IMPERIAL COLLEGE LONDON

B.Sc. Examination 2016

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

BIOLOGICAL CHEMISTRY

Wednesday 3 February 2016 10.00 - 13.00

FOR FIRST YEAR STUDENTS IN BIOCHEMISTRY AND BIOTECHNOLOGY

Please use the top answer book for Section A and separate answer books for each question in Section B. Parts of a question carry equal weighting unless otherwise specified.

A number of equations from physical chemistry are included at the end of the paper.

SECTION A

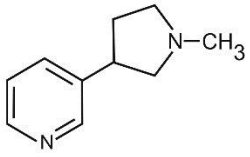
ANSWER ALL QUESTIONS. This section is worth 40% of the total marks. Each question is worth 4% of the total marks. Candidates should allow about 70 minutes for this section.

1. State what needs to be done to interconvert the following pairs:
   (a) two conformations
   (b) two configurations
   (c) two tautomers
   (d) two enantiomers.

2. Show the reaction catalysed by phosphoglucomutase. Why is this reaction important in contracting muscle?

3. For the hydrolysis of ATP to form ADP and P_i at 37 ºC, the Gibbs free energy change is -30.5 kJ/mol and the enthalpy change is -22.2 kJ/mol.
   (a) Calculate the entropy change associated with the reaction at 37 ºC. (2 pt)
   (b) Explain whether the reaction is endothermic or exothermic. (1 pt)
   (c) Explain whether the reaction is enthalpy driven and/or entropy driven. (1 pt)

4. The covalent structure of nicotine is shown below.
   (a) What is the hybridisation of each of the two nitrogens? (2 pt)
   (b) Which, if any, of the nitrogens is/are basic? Briefly explain your answer. (2 pt)



5. Draw an energy level diagram for how molecular orbitals can be formed from atomic orbitals to make a double bond in ethene.

6. Indicate what factors are responsible for the 'high energy' character of the phospho-anhydride bonds in ATP.

7. Name two amino acids that can each exist in two different structural forms under physiological conditions and give the structure of both forms of each.

8. Explain why the pentose phosphate pathway needs to be more active in cells that are dividing than in cells that are not.

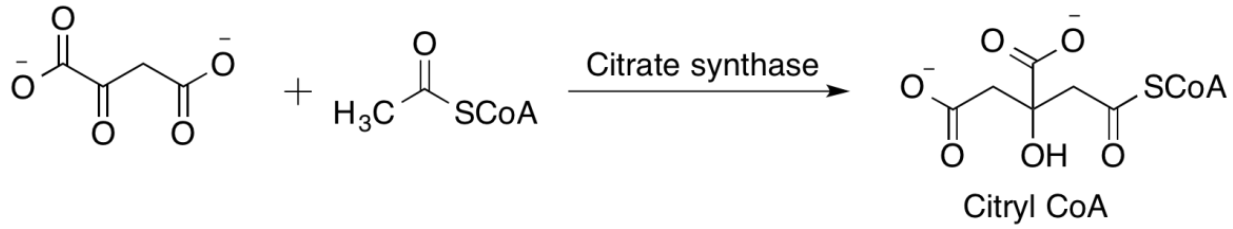
9. Bicarbonate, HCO_3^-, can become protonated to form H_2CO_3 with a pK_a of 6.35. Calculate the ratio of H_2CO_3 to HCO_3^- at a pH of 7.20 and comment on whether bicarbonate would be a useful buffer in blood at this pH.

10. Bicarbonate, HCO_3^-, is transported through the red cell membrane by passive transport. If the membrane potential is -12 mV and the serum concentration of bicarbonate ion is 30 mM at 37 ºC, what is the concentration of bicarbonate in the cytoplasm of the red blood cell?

SECTION B

ANSWER FOUR QUESTIONS. This section is worth 60% of the total marks. Each question is worth 15% of the total marks. Candidates should allow about 110 minutes for this section. USE A SEPARATE ANSWER BOOK FOR EACH QUESTION.

11. In the first step of the citric acid (Krebs) cycle, oxaloacetate condenses with acetyl CoA to form citryl CoA:



Citryl CoA

(a) Use diagrams to explain why the alpha-carbon of acetyl CoA can behave as a nucleophile. (20%)

(b) What is the most reactive carbonyl group in oxaloacetate? Explain your answer. (20%)

(c) With the help of curly arrows propose a complete mechanism for formation of citryl CoA from oxaloacetate and acetyl CoA. Citryl CoA is formed as an intermediate in the citrate synthase reaction. Name two amino acids that could be involved in this enzymic formation of citryl CoA. Explain your choices. (60%)

12. (a) Describe the origin of van der Waals interactions based on the behaviour of electrons in atoms and indicate which types of atoms can participate in these interactions. (25%)

(b) Contrast the nature of interactions involved in hydrogen bonds with those involved in van der Waals interactions. (10%)

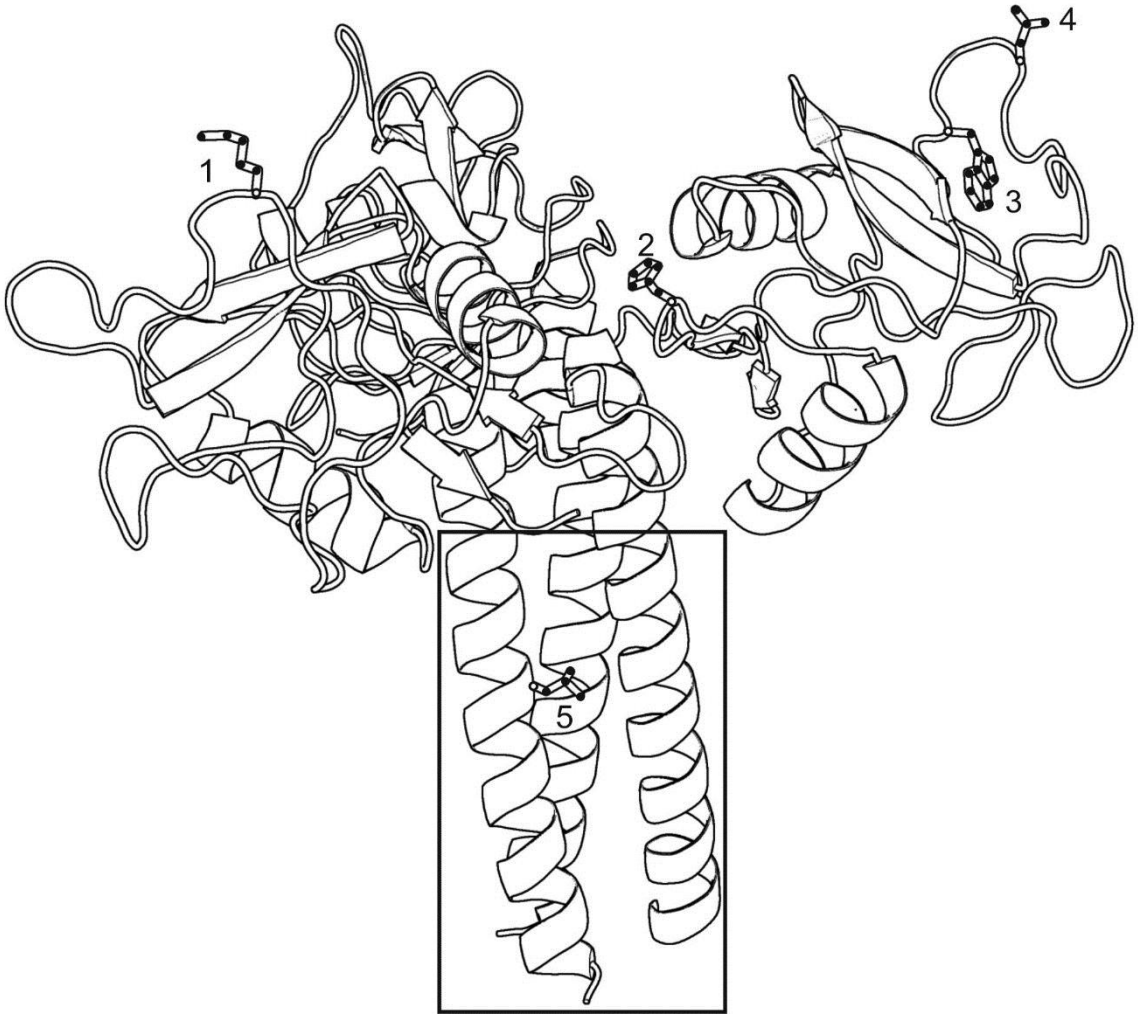
(c) Use an energy diagram to explain why van der Waals interactions can be favourable over a range of inter-atomic distances but are most favourable at an optimal inter-atomic distance. (35%)

(d) Based on your answer in part (b), indicate how a van der Waals radius is defined. (15%)

(e) Explain the concept of a steric clash and discuss whether it is accurate to represent an atom as a hard sphere with a radius equal to the van der Waals radius. (15%)

13. Explain the roles of the following in lipid metabolism:
    (a) Malonyl CoA
    (b) Carnitine acyl transferase I
    (c) Hormone-sensitive lipase
    (d) NADPH

14. The structure of a protein called MBP is shown below.



(a) Describe the secondary structure and super-secondary structure of the portion of the protein highlighted in the box. Indicate what pattern of amino acids might be present in this region and why this would stabilize this part of the molecule. (25%)

(b) For some of the amino acid residues in MBP, α carbons are denoted by open circles and side chains are shown with black dots representing C, N, or O atoms. H atoms are not shown. Identify the amino acids indicated by 1, 2 and 3 and draw full structures of the side chains. (15%)

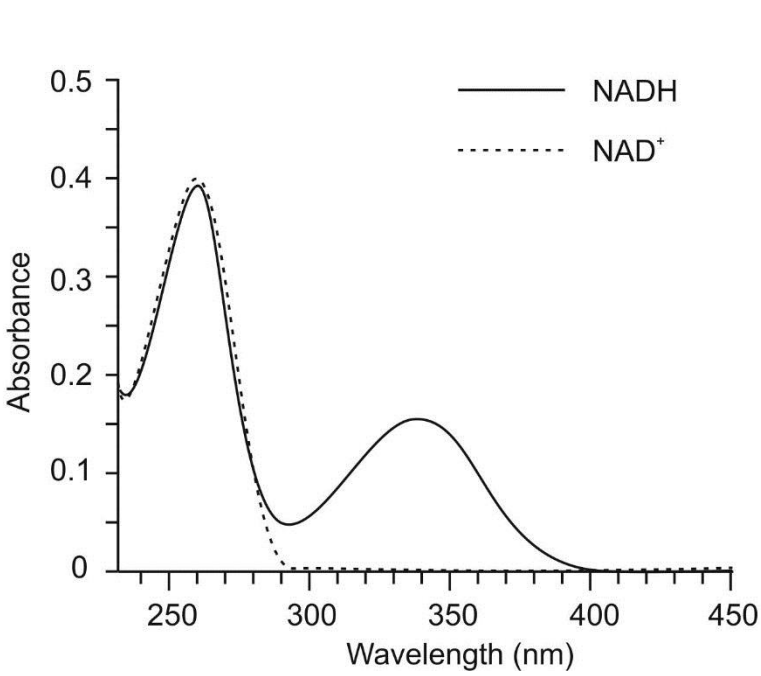
(c) Draw the structures of all of the possible side chains that might correspond to the amino acids labelled 4 and 5. If residue 4 carries a negative charge, indicate which of these amino acids it must be. Based on its location, indicate the most likely identity of residue 5, explaining your reasoning. (20%)

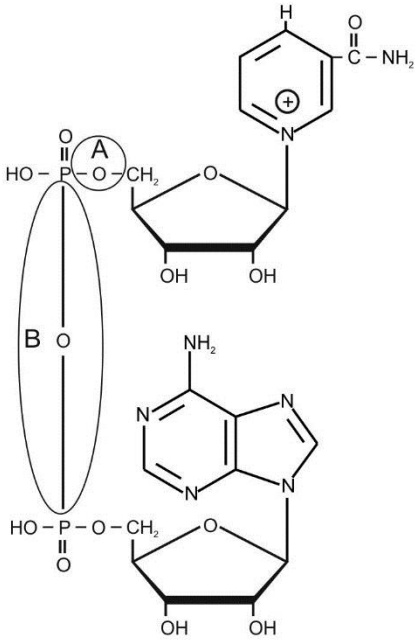
(d) Indicate what is meant by quaternary structure and describe the quaternary structure of MBP. (10%)

(e) Indicate which highlighted amino acid residue(s) contribute to absorption of UV light at 280 nm by MBP. (10%)

(f) MBP loses activity when it shifts from pH 7.2 to pH 5.4 in lysosomes. Indicate which of the highlighted amino acids might be responsible for this change and explain why. (20%)

15. A common way to monitor the progress of reactions involving NAD^+ is to measure absorbance at 340 nm, based on the absorption spectra for 1 mM solutions of NAD^+ and NADH in a 1-cm cuvette shown below.





(a) Determine the extinction coefficient of NADH at 340 nm. (15%)

(b) Indicate the types of linkages present at A and B in the right hand figure above. (10%)

(c) Based on the dissociation properties of ATP, indicate what the actual charge on the molecule that we usually designate as NAD^+ would probably be. (10%)

(d) Based on the structure of NADPH, would you expect it to have an extinction coefficient and charge similar to that for NADH? Explain your answer. (20%)

(e) Use the following half reactions to determine the ΔG^o for reduction of pyruvate to lactate. (20%)

$NAD^+ + H^+ + 2e^- \rightarrow NADH^+$ $\quad \Delta\mathcal{E}^o = -320$ mV

Pyruvate + NADH + $H^+ \rightarrow$ Lactate + NAD^+ $\quad \Delta\mathcal{E}^o = -190$ mV

(f) The enzyme lactate dehydrogenase is added to a solution containing 5 mM NADH and 5 mM pyruvate and incubated at 37ºC until the absorbance at 340 nm is not changing any more. Determine the final ratio of $[NAD^+]$ to [NADH]. You may assume that ΔG^o does not change between 25 ºC and 37 ºC. (25%)

16. (a) Draw a diagram of the peptide Ala-Ala, indicating the hybridization state of the C, N and O atoms associated with the peptide bond and showing relevant orbitals on these atoms. Using the diagram as a basis, explain why a peptide bond is planar and why it is polar. (50%)

    (b) Using alanine as a specific example, explain why a deficiency in pyridoxal phosphate (vitamin B6) would decrease incorporation of nitrogen from amino acids into urea. (50%)

17. Describe the reaction sequence occurring within cytochrome oxidase (Complex IV) for the reduction of oxygen by cytochrome c.

18. A mutation in the insulin gene is found in patients who express 'Insulin Los Angeles' which contains a serine residue at position 48 rather than the phenylalanine residue usually found at this position.

    (a) On one set of axes, plot the data below to obtain binding curves for binding of normal (N) insulin and insulin LA to the insulin receptor on liver cells. In the table, binding is expressed as the fraction of the maximal amount of binding which was obtained at very high insulin concentration (100 μM). Use the plot to estimate the K_D of each form of insulin for the insulin receptor. Show how you have obtained these values. (25%)

Free insulin normal or LA (nM)	Normal insulin bound to receptor	Insulin LA bound to receptor
0	0	0
0.1	0.17	0.010
0.3	0.38	0.030
1.0	0.67	0.095
3.0	0.86	0.24
10.0	0.95	0.51
20.0	0.98	0.68
30.0	0.99	0.76

    (b) From the K_D values, calculate the difference in the free energy of binding of N and LA forms of insulin to the receptor at 37 ºC. Speculate on where in the normal insulin molecule the phenylalanine residue is likely to be and discuss why the change to a serine residue might change the tertiary structure of insulin. (25%)

    (c) The blood insulin concentration both in patients with insulin LA and in individuals without the mutation is about 0.3 nM, so there is reduced stimulation of the receptor by insulin LA compared to normal insulin. What would be the effect of this difference in activity on: (i) glycogen metabolism in the liver and (ii) gluconeogenesis in liver? (50%)

Equations and constants from physical chemistry

Ideal gas law:	$PV = nRT$
Free energy changes:	$\Delta G = \Delta H - T\Delta S$ (at constant temperature)
	$\Delta G = \Delta G^\circ + RT \cdot \ln(\Gamma)$
Chemical potential:	$\mu_A = \mu_A^\circ + RT \cdot \ln[A]$
Relationship of standard chemical potential and equilibrium constant:	$(\Delta\overline{G}^\circ) = \Delta\mu^\circ = - RT \cdot \ln(K_{eq})$
Relationship of oxidation potential and free energy (n = number of electrons):	$\Delta\mathcal{E} = - \Delta G/nF$
Free energy and binding:	$\Delta G^\circ_{Association} = - RT \cdot \ln(K_A) = RT \cdot \ln(K_D)$
Nernst equation (z = charge on ionic species):	$\Delta\Psi = - RT/zF \cdot \ln([A]_{in}/[A]_{out})$
Henderson-Hasselbalch equation:	$pH = pK_a + \log([\text{unprotonated}]/[\text{protonated}])$
Light energy:	$\Delta E = h \cdot \nu = h \cdot c / \lambda$
Beer-Lambert Law:	$A = \varepsilon \cdot c \cdot l$
Coulomb potential:	$E \propto q_1 \cdot q_2 / r$
Gas constant:	$R = 8.314\ J\ mol^{-1}\ K^{-1}$
Faraday:	$F = 96.48\ kJ\ mol^{-1}\ V^{-1}$

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