IMPERIAL COLLEGE LONDON

B.Sc. Examination 2017

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

PROTEINS AND ENZYMES

Thursday 15 June 2017

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:
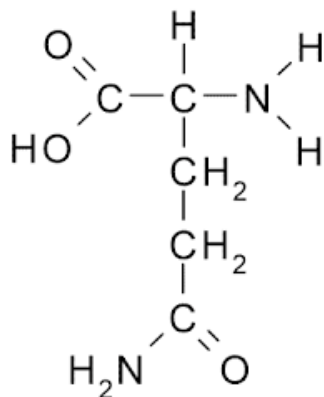
    A) R
    B) D
    C) N
    D) E
    E) Q

2. Which of the following statements about disulphide bridges is/are true?
    A) They result from formation of a covalent bond
    B) They are formed between two Cys residues located next to each other in the primary structure
    C) They are found in Fe_2S_2 clusters in the chloroplast
    D) They cannot typically form in the cytoplasm of cells
    E) They can be reduced by the addition of SDS

1

3. **Which of the following amino acids absorb at 280 nm?**
   A) P
   B) T
   C) H
   D) Y
   E) W

4. **You have been given 40 µl of a concentrated solution of Protein B. You prepare a 1:100 dilution of the sample and obtain an OD_{280} value of 0.263. The extinction coefficient (ε_{280}) and molecular mass of Protein B are 0.86 $(mg.mL^{-1})^{-1}.cm^{-1}$ and 15.2 kDa, respectively. What is the molar concentration of Protein B in the original concentrated solution?**
   A) 201 mM
   B) 20.1 mM
   C) 2.01 mM
   D) 0.20 mM
   E) 20.1 µM

5. **Three pK_a values describe protonation/deprotonation events of chemical groups of Arg: $pK_1 = 1.8$, $pK_2 = 9.0$ and $pK_3 = 12.5$. Which of the following statements about Arg is/are correct?**
   A) At pH 12.5 the overall charge on Arg is +0.5
   B) At pH 6 the overall charge on Arg is +1
   C) At pH 3 the overall charge on Arg is +2
   D) At pH 1.8 the overall charge on Arg is +1.5
   E) The pI value of Arg is 7.8

6. **Which of the following statements about NMR spectroscopy is/are correct?**
   A) It can be used to determine the structure of proteins
   B) It relies on the formation of crystals
   C) It requires treatment of the protein with β-mercaptoethanol
   D) It exploits the spin properties of certain nuclei found in proteins
   E) It is used to detect the elution of proteins during column chromatography

7. **Hydrogen peroxide decomposes in a first-order reaction to water and oxygen with a rate constant of 0.015 min^{-1} at 200 °C. Suppose you start with a 0.500 M solution of hydrogen peroxide, what is the molarity of the hydrogen peroxide remaining after 600 seconds?**
   A) 33.3 mM
   B) 75.0 mM
   C) 333 mM
   D) 425 mM
   E) 430 mM

8. **A mutant form of an enzyme displaying Michaelis-Menten kinetics was made in which the ES state only was destabilised by mutagenesis. Which of the following parameters will show an increase in value in the mutant enzyme compared to the original WT enzyme?**
   A) k_1
   B) k_{-1}
   C) k_{cat}
   D) k_{cat}/K_m
   E) $\Delta G_{reaction}$

9. **An enzyme with a single substrate ($K_M = 2.0 \times 10^{-4}$ M) was assayed in the presence of 2.0×10^{-4} M substrate and 5.0×10^{-3} M competitive inhibitor ($K_I = 2.5 \times 10^{-3}$ M). The V_{max} for the uninhibited reaction is 60 µM.min^{-1}. What is the initial velocity in the presence of the competitive inhibitor?**
   A) 15 µM.min^{-1}
   B) 0.24 mM.min^{-1}
   C) 0.15 µM.min^{-1}
   D) 24 µM.min^{-1}
   E) 2.4×10^4 nM.min^{-1}

10. **Assuming that [S]<<K_M, what is the value of k_{cat}/K_M, if [S] = 0.20 mM, [E] = 0.4 µM and initial velocity of reaction = 50 mM/min?**
    A) 1.0×10^7 M^{-1}s^{-1}
    B) 6.3×10^5 M^{-1}s^{-1}
    C) 6.3×10^2 M^{-1}s^{-1}
    D) 1.0×10^6 M^{-1}s^{-1}
    E) 3.8×10^{10} M^{-1}s^{-1}

11. **The rate constant for a chemical reaction is 6.0×10^{-2} s^{-1} at 9°C. The activation energy (E_a) is 50 kJmol^{-1}. If the temperature is raised by 12 °C, what is the new rate constant? Assume that the Gas constant, R = 8.31 J mol^{-1} K^{-1}.**
    A) 0.060 s^{-1}
    B) 0.063 s^{-1}
    C) 0.080 s^{-1}
    D) 0.14 s^{-1}
    E) 0.45 s^{-1}

12. **In the presence of 0.50 mM uncompetitive inhibitor the V_{max} was reduced by 25% relative to the uninhibited reaction. What is the K_I for the inhibitor?**
    A) 0.38 mM
    B) 0.55 mM
    C) 2.0 mM
    D) 1.5 mM
    E) 0.17 mM

13. **Which of the following statement(s) is/are true about mechanism-based inhibitors?**
    A) They have higher affinity for the enzyme-substrate complex than for the free enzyme
    B) They covalently modify the active site
    C) They are substrates for the enzyme
    D) They bind to an allosteric site
    E) They bind reversibly

14. **The pK_a of an aspartic acid side chain in pepsin is 4.0. What percentage is in the protonated form at pH 5.0?**
    A) 9.1
   B) 10
    C) 80
    D) 90
    E) 91

15. **Which of the following statements about ClustalW is/are true?**
    A) It is a global alignment program
    B) The output can be used to build phylogenetic trees
    C) It is a local alignment program
    D) It can be used to search an unknown sequence against the RefSeqP database
    E) It can be used to identify conserved residues in related proteins

16. **Of the following BLAST E-values, which ONE is the most significant?**
    A) 0.2
    B) 2e-10
    C) 10
    D) 3e-127
    E) 2

17. **What is the common name of the molecule shown below?**
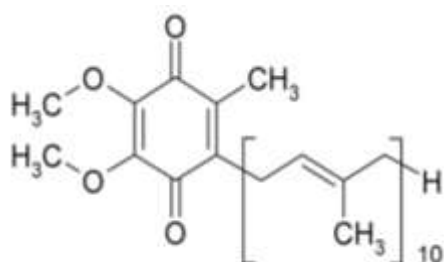
    A) Chlorophyll a
    B) Ubiquinone
    C) Pyridoxine
    D) Niacin
    E) Riboflavin

18. **Which of the following is/are true about trypsin?**
    A) It is closely related to subtilisin through divergent evolution
    B) It cleaves on the C-terminal side of lysine and arginine residues
    C) It is classified as a lyase
    D) It does not require water as a substrate in the reaction
    E) The His residue of the catalytic triad acts both as a general acid and general base during catalysis

19. **Which of the following co-enzymes can carry two-carbon units?**
    A) Coenzyme A
    B) Coenzyme Q
    C) Thymidine pyrophosphate
    D) Biotin
    E) Lipoamide

20. **Which of the following is/are true about pyridoxal phosphate?**
    A) It can phosphorylate substrates
    B) It is derived from the vitamin pyridoxine
    C) It is covalently attached to enzymes via an amide linkage
    D) It acts as an electron sink to stabilise carbanions
    E) It can catalyse isomerase reactions

21. The pyruvate dehydrogenase complex:
   A) is involved in formation of acetyl-CoA in the mitochondrion
   B) is a membrane-bound respiratory enzyme involved in quinone reduction
   C) contains an FMN cofactor
   D) contains a CoA prosthetic group
   E) is a trimer

22. Which of the following is/are true about citrate synthase?
   A) It is a glycolytic enzyme
   B) It catalyses a condensation reaction between acetyl-CoA and acetoacetate
   C) The mechanism involves nucleophilic attack by the enolate form of acetyl-CoA
   D) The enzyme displays ping-pong kinetics
   E) It is an example of induced fit

23. Which of following is/are true about the role of metal ions in enzyme catalysis?
   A) They stabilise positive charge in the transition state
   B) They can decrease the pK_a of a bound water molecule
   C) The can act as Lewis bases
   D) They only bind weakly to enzymes
   E) They can act as reducing agents

24. Which of the following is/are true for allosteric enzymes?
   A) They are regulated primarily by covalent modification
   B) They each catalyze several different reactions within a metabolic pathway
   C) They have more than one polypeptide chain
   D) They have only one active site in the holoenzyme
   E) They show strict Michaelis-Menten kinetics

25. Which of the following is/are true for myoglobin?
   A) Myoglobin is a tetramer
   B) Myoglobin is allosterically regulated by oxygen binding
   C) Upon oxygen binding the haem group becomes planar
   D) Myoglobin is found in both red blood cells and muscle cells
   E) The 'Hill coefficient' for oxygen binding by myoglobin equals 1

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. You have attempted the recombinant expression of a 45-kDa His-tagged derivative of Protein E in *Escherichia coli*.

    (a) Prior to purification, you first decide to check whether His-tagged Protein E is present in the crude cell lysate. With the aid of diagrams explain how you would do this. (35%)
    (b) You confirm that His-tagged Protein E is present in the soluble lysate. With the aid of diagrams describe how you would carry out an effective one-step purification of His-tagged Protein E using column chromatography, including details of the column and how the target protein both binds to and is eluted from the column. (35%)
    (c) SDS-PAGE analysis indicates the presence of three species in your purified sample: a 100-kDa protein, a 45-kDa protein and a 25-kDa protein. Suggest what these different proteins might represent. (20%)
    (d) Briefly describe how you might separate these different species. (10%)

27. A PhD student is performing kinetic measurements to characterise a new inhibitor, termed X54, of their enzyme. The initial rates of reaction measured with substrate S13 in the presence and absence of inhibitor X54 are summarised in the table below.

| [S13] (mM)	Initial rate of reaction in absence of inhibitor (nM/s)	Initial rate of reaction in presence of X54 (nM/s)
0.0666	0.3030	0.0890
0.1333	0.4405	0.1397
0.2666	0.5714	0.1940
0.4000	0.6329	0.2166
0.8333	0.7092	0.2599
1.6666	0.7462	0.2826

    (a) Determine the V_{max} and K_M values for substrate S13 using a **Lineweaver-Burk plot**. (40%)
    (b) Determine the type of inhibition that inhibitor X54 exerts on the enzyme. Give your reasoning by comparing the V_{max} and K_M values with those of the uninhibited reaction. What is the mode of action of this type of inhibitor? (30%)
    (c) What is the dissociation constant (or constants) for inhibitor X54 bound to the enzyme? The amount of X54 that was used in the experiment was 0.03 nM. (30%)

28. Ribonuclease A is a hydrolytic enzyme that cleaves RNA via formation of a 2'3' cyclic nucleotide. Two histidine residues, His-12 and His-119, displaying slightly different pK_a values, are required for enzyme activity.

(a) Define general acid/base catalysis. (10%)

(b) Using curly arrows, suggest a possible reaction mechanism for ribonuclease A indicating the structures of all intermediates, including the structures of the histidine side chains. (60%)

(c) Sketch a likely plot of V_{MAX} versus pH for ribonuclease A and indicate how you would use this to determine the pK_a values of His-12 and His-119. (20%)

(d) Suggest one reason why the two histidine side chains show different pK_a values. (10%)
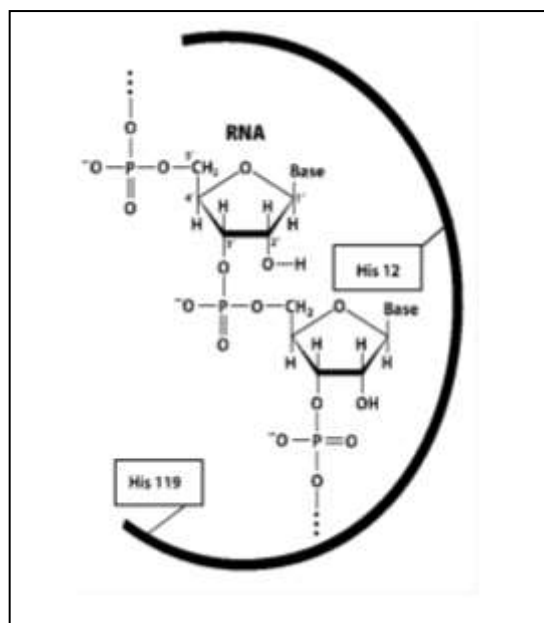
29. The enzyme 3-dehydroquinate dehydratase (DHQD) found in the shikimate pathway catalyses the conversion of 3-dehydroquinate to 3-dehydroshikimate. The reaction is thought to involve: (i) the formation of a Schiff base between the enzyme and substrate (ii) abstraction of a proton from the enzyme-bound intermediate by an active-site histidine (iii) elimination of water and (iv) release of the product from the enzyme. Using curly arrows propose a detailed mechanism, explaining your reasoning and including the structures of amino-acid side chains.

3-Dehydroquinate

3-Dehydroshikimate
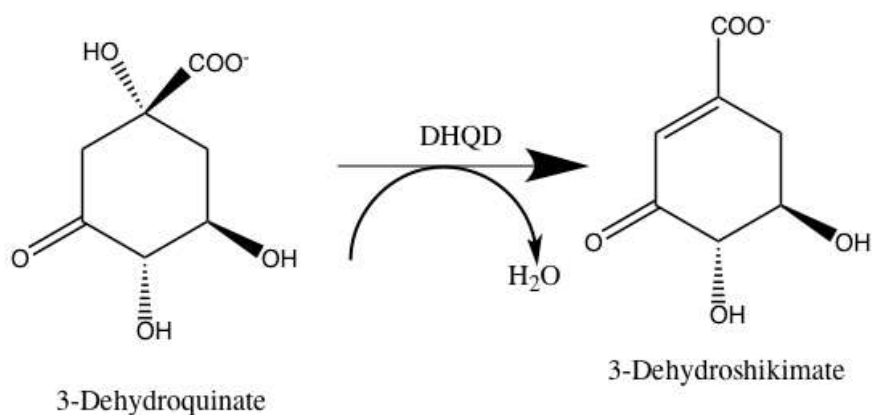
SECTION C

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. With the aid of diagrams explain the features of the α-helical and β-sheet secondary structures seen in proteins. Include in your answer details of how these structures are stabilised. Show where residues found in each structure will lie on a Ramachandran plot.

31. AMP is a heterotropic positive modulator of the enzyme glycogen phosphorylase *b*, which is found as a non-phosphorylated homodimer in muscle tissue. With the aid of diagrams, explain how AMP regulates glycogen phosphorylase b activity in terms of the model proposed by Monod, Wyman, and Changeux, clearly defining the parameters used to describe allosteric behaviour. From a physiological perspective, suggest why AMP is a suitable molecule to activate glycogen phosphorylase *b*.

32. Describe the structure and function of the pyruvate dehydrogenase complex including the roles of the **five** different cofactors in catalysis.

33. Explain, using transition state theory, why enzymes act as catalysts and discuss how this is achieved at the **molecular level**, using specific examples to illustrate your answer.

End of paper

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