FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY (APK)

MODULE: BIC2A01: BIOCHEMICAL TECHNIQUES AND ENZYMOLOGY

JUNE EXAMINATION

DATE: 10 June 2021   **TIME: 08:30-11:30**

EXAMINER 1 (Section A)   **Prof LR Motadi**
EXAMINER 2 (Section B)   **Dr M Choene**

INTERNAL MODERATORS   Dr MBC Simelane

TIME   3 HOURS   MARKS   100

NUMBER OF PAGES: 9 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

<u>**Additional Information**</u>:
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)

Question 1 [10]

Multiple Choice:

1. **The process of passing a mobile phase through a chromatography column is called which one of the following?**
a) Partitioning
b) Elution
c) Flushing
d) Washing

2. **The pH at which a protein carries a net zero charge is termed which of the following?**

a) pI
b) pK_a
c) pK_b
d) K

3. **What is the first stage of the two-stage two-dimensional PAGE?**

a) HPLC
b) SDS-PAGE
c) isoelectric focussing
d) Sedimentation

4. **Spectroscopy measures the change in behaviour of a molecule when it is exposed to which of the following?**
a) An electrical charge
b) Acidic conditions
c) A centrifugal force
d) Electromagnetic radiation

5. **Which of the following types of spectroscopy can tell us the most about the carbon framework of an organic compound?**

a) Mass Spectroscopy

b) X-ray crystallography

c) NMR-Spectroscopy

d) Centrifugation through isolation of Carbon

6. Which of the following techniques is used to study the three-dimensional structure of a molecule?

a) Mass Spectroscopy

b) X-ray crystallography

c) NMR-spectroscopy

d) UV-visible spectroscopy

7. HPLC stands for

a) High Pressure Liquid Chromatography

b) High Performance Liquid Chromatography

c) both (a) and (b)

d) Highly Placed Liquid Chromatography

8. The eluent strength is a measure of

a) solvent adsorption energy

b) solvent absorption energy

c) solvent diffusivity

d) solvent mixing index

9. Column efficiency is measured in terms of number of plates which is

a) inversely related to the square of the peak width

b) inversely related to the cube root of the peak width

c) directly related to the square of the peak width

d) directly related to the square of the peak width

10. For a typical adsorbent such as silica gel, the most popular pore diameters are

a) 100 and 150 A°

b) 150 and 200 A°

c) 60 and 100 A°

d) 10 and 50 A°

Question 2   [10]

1. During photosynthesis water molecule is split into $2H^+ + 2e^- + 1/2O_2$. If the e^- moves from its ground zero energy level to a 4th energy level state, Draw the Jabloski diagram showing all the absorption and emission of energy by the electron.   [7]

2. Explain the phenomenon triplet state.   [3]

Question 3   [10]

1. Provide a structure of a compound having a molecular formula of C5H10O2 that is consistent with the following spectra. SHOW your work and assign all relevant peaks in 1 H NMR spectra. To confirm your choice, predict the splitting patterns for the protons in your proposed structure and estimate and/or calculate their chemical shifts.   [7]

2. Draw the spectrum of C6H13ClO of H-NMR   [3]

Question 4   [10]

1. While cleaning a stockroom, a student found a bottle of ethanol labelled "Denatured with Benzene". He decided to determine the concentration of benzene in the ethanol by obtaining the UV spectrum of the liquid in a 2.0cm cell. There was a particular peak in the spectrum at 260nm that wa attributable to benzene, and the absorbance of this peak was 0.69. In a reference source, the student found that the molar absorptivity of the benzene in ethanol was $203M^{-1}cm^{-1}$.

a) If the student has run the Mass spec of benzene, Show all the picks that would be present on the spectrum with their M/Z.   [5]

b) What is the concentration of benzene in the sample?   [3]

c) What is centrifugation? Give examples of centrifuges and explain their principles.   [2]

Question 5 [10]

1. During protein folding the following bonds are formed, hydrophobic, hydrophilic interactions, sulphide, hyprogen and ionic bonds. In your own words and understanding explain how each are formed.   [5]

2. Bax protein has its known receptor on the mitochondrial wall. If you were tasked to isolate and purify Bax using HPLC. Which type of column HPLC would you use and explain using the diagram how you will carry out the purification. Label your work   [5]

Question 1   [10]

Multiple choice:

1. On a Lineweaver-Burk or double-reciprocal plot, the following observations can be made:

a) Slope of line = K_M / V_{max}

b) $1 / v_o$ intercept is $1 / V_{max}$

c) extrapolated $1/[S]$ intercept $= -1/K_M$

d) (1), (2) and (3)

2. Allosteric enzymes have an allosteric site which is...

a) It is a binding site containing amino acids with aliphatic side chains

b) It is a binding site that can accept a wide variety of differently shaped molecules

c) It is a binding site, which is separate from the active site, and affects the activity of an enzyme when it is occupied by a ligand

d) It is a description of an active site which has undergone an induced fit

3. The catalytic mechanisms employed by Serine protease include:

a) Acid; Base; Covalent; Proximity and orientation; Electrostatic; Preferential transition state binding

b) Acid; Base; Covalent; Proximity and orientation; Electrostatic; Metal ion

c) Acid; Electrostatic; Preferential transition state binding

d) Acid; Base; Covalent; Proximity and orientation

4. Conversion of substrate present in excess amounts to product by an enzyme increased in a linear fashion for about 15 minutes, after which it was seen to plateau. This is because the enzyme has:

a) Reached it Km

b) Reached its Vmax

c) Been inactivated after 15 minutes of activity

d) Switched its substrate specificity

5. Inhibitors blocking the active site by making covalent bonds are specifically known as:

a) reversible inhibitors

b) irreversible inhibitor

c) competitive inhibitor

d) non-competitive inhibitors

6. The rate constant of a reaction depends on the:

a) Concentration

b) Temperature

c) Pressure

d) Nature of medium

7. When the rate of a reaction does not depend on the concentration of reactant molecules, the order of reaction is:

a) 1

b) Zero

c) 2

d) 3

8. Which one of the following conditions is least likely to denature an enzyme?

a) a high temperature

b) an extreme pH

c) heavy metal ions

d) a low temperature

9. The relationship between an enzyme and substrate can be described as:

a) One which the enzyme changes permanently.

b) A temporary association.

c) A non-complementary binding.

d) An association stabilized by covalent bonds.

10. How is substrate specificity determined by chymotrypsin?

a) binding of the proper amino acid into a deep pocket on the enzyme

b) binding of the N-terminus amino acid at the active site

c) interaction of the active site amino acids with the substrate

d) covalent binding of a His residue to the substrate

Question 2 [13]

1. How different are the curves of the graph of the variation in the speed of a reaction as function of substrate concentration and the graph of the variation in the speed of a reaction as function of temperature? [4]

2. In an enzymatic reaction, what is the effect of a substance with the same spatial conformation as the enzyme substrate? How is this type of substance recognized [3]

3. Cofactors are required for enzyme-catalysed reactions. Provide the different types of cofactors and give examples. [6]

Question 3 [14]

1. (a) Sketch the general mechanism of a double displacement reaction (a ping-pong bisubstrate reaction), using the A, B, P, Q, E, EA-FP, FB-FQ nomenclature (It is not necessary to label the horizontal axis). [4]

(b) Chymotrypsin acts via a ping-pong mechanism. Using the mechanism in (a) state the correspondence between A, B, P, Q and the reactants/products of this serine protease.[4]

2. The reaction indicated below is catalysed by the enzyme lactate dehydrogenase:

$$\text{L-Lactate} + \text{NAD}^+ \leftrightarrow \text{pyruvate} + \text{NADH} + \text{H}^+$$
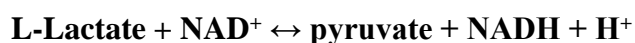
In this reaction, it is found that NAD+ binds tightly to the enzyme but there is no detectable binding of lactate.

(a) What does this suggest about the reaction mechanism [3]

(b) What is the function of NAD+ in this reaction? [1]

(c) What would the effect of Oxamate, a molecule resembling pyruvate, be on the activity of the enzyme? Explain/motivate your answer. [2]

Question 4 [13]

1. Aspartate transcarbamyolase (ATCase) is an allosteric enzyme.
   (a) What are the different activity states of this enzyme? [1]
   (b) What is the essential difference between these two states? [1]
   (c) Explain the difference in activity between the two states in terms of Michaelis-Menten Kinetics [1]
2. "Allosteric enzymes operate in a feedback mechanism in which one of the intermediates formed in the series of enzymatic reactions acts as an effector molecule and binds to the enzyme". Explain this statement using enzymes involved in glycolysis: hexokinase and phoshofructokinase. [10]