Chemistry 5.07
Problem Set 2 (weak non-covalent interactions and protein primary structure)

Problem on primary structure:
1. Proteins are often associated with membranes by attachment of fatty acids such as palmitate ($CH_3(CH_2)_{14}CO_2^-$) to their amino terminus in a post-translational modification step. A group of investigators was interested in a peripheral membrane protein (a protein weakly associated with the membrane) that plays an important role in a signaling pathway inside the cell. The molecular weight of the protein was determined to be 35 kDa by electrospray ionization mass spectrometry (ESI MS) and in an effort to obtain some sequence information **the protein was initially cleaved with the protease trypsin**. [Trypsin is a protease that cleaves adjacent to positively charged amino acids, see your text). The resulting peptides were separated and isolated by HPLC (high pressure liquid chromatography) and one of these peptides was investigated in detail. You are assigned the task of determining the primary sequence of this peptide given the following pieces of information.

a. Total acid hydrolysis of this peptide in 6N HCl, subsequent to reduction of the peptide with dithiothreitol, resulted in the following amino acid composition:
   2 F, M, A, V, 2 K, S, 2 C and D
   Recall that some amino acids are destroyed during this process and may not be detectable.

b. One round of Edman sequencing gave V.

c. The intact peptide was reduced with dithiothreitol and then treated with the protease called chymotrypsin. Chymotrypsin cleaves peptide bonds after aromatic amino acids or of **very** large hydrophobic amino acids. Five peptides were isolated subsequent to this cleavage and the amino acid composition of each was determined: (W, V); (C, F); (D, K); (M, A, K, C) and a fifth peptide which upon total acid hydrolysis gave S, F and a third product that is not an amino acid. This third product is hydrophobic can be extracted into ether if the solution is acidified to pH2 and upon examination by MALDI TOF mass spectrometric analysis it was shown to have a molecular weight of 256 Da.

d. The intact peptide was also cleaved with the chemical reagent cyanogen bromide (CNBr). This reagent cleaves adjacent to methionines and leaves an unusual amino acid (a homoserine lactone). Initially the intact peptide was reduced with dithiothreitol and then treated with CNBr. HPLC analysis of the products revealed three peptides which could be separated. The amino acid composition of each determined:
   (A, K); (W, F, V, D, K, C); and (F, S, C, and an unnatural amino acid)

Questions:
i. To obtain the peptide of interest, the intact protein initially was cleaved with the protease trypsin. What is the key characteristic common to each of these peptides? Why? To answer this question look at the chemistry of trypsin.

ii. Provide several reasons why the investigators might have decided to treat their HPLC purified peptide with dithiothreitol? Show the chemistry with structures. Why is this reagent important in determination of a peptide sequence?

iii. In part d above in which three peptides were isolated using HPLC, how would you identify the following two peptides [(W, F, V, D, K, C); and (F, S, C, and an unnatural amino acid)] to know which fractions to collect during the elution process for further analysis? How would you identify the third peptide (A, K) ?

iv. Using the information in a-d and the information in the introduction to the problem, draw the chemical structure of the primary sequence of this isolated peptide and underneath each residue put the one letter amino acid code. **Show how each piece of information gave you insight that led to the final structure.** This analysis (telling us what you learned from the information provide in a through d etc) and the role of trypsin is critical in case you get the incorrect structure. It will help you understand where your analysis had problems.

2. Problem on weak non-covalent interactions: OMP decarboxylase is a protein catalyst, enzyme, that catalyzes the conversion of OMP to UMP shown below. To obtain a structure of this enzyme it was co-crystallized with 6-azaUMP

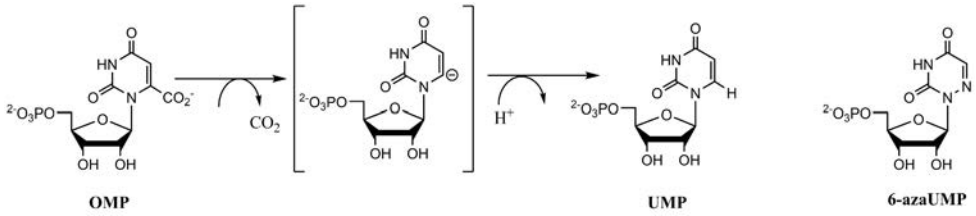
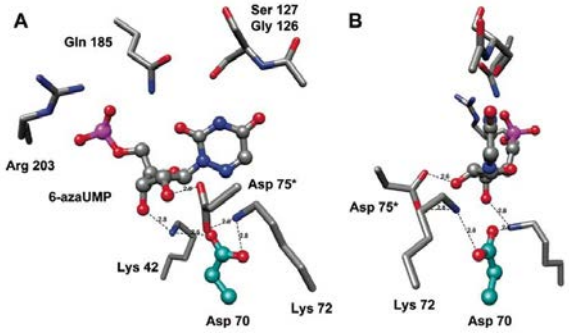


Figure 1. Structure of OMP decarboxylase with 6-azaUMP bound in the active site. A and B are the same structure examined from a different perspective. Note in 6-azaUMP there is NO carboxylate at C-6 as is observed in OMP. Thus to imagine the interactions of OMP to the active site using this inhibited structure, you need to add in a carboxylate at C-6. In most programs available to look at structures (ex PYMOL), on can easily add in this group, energy minimize and look at the interactions.



Questions:

i. Redraw the Figure in A putting in all the appropriate charges. What are the pKas of the side chains of the amino acids that you have drawn in your figure?

ii. Weak non-covalent interactions within macromolecules and between small molecules and macromolecules play an essential role in structure and function. Show with structure two types of weak non-covalent interactions in the structure.
iii. From a chemical perspective, using your drawing in part ii, what is unusual about the amino acid side chains that are adjacent to the site where the chemistry occurs (Eq. 1)?

3. Problem on cooperativity

2, 3Bisphosphoglycerate (BPG, Fig. 2) is present in red blood cells at 4 mM concentrations, about the same concentration as hemoglobin (Hb, 2 mM). Without BPG, Hb is an extremely inefficient O_2 transporter, releasing only about 8% of its cargo to the tissues (Fig. 3). A structure of deoxyHb bound to BPG is shown in Fig. 4. BPG does not bind to oxyHb.

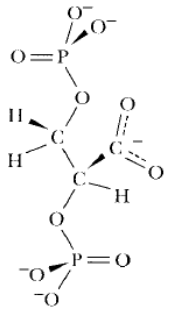


Fig. 2 BPG

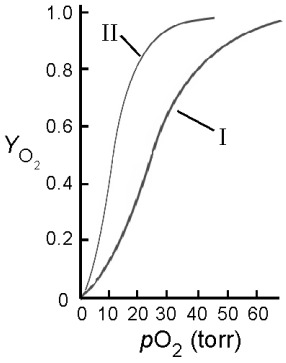


Fig. 3 Purified Hb and Hb in whole blood

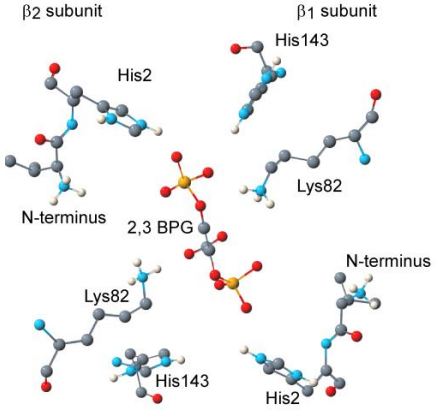


Fig. 4 BPG bound to deoxyHb

The globin gene in fetuses differs from adult Hb in that it has two α and two γ chains. Recall that adult Hb has two α and two β chains. The fetal γ chain is thought to have arisen from the β chain via gene duplication and mutation. Noteworthy is the substitution of His143 in the β chain with a serine in the γ chain (see Fig. 4).

Questions:

i. Given the information above, label purified Hb (purified implies the small molecules have been removed from the protein) and Hb in whole blood in Fig. 3. Describe the basis for your labeling assignment.

ii. From Fig. 4 what is (are) the major type of weak non-covalent forces that allow tight binding of BPG to deoxyHb? Clearly show all the charges in Fig. 4.

iii. The binding curves for O_2 to adult and fetal Hb are shown in Fig. 5.

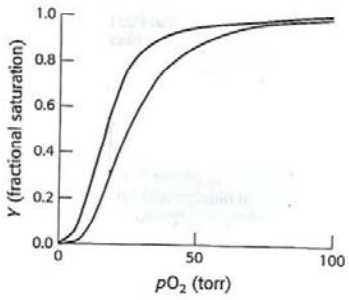


Fig. 5

Label the curve associated with HbF (fetal) and HbA (adult). Explain in one sentence the basis for your choice of labeling. Explain why the mutation of His143 to a Ser, could have the observed effect.

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