CHEM-342 Introduction to Biochemistry
Mid-term Examination - Group Part
Friday, 26 March 2010
H. B. White - Instructor
25 Points

Group Members ____________________________

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Group Average 20.0, Range 13-23, N = 7

Important - Please read this before you turn the page.

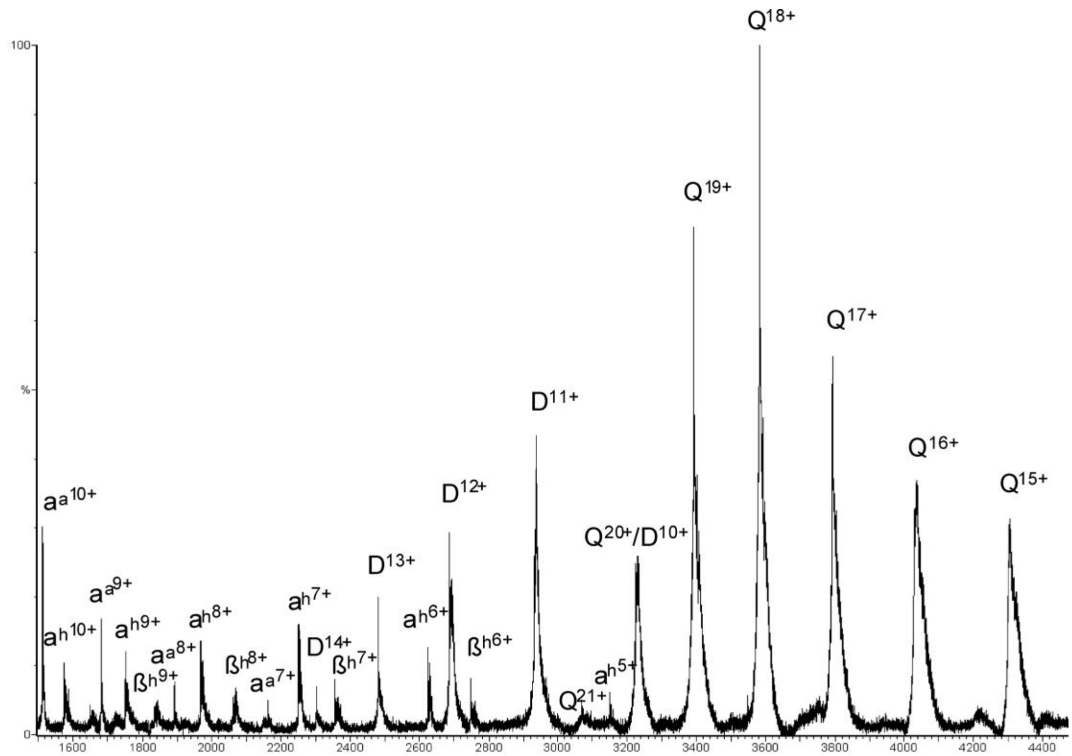
1. Write your names or group number on each page of the exam you turn in.
2. This part of the examination is closed book and closed notes.
3. Please read each question carefully and make sure that you have thought it through with **everyone's** input **before** converging on a solution.
4. If you do not agree with your group, you may submit the examination under your own name for separate grading.

1. (10 Points) Based on the presumption that hemoglobin contains a single iron atom, Zinoffsky (1886) calculated an empirical formula for hemoglobin which corresponds to a molecular weight of ~16,700 Da. Forty years later, Svedberg and Fåhraeus (1926) used equilibrium ultracentrifugation to determine a molecular weight of ~66,800Da. Subsequently in the 1960's the complete amino acid sequences of the α and β subunits of hemoglobin were determined from which a precise molecular weight could be calculated. Now, yet another 40 years later, it is possible to directly determine the molecular weight of hemoglobin by mass spectrometry. The figure on the next page [*J. Am. Soc. Mass Spectrometry* **30**, 625-631 (2009)] displays the mass spectra obtained for human normal (HbA) at the top and sickle cell hemoglobin (HbS) at the bottom. The molecular weights of 64,454.7Da for HbA and 64,395.8Da for HbS are respectively within 1.5 and 2.4 mass units of the theoretical masses. The peaks correspond to the mass/charge ratio, e.g. Q^{18+} for HbA = 64,454/18 = 3581.

   a. Although one can calculate the average molecular weight for hemoglobin from these data, the broadness of the peaks indicates that there is a distribution of molecular weights. Thus, although HbS is theoretically 60 atomic mass units smaller than HbA, there are some molecules of HbS that are larger than some molecules of HbA. How is this possible?

   b. From these data, approximately what are the molecular weights of the α and β subunits of human hemoglobin? Present your reasoning.

   c. Although some dissociation into dimers (D^{13+}, D^{12+}, D^{11+}, etc.), subunits (e.g α^{h10+}), and loss of heme (e.g. α^{a10+}) can be detected by this method, the noncovalent association of hemoglobin subunits as a $\alpha_2\beta_2$ tetramer is remarkably stable as positively charged gaseous ions (Q^{19+}, Q^{18+}, Q^{17+}, etc.). From these data, generate a model/diagram depicting the sequence of steps in which the hemoglobin tetramer would generate the α subunit lacking its heme group.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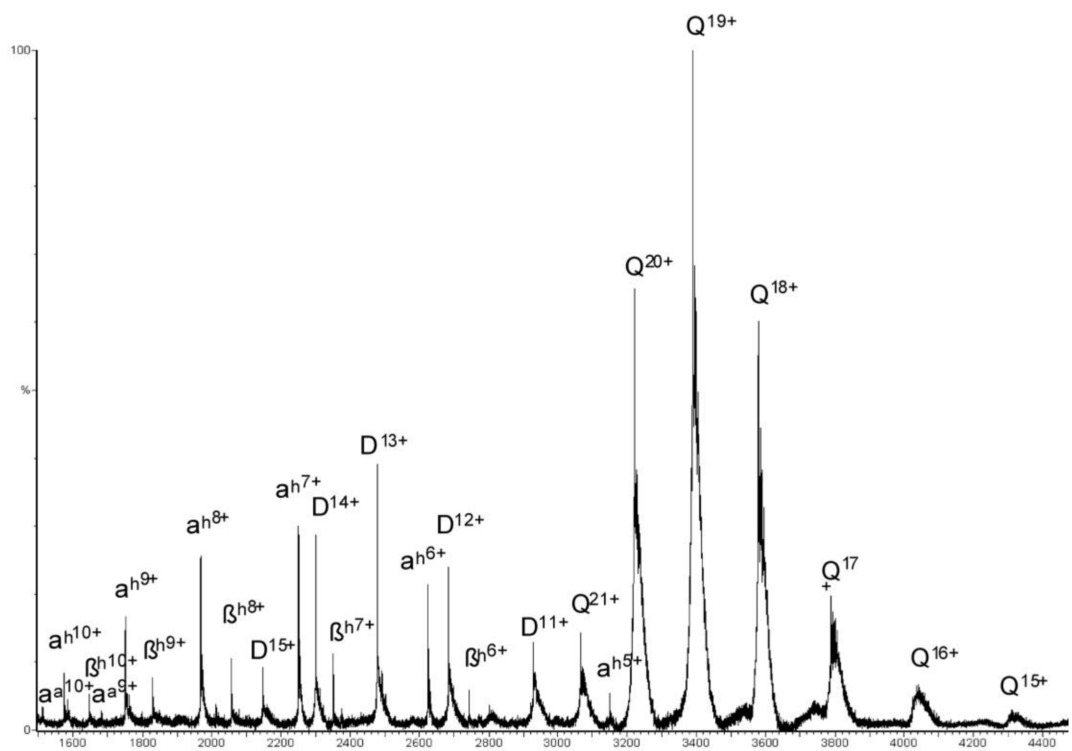
(a)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(b)

Complete the Concept Map (15 Points) Most of the terms have been removed from the concept map below. Based on the linking words associated with the arrows, fill in the missing terms in the boxes provided