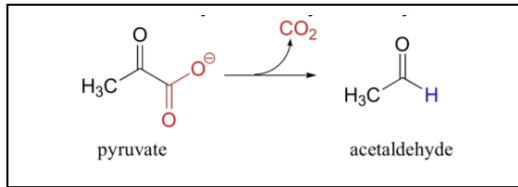
- a) Coenzymes
- b) Cofactors
- c) Isoenzymes
- d) Apoenzymes

5. Which of the following is the best description of an enzyme

- a) They allow chemical reactions to proceed very quickly
- b) They increase the rate at which a chemical reaction approaches equilibrium
- c) They make a reaction thermodynamically favorable
- d) All of the above

e) None of the above

6. Pyruvate decarboxylase catalyzes the reaction shown below. Based on this information, you can say this enzyme is a



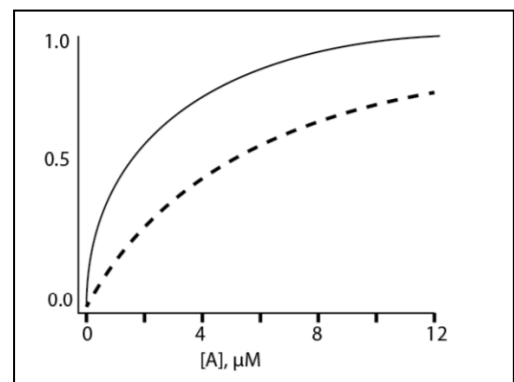
- a) oxidoreductase
b) transferase
c) hydrolase
d) lyase
e) ligase
7. Enzymes make reactions go _____ by _____ the activation energy of the reaction
a) Faster: raising
b) Slower: raising
c) Faster: lowering
d) Slower: lowering
8. Which of the following statements is false with respect to an enzyme's ability to catalyze a reaction?
a) An enzyme provides a reaction surface and a suitable environment for the reaction to take place
b) An enzyme binds reactants such that they are positioned correctly and can attain their transition-state configurations
c) An enzyme allows the reaction to go through a less stable transition state than would normally be the case
d) An enzyme can weaken bonds in reactants through the binding process
9. Which of the following statements about a plot of V_0 vs. $[S]$ for an enzyme that follows Michaelis-Menten kinetics is false?
a) K_m is the $[S]$ at which $V_0 = \frac{1}{2} V_{max}$
b) The shape of the curve is a hyperbola
c) The y-axis is a rate term with units of $\mu\text{m}/\text{min}$
d) At very high $[S]$, the velocity curve becomes a horizontal line that intersects the y-axis at K_m
10. The Michaelis-Menten equation relates the rate of an enzyme-catalyzed reaction to which of the following?
a) Substrate concentration
b) Product concentration
c) Activation energy
d) Inhibitor concentration
-

Question 2**[20]**

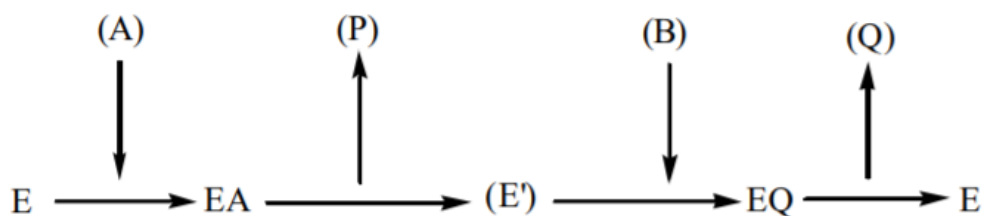
1. Explain the strategies commonly employed by enzymes to accelerate the rates of biochemical reactions. [14]
2. Cofactors are required for enzyme-catalysed reactions. Provide the different types of cofactors. [6]

Question 3**[6]**

1. The lineweaver-Burk plot is a linearization of the Michaelis Menten equation. Derive the Lineweaver-Burk Relationship starting from Michaelis Menten equation [4]
2. You are studying two enzymes X (solid line) and Y (dotted line) that utilize the same substrate and collect the following data:
 - a) Estimate the K_m of enzyme X [1]
 - b) Which enzyme has a higher affinity for the substrate? [1]

**Question 4****[12]**

1. The mechanism of chymotrypsin illustrates several of the factors that are believed to contribute to the rate acceleration obtained by enzymes. Answer the following with respect to the chymotrypsin mechanism.
 - a) A reaction model that shows ping-pong kinetics. (Identify A, B, P, Q, and E, in the following schemes) [5]



- b) Transition state stabilization occurs by bonds formed between the enzyme and the transition state that are not found when binding the substrate or product. Explain this occurrence. [7]

Question 5**[12]**

1. Describe the different types of sequential bisubstrate reactions that occur inside our cells.

[12]
