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

BIOLOGICAL CHEMISTRY

Tuesday 30 January 2018 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.

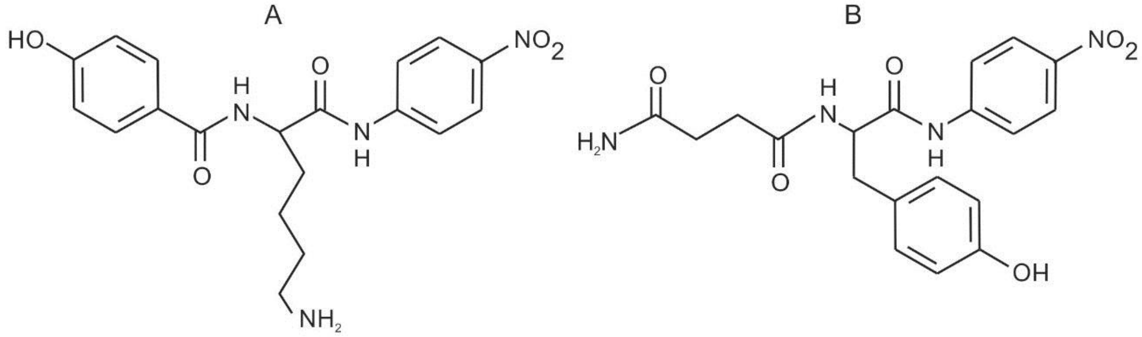
A number of equations from physical chemistry are included at the end of the paper.

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.

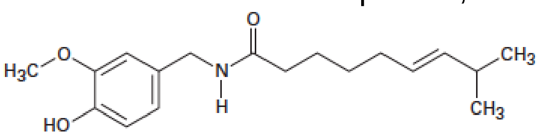
1. Indicate which proteases you would expect to cleave each of the following two substrates, explaining your reasoning.

A B



2. Discuss why the pK_a of arginine is significantly higher than the pK_a of lysine.

3. The covalent structure of capsaicin, which is found in chilli peppers, is shown below.



   (a) Name all the functional groups that contain oxygen. (1.5 pts)

   (b) Indicate which atoms lie in the same plane as the nitrogen. Briefly explain your answer. (2.5 pts)

4. Describe a common element of covalent structure used in both DNA and phospholipids.

5. Explain how a dramatic decrease in the concentration of malonyl CoA in the cytoplasm of a liver cell would affect fatty acid degradation.

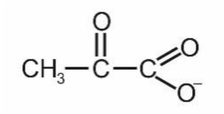
6. Explain why two of the standard amino acids can exist as diastereomers.

7. Draw the structure of valine at pH 7.2 and use appropriate calculations to show why it is neutral but cannot diffuse directly through a lipid bilayer.

8. Indicate the functions of the F_0 component and the F_1 component of the ATP synthase complex.

9. Define the kinetic (rate) constant k_{off} in the context of a hormone binding to its receptor and explain why decreasing the value of k_{off} results in higher affinity binding.

10. Pyruvate (shown below) has several metabolic fates in a mammalian cell. Show *two* reactions involving pyruvate that occur in cells and indicate what type of chemical conversion occurs.



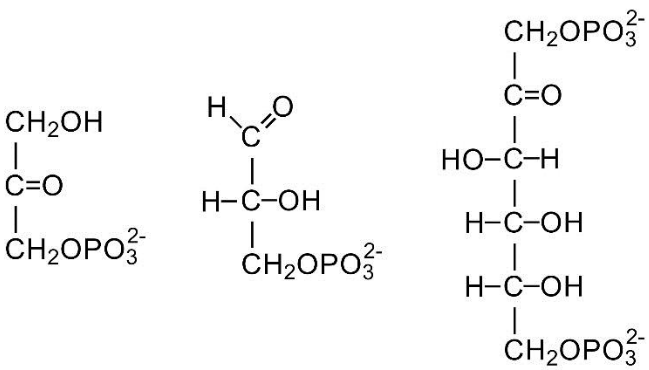
SECTION B

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. Explain how the Bohr radius and the van der Waals radius are two different ways to describe the size of an atom.

12. (a) Using diagrams explain what is meant by S_N1 and S_N2 reactions. (40%)

    (b) During gluconeogenesis, dihydroxyacetone phosphate (I) reacts with glyceraldehyde-3-phosphate (II) in an aldol reaction to produce the linear isomer of fructose 1,6-bisphosphate (III). Using curly arrows, show and explain the mechanism of this enzyme catalysed reaction. (60%)



I II III

13. The normal or resting concentration of Ca^{2+} in the cytoplasm of a liver cell is 0.1 μM. The concentration of Ca^{2+} outside the cell is constant at 2 mM.

(a) The normal concentration gradient is maintained by a Ca^{2+}-pumping ATPase (active transport). Assuming that the ΔG for hydrolysis of ATP to ADP is -54 kJ/mol, calculate how many Ca^{2+} can be pumped out of the cell for each ATP hydrolysed. (25%)

(b) Ca^{2+} in the cytoplasm binds to a protein kinase. Ca^{2+} is an allosteric activator of the kinase, which is inactive when no Ca^{2+} is bound to it. Discuss what type of reaction the kinase performs and what is meant by allosteric activation. (25%)

(c) Protein kinase activity is measured by incubating the kinase with a protein substrate for 15 min at 37 ºC and then determining how much product is produced. Use the data below to draw a graph showing the activity of the kinase, expressed in nmole product/min, as a function of Ca^{2+} concentration. (25%)

Ca^{2+} concentration (μM)	Kinase activity (nmole product)
0.0	0
0.1	46
0.2	73
0.4	106
0.8	133
1.2	148
1.6	156
2.0	160
2.4	163
2.8	166

(d) From your graph, determine the dissociation constant (K_D) for binding of Ca^{2+} to the kinase (15%). Show how you determined this value.

(e) In response to certain hormones, a channel in the plasma membrane temporarily opens to allow Ca^{2+} to enter the cell until the concentration reaches 10 μM in the cytoplasm. Estimate the approximate percentage of the maximum possible kinase activity that would be achieved at this level of Ca^{2+}. (10%)

14. Describe ***two*** of the following (50% each):

(a) The reaction catalyzed by acetyl CoA carboxylase and how this enzyme is regulated to control the rate of fatty acid synthesis.

(b) How fructose 2,6 bisphosphate is produced and the mechanisms by which it regulates the rates of gluconeogenesis and glycolysis.

(c) The reactions catalyzed by aminotransferases and glutamate dehydrogenase and their roles in metabolism of amino acids.

15. In the final step of the oxidative phosphorylation pathway, reduced cytochrome c (Cyt c_{red}) reduces molecular oxygen to water. The overall reaction is:

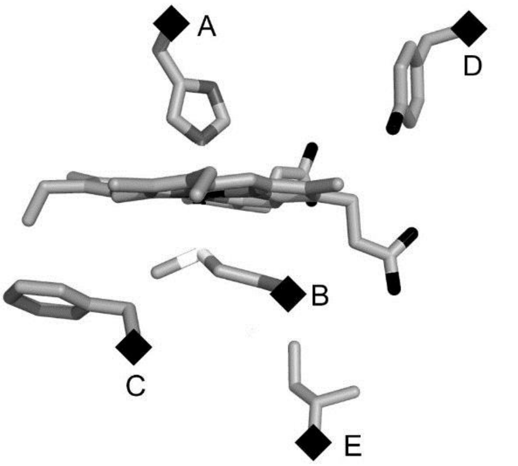
$$4 \text{ Cyt } c_{red} + O_2 \rightarrow 4 \text{ Cyt } c_{ox} + 2H_2O$$

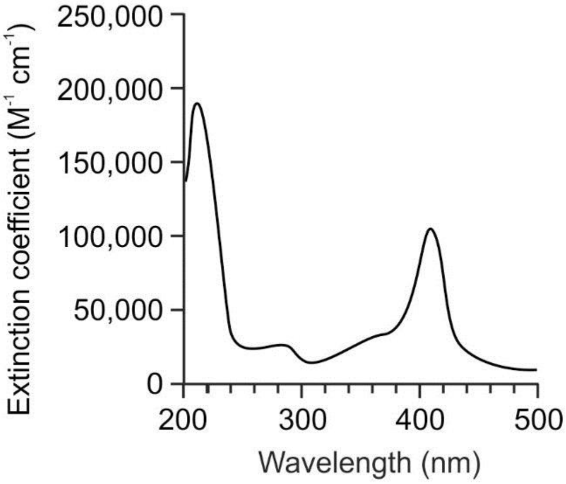
(a) During this process, 8 protons are transferred across the membrane. Given the following information about the half reactions involved, determine the free energy change under standard conditions that is made available for each proton transfer (25%):

Cyt c_{ox} + e- → Cyt c_{red} $\mathcal{E}^o = 0.22$ V

$1/2\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$ $\mathcal{E}^o = 0.82$ V

The molecular weight of cytochrome c is 12,500 Da. The structure of cytochrome c near the haem group and the absorbance spectrum are shown below:





(b) Identify the amino acid side chains indicated by A, B, C, D and E. The diamonds indicate the positions of the α-carbons and only the heavy atoms C, N and O in the side chains are shown, without H atoms. (20%)

(c) The structure of cytochrome c is shown at pH 7.5. At pH 5.5 the structure changes significantly. From the information shown in the structure, discuss why the properties of cytochrome c would be sensitive to pH over this range. (15%)

(d) The absorbance at 410 nm is due to the haem group and gives cytochrome c its red colour. Indicate the likely sources of the absorbance at 280 nm and explain your reasoning. (15%)

(e) From your experience with the spectrophotometers in the practical laboratories, estimate the lowest absorbance value that you could reliably detect with at least ±10% accuracy. Based on this value, calculate the minimal concentration of cytochrome c, expressed in mg/ml, that you would be able to detect by reading the absorbance of a solution at 410 nm. (25%)

16. (a) Summarise how each of the three complexes (Complex I, III and IV) in the electron transport chain move protons between the matrix and the intermembrane space. (75%)

(b) Describe two ways by which electrons are transported between the complexes. (25%)

17. Explain the biochemical basis for the following observations about inherited human diseases that result from deficiencies in enzymes involved in carbohydrate metabolism.

(a) Symptoms of phosphorylase kinase deficiency include an enlarged liver and hypoglycaemia (low blood sugar). (35%)

(b) Low levels of glycogen branching enzyme result in glycogen with an abnormal structure. When this glycogen is degraded, the products, glucose 1-phosphate and free glucose, are at a ratio of 100:1 compared to a ratio of 10:1 when normal glycogen is degraded. (35%)

(c) In individuals with fructose-1,6-bisphosphatase deficiency, blood glucose levels are normal at the beginning of a period without eating but very low after 24 hours without food. (30%)

18. The folding of hexokinase from its unfolded state has a ΔG of -55 kJ/mole and ΔH of -25 kJ/mole.

(a) A solution of hexokinase in a beaker can be described as a closed system. Indicate what this means and calculate the ratio of native to denatured hexokinase at equilibrium in the beaker. (20%)

(b) For a closed system, heat entering or leaving the system results in an increase or decrease in enthalpy of the system. Indicate whether the folding reaction is endothermic or exothermic. Describe what forms the enthalpy of the system takes when the system absorbs or releases heat. (15%)

(c) Calculate the entropy change, ΔS, for folding of hexokinase. Discuss whether the entropy change is favourable and what features of the folded protein might result in the ΔS value that you have found. (25%)

(d) Explain how the terms 'random coils' and 'turns' are used to describe characteristics of the denatured and native forms of hexokinase. (20%)

(e) In the denatured state, if one of the peptide bonds is in the *cis* configuration it must usually be converted to the *trans* configuration during the folding process. Indicate the energy barrier for this reaction, explaining your reasoning. (20%)

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:	$pH = pK_a + \log([unprotonated]/[protonated])$
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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