Chemistry 2740 Spring 2011 Test 2

Time: 50 minutes
Marks: 32
Aids allowed: calculator, 8.5×11-inch formula sheet
Useful data:
$R = 8.314\,472\,\mathrm{J\,K^{-1}mol^{-1}}$
To convert degrees Celsius to Kelvin, add 273.15.
Instructions: You can answer the questions in any order, but make sure that you clearly label each of your answers with the question number in your exam booklet(s).

1. Name one practical consequence of the third law of thermodynamics. [2 marks]

2. Laboratory freezers used to keep biological samples operate at much lower temperatures than household freezers. One particular freezer keeps a temperature of $-86\,^\circ\mathrm{C}$ in a room at $20\,^\circ\mathrm{C}$ while consuming $31\,\mathrm{MJ/day}$ of electricity.

   (a) Assuming an ideal refrigerator, how much heat would be expelled into the room per day? [6 marks]

   (b) How will the heat expelled into the room by the real freezer differ from the figure calculated above? Why? What is the underlying principle? [3 marks]

   Note: The electricity usage figure given above is the actual figure for a real freezer as measured by the manufacturer.

3. Haemoglobin (Hb) is the major oxygen carrier in the blood of vertebrates. It is made up of two α and two β subunits (i.e. two copies of each of two different kinds of metalloproteins). Each of the four subunits can carry one oxygen molecule. The equilibrium constants for binding of oxygen to human haemoglobin at 21.5 °C and pH 7 are as follows:[1]

$$
\begin{array}{rcll}
\mathrm{Hb}_{(\mathrm{aq})} + \mathrm{O}_{2(\mathrm{aq})} & \rightleftharpoons & \mathrm{HbO}_{2(\mathrm{aq})} & K_1 = 9.32 \times 10^3 \\
\mathrm{HbO}_{2(\mathrm{aq})} + \mathrm{O}_{2(\mathrm{aq})} & \rightleftharpoons & \mathrm{Hb(O_2)}_{2(\mathrm{aq})} & K_2 = 2.10 \times 10^5 \\
\mathrm{Hb(O_2)}_{2(\mathrm{aq})} + \mathrm{O}_{2(\mathrm{aq})} & \rightleftharpoons & \mathrm{Hb(O_2)}_{3(\mathrm{aq})} & K_3 = 9.07 \times 10^3 \\
\mathrm{Hb(O_2)}_{3(\mathrm{aq})} + \mathrm{O}_{2(\mathrm{aq})} & \rightleftharpoons & \mathrm{Hb(O_2)}_{4(\mathrm{aq})} & K_4 = 6.60 \times 10^5
\end{array}
$$

(a) Calculate the standard free energy of reaction for the first binding step at 21.5 °C. [2 marks]

(b) From experiments carried out with purified subunits under conditions where they do not associate, it is known that the equilibrium constants for binding of oxygen to isolated α and β subunits are both similar to K_1 and K_3. If the subunits did not interact with each other, all four equilibrium constants for binding of oxygen to haemoglobin would also be similar to K_1 and K_3. The larger equilibrium constants in steps 2 and 4 are examples of **cooperativity**, where binding of a ligand (in this case, oxygen) to a multisubunit protein alters the affinity of the protein for the ligand at additional binding sites.

i. What is the free energy change for the overall reaction for the first two steps, $\mathrm{Hb}_{(\mathrm{aq})} + 2\mathrm{O}_{2(\mathrm{aq})} \rightleftharpoons \mathrm{Hb(O_2)}_{2(\mathrm{aq})}$? Call this quantity $\Delta_{r(i)}G^\circ_m$. [4 marks]

ii. Suppose that there was no cooperativity, i.e. that the equilibrium constant for binding oxygen was the same in step 2 as in step 1. What would the free energy change be for the overall reaction $\mathrm{Hb}_{(\mathrm{aq})} + 2\mathrm{O}_{2(\mathrm{aq})} \rightleftharpoons \mathrm{Hb(O_2)}_{2(\mathrm{aq})}$ in this case? Call this quantity $\Delta_{r(ii)}G^\circ_m$. [2 marks]

iii. The difference between these two values, i.e. $\Delta_{r(i)}G^\circ_m - \Delta_{r(ii)}G^\circ_m$, is the free energy of cooperativity, i.e. the free energy decrease due to the cooperative binding. Calculate this quantity. [2 marks]

(c) Now consider a solution containing haemoglobin in which there is a large excess of oxygen at a concentration of 300 μmol/L. Using the equilibrium relationships, show that almost all of the haemoglobin will be in the fully oxygenated form ($\mathrm{Hb(O_2)}_{4(\mathrm{aq})}$) under these conditions. [8 marks]

Note: While you need to do a few calculations, you don't have to be precise about the expected fraction that will be in the fully oxygenated form, provided you explain clearly how the numbers indicate that this form will be strongly dominant.

(d) Would the fully oxygenated form still be overwhelmingly dominant if all the equilibrium constants were similar to K_1 and K_3, i.e. if there was no cooperativity? Explain briefly. [3 marks]

[1] Q. H. Gibson, *J. Biol. Chem.* **245**, 3285 (1970).