IMPERIAL COLLEGE LONDON

B.Sc. Examination 2018

This paper is also taken for the relevant examination for the Associateship of the Royal College of Science

PROTEINS AND ENZYMES

Thursday 14 June 2018 10.00 - 13.00

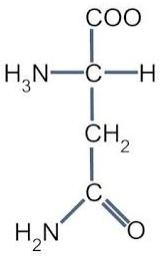
FOR FIRST YEAR STUDENTS IN BIOCHEMISTRY AND BIOTECHNOLOGY

Please use the **MCQ ANSWER SHEET** provided **FOR SECTION A** and a **SEPARATE ANSWER BOOK FOR EACH QUESTION IN SECTION B AND SECTION C**. All parts of a question carry equal weighting unless otherwise specified.

SECTION A

This section consists of 25 compulsory multiple choice questions. Using the answer sheet provided, mark the box or boxes to indicate your answer. Some questions in this section have more than one correct answer. Credit will be given for all correct answers but you will be penalised with a negative mark for incorrect choices. You will not be penalised if you do not select an answer. This section carries 25% of the marks. You should allow approximately 45 minutes to answer this section.

1. Identify the following amino acid:



A) I
B) V
C) L
D) D
E) N

2. Given that histidine has pK_1 = 1.82, pK_2 = 6.00 and pK_3 = 9.17 values, which of the following statements is/are correct?

A) At pH 4 the overall charge on the histidine molecule is +1
B) The overall charge on the histidine molecule at pH 12 is -2
C) The pI of histidine is 7.59
D) At pH 1 all the NH_3^+ groups have donated their proton to the surrounding solution
E) At pH 1.82 the overall charge on the histidine molecule is +0.5

3. Which of the following is/are true about β-sheets?

A) The amino acid side chains that comprise the β-strands all point in the same direction from the peptide backbone
B) They are stabilized by inter-strand H-bonding networks
C) In anti-parallel β-sheets, the individual strands are typically connected by α-helices
D) The residues that form β-strands have phi/psi angle pairs that fall in the upper left quadrant of the Ramachandran plot
E) The distance between adjacent amino acid residues in a β-strand is typically 1.5 Å

4. Which of the following is/are true about cyanogen bromide cleavage?

A) It is an enzyme-based cleavage method
B) It cuts on the C-terminal side of Met residues
C) It only ever cuts once in a protein
D) It results in the formation of homoserine lactone residues
E) It has a similar substrate profile to carboxypeptidase

5. Which of the following is/are true about SDS-PAGE analysis?

A) SDS is a non-polar detergent used to denature proteins
B) Smaller molecules migrate further than larger molecules
C) Proteins are visualized using an ethidium bromide stain
D) Proteins move under the influence of an electric field from the cathode to the anode
E) Proteins move through an agarose gel

6. An enzyme displays a K_M of 1 mM and V_{max} of 5.1 nmol s^{-1}. In the presence of 0.35 mM inhibitor, the K_M was measured to be 3 mM and the V_{max} was unchanged. What is the dissociation constant for the inhibitor?

A) 0.117 mM
B) 0.175 mM
C) 1.00 mM
D) 3.00 mM
E) 5.10 mM

7. The pKa of an aspartate residue is 4.00. What is the pH if 10% is protonated?

A) 3.00
B) 4.95
C) 5.00
D) 6.20
E) 6.30

8. Which of the following statements is/are true about catalytically perfect enzymes?

A) The enzyme's tertiary structure guides the substrate to the active site
B) The rate of product formation is assumed to be negligible
C) They turnover a substrate every time they encounter one
D) Their catalytic efficiency is around 10^8-10^9 M^{-1} s^{-1}
E) They can only function at elevated temperature and pH

9. Which of the following amino-acid residues can act as an acid in catalysis?

A) Phe
B) Arg
C) Asp
D) Leu
E) His

10. The rate constant for a chemical reaction is 8×10^{-1} s^{-1} at 6˚C. The activation energy is 30 kJ mol^{-1}. If the temperature is raised by 5˚C, what is the new rate constant? Assume that the Gas constant, R = 8.31 J mol^{-1} K^{-1}.

A) 8.0×10^{-1} s^{-1}
B) 1.0 s^{-1}
C) 3.0 s^{-1}
D) 38 s^{-1}
E) 40 s^{-1}

11. What concentration of enzyme was used in an enzyme assay that gave an initial rate of reaction of 240 mM min^{-1} at a [S] of 2.0×10^{-3} M if the K_m = 3.0 mM and $k_{cat} = 5.0 \times 10^3$ s^{-1}? Assume that the enzyme displays Michaelis-Menten kinetics.

A) 2000 nM
B) 2.0 µM
C) 120 µM
D) 2.0 mM
E) 120 mM

12. To what type of inhibition is an enzyme subjected if the V_{max} is reduced from 15.0 nM s^{-1} to 7.5 nM s^{-1} and the K_m is decreased from 1.6 nM to 0.8 nM? Assume that the enzyme displays Michaelis-Menten kinetics and that the inhibition is reversible.

A) Mixed
B) Pure competitive
C) Pure non-competitive
D) Uncompetitive
E) Competitive

13. Which of the following statements about local sequence alignments is/are true?

A) BLAST produces local alignments
B) Local alignments identify the best regions within sequences that match
C) A local alignment always finds the best alignment over the whole length of 2 sequences
D) InterPro produces local alignments
E) Local alignments can identify common domains in different protein sequences

14. In the binding of oxygen to myoglobin, the relationship between the concentration of oxygen and the fraction of binding sites occupied can best be described as:

A) hyperbolic
B) linear with a negative slope
C) linear with a positive slope
D) random
E) sigmoidal

15. What is the Bohr effect?

A) The ability of hemoglobin to retain oxygen when in competition with myoglobin
B) The regulation of oxygen-binding to haemoglobin by hydrogen ions and carbon dioxide
C) The alteration of hemoglobin conformation during low oxygen stress
D) The dissociation of hemoglobin when exposed to low pH
E) The regulation of oxygen-binding to myoglobin in the lungs

16. Which of the following statements is/are true concerning the concerted model of allosteric regulation?

A) The T state is the most active form of an enzyme
B) The T state is the dominant form of the enzyme in the absence of substrate
C) Positive regulators switch the R state to the T state
D) Oligomeric complexes can contain a mixture of protomers in the R and T states
E) The allosteric constant (L) = [T]/[R] in the absence of substrate

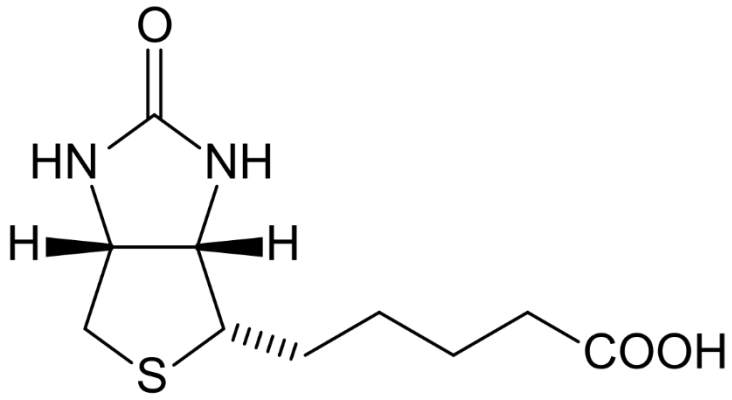
17. Which of the following statements is/are true for aspartate transcarbamoylase?

A) It catalyses the first step of the purine biosynthetic pathway
B) It is a dodecamer
C) It is made up of regulatory and catalytic subunits
D) Regulation by CTP is an example of 'homotropic' allosteric regulation
E) Both purine and pyrimidine nucleotides inhibit the enzyme

18. The Coulombic energy of interaction between two point charges:

A) has a positive value when the charges are either both positive or both negative
B) is proportional to the inverse of the square of the distance between them
C) is greater in a polar environment than a non-polar environment
D) decreases when the charges are moved into a medium with a higher relative permittivity
E) is zero in a vacuum

19. What is the common name of the molecule shown below?



A) Thiamine
B) Lipoic acid
C) Biotin
D) Niacin
E) Riboflavin

20. Which of the following is/are true about chymotrypsin?

A) It is a serine protease closely related to elastase
B) It cleaves on the C-terminal side of lysine and arginine residues
C) It is classified as a lyase
D) It contains a catalytic triad of Asp, His, Ser
E) It is synthesised in the duodenum as an inactive precursor or zymogen

21. Which of the following intermediates in the TCA cycle are synthesised using one or more co-enzymes?

A) Citrate
B) Isocitrate
C) Fumarate
D) Malate
E) Oxaloacetate

22. Which of the following co-enzymes carry electrons and protons?

A) CoA
B) CoQ
C) FAD
D) Biotin
E) Lipoamide

23. Trypsin uses which of the following catalytic mechanisms?

A) General acid-base catalysis
B) Covalent catalysis
C) Metal ion catalysis
D) Electrostatic catalysis
E) Preferential binding of the product

24. Lactate dehydrogenase:

A) is involved in fermentation
B) reduces lactate to pyruvate
C) typically uses both NADH and NADPH
D) contains the Rossmann fold
E) involves stereospecific proton transfer

25. Which of the following is/are true about pyridoxal phosphate?

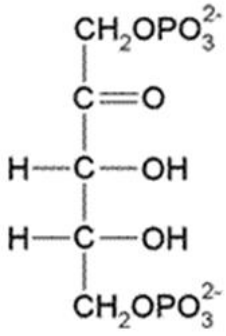
A) It is found in phosphotransferases
B) It is derived from the vitamin pyridine
C) It is covalently attached to enzymes via an imine linkage
D) It acts as an electron sink to stabilise carbocations
E) It is found in aminotransferases

SECTION B

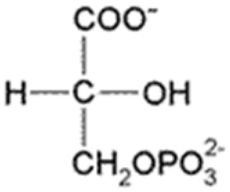
A TOTAL OF FOUR QUESTIONS MUST BE ANSWERED FROM SECTIONS B AND C, WITH AT LEAST ONE QUESTION ANSWERED FROM SECTION B AND AT LEAST ONE QUESTION ANSWERED FROM SECTION C. Each question is worth 18.75% of the total marks. Candidates should allow about 135 minutes for sections B and C. **USE A SEPARATE ANSWER BOOK FOR EACH QUESTION.**

26. The enzyme ribulose bisphosphate carboxylase catalyses the conversion of ribulose bisphosphate (RuBP) to 2 molecules of 3-phosphoglycerate (3-PG). The mechanism is thought to involve (1) the abstraction of a proton from C3 of RuBP to form an enediolate intermediate (2) the reaction of the enediolate intermediate with CO_2 to form a β-keto acid and (3) hydration of the β-keto acid and subsequent bond cleavage to generate 2 molecules of 3-PG. In the active site are a Mg^{2+} ion and a **stable** carbamate species which is formed from an addition reaction between a Lys residue and a **second molecule** of CO_2.

    (a) Using curly arrows, propose a mechanism for the formation of the carbamate species, including the structure of the side chain. (15%)
    (b) Using curly arrows, propose a possible mechanism for the formation of 3-PG from RuBP explaining possible roles for the Mg^{2+} ion and the carbamate. (75%)
    (c) What is the physiological significance of the carboxylation reaction? (10%)



RuBP



3-PG

27. The endopeptidase, thermolysin, contains in its active site a glutamate residue (Glu-143) that is thought to act as a general base and a zinc ion which is coordinated by two histidine residues, one glutamic acid residue and a single water molecule.

    (a) Using curly arrows suggest a possible mechanism for thermolysin, including the structures of all amino-acid side chains and the zinc centre. (50%)
    (b) Suggest three possible roles for the zinc ion during catalysis. (30%)
    (c) How would you experimentally test the importance of the zinc ion and Glu-143 for catalysis? (20%)

28. A biochemist is designing inhibitors against an enzyme activated in cancer cells. The biochemist has collected kinetic data for the enzyme in the presence of the substrate and has also designed two inhibitors (M1 and M2) and tested their ability to inhibit the enzyme-catalysed reaction.

[S] (μM)	v_o (μM/s) (no inhibitor)	v_o (μM/s) (+4 nM inhibitor M1)	v_o (μM/s) (+0.8 nM inhibitor M2)
0.050	0.1715	0.0375	0.05725
0.100	0.2675	0.0705	0.0890
0.200	0.3700	0.1250	0.1230
0.300	0.4225	0.1715	0.14125
0.625	0.500	0.2875	0.16675

(a) Determine the values of K_M and V_{max} for all three reactions using a Lineweaver-Burk plot. (50%)
(b) Determine the type of inhibition imposed by inhibitors M1 and M2 and explain your reasoning. (20%)
(c) Calculate the dissociation constant(s) for each inhibitor. (30%)

29. You obtain the following three sets of peptide fragments produced by cleavage of a larger protein using three different methods:

Peptide fragment Set 1
Cys-Ser-Ala-Lys
His-Asp-Ala-Gly-Glu-Ile-Phe-Gly-Ala-Ser-Val-Arg
Ile-Ala-Gly-Tyr-Val-Met-Gln-Glu-Trp-Ala-Lys-Pro-Cys-Glu-Asp-Arg
Trp-Ala-Ser-Ser-Met-Asn-Arg
Ser-Thr-Gly-Val-Cys-Thr-Ser
His-Asn-Met-Glu-Ser-Thr-Ile-Ile-Cys-Pro-Phe-Ala-Lys

Peptide fragment Set 2
Gly-Ala-Ser-Val-Arg-His-Asn-Met-Glu-Ser-Thr-Ile-Ile-Cys-Pro-Phe
Val-Met-Gln-Glu-Trp
Ala-Lys-Pro-Cys-Glu-Asp-Arg-Trp
Ala-Ser-Ser-Met-Asn-Arg-His-Asp-Ala-Gly-Glu-Ile-Phe
Cys-Ser-Ala-Lys-Ile-Ala-Gly-Tyr
Ala-Lys-Ser-Thr-Gly-Val-Cys-Thr-Ser

Peptide fragment Set 3
Glu-Ser-Thr-Ile-Ile-Cys-Pro-Phe-Ala-Lys-Ser-Thr-Gly-Val-Cys-Thr-Ser
Gln-Glu-Trp-Ala-Lys-Pro-Cys-Glu-Asp-Arg-Trp-Ala-Ser-Ser-Met
Asn-Arg-His-Asp-Ala-Gly-Glu-Ile-Phe-Gly-Ala-Ser-Val-Arg-His-Asn-Met
Cys-Ser-Ala-Lys-Ile-Ala-Gly-Tyr-Val-Met

(a) How have the peptides been generated? (15%)
(b) Why were only 6 fragments generated for the Set 1 digestions? (15%)
(c) Give the full sequence of the protein. (10%)

Question continues on next page

(d) You need to isolate the individual peptides produced in Set 3 prior to further analysis. What pH/pHs could you use to effectively separate the peptides based on charge. Assume you can get effective separation of peptides that differ in charge by 1 pH unit. Explain your answer. (Note: the pK_a of the protonated His side chain can be assumed to be 6.0). (20%)

(e) Describe the principles of a method you would use to obtain N-terminal sequence information for each peptide. (40%)

SECTION C

A TOTAL OF FOUR QUESTIONS MUST BE ANSWERED FROM SECTIONS B AND C, WITH AT LEAST ONE QUESTION ANSWERED FROM SECTION C AND ONE QUESTION FROM SECTION B. Each question is worth 18.75% of the total marks. Candidates should allow about 135 minutes for sections B and C. **USE A SEPARATE ANSWER BOOK FOR EACH QUESTION.**

30. Given an unknown protein sequence discuss the bioinformatics programs you would use to annotate it. Your answer should include one tool that could identify the overall function by searching a relevant sequence database and state which database you would search and why. You also need to describe a second program you would use that can identify component features in the sequence, such as domains. You should explain how you would interpret the information produced by both programs and what each can tell you about the protein.

31. Why are co-enzymes used in enzyme catalysis, choosing **four enzymes** to illustrate your answer?

32. Discuss with examples how enzyme activity is controlled in the cell at the post-translational level.

33. With the use of diagrams outline the features of the peptide bond, explaining how this limits the way in which the proteins can fold. Explain in detail the way in which the polypeptide chain folds in order to adopt the two common secondary structures. Explain how information on the fold of a protein is represented on a Ramachandran plot.

End of paper