

BIOC 463A
2007-2008

Alkaline Phosphatase Structure Homework Exercise

In this exercise you will be required to go to the ExPaSY and Protein Data Bank websites to increase your understanding of the structure and function of alkaline phosphatase (AP). These websites can be accessed via the Mass Spec.html link on BIOC 463A homepage. *The results from this exercise will be handed in as homework AND should be included in an overall description of the enzyme, either in the Introduction or Discussion portion of your final manuscript.*

WARNING: DO NOT PROCRASTINATE ON BEGINNING TO DO THIS ASSIGNMENT. NOT BEING ABLE TO GET A GRAPHICS PROGRAM TO DOWNLOAD AND WORK PROPERLY ON YOUR HOME PC AT THE LAST MINUTE IS NOT A VALID EXCUSE FOR NOT DOING THIS PROBLEM SET.

1. Go to the ExPaSY website and determine the calculated molecular weight and pI for AP. Remember that AP is expressed with a signal peptide that is cleaved once the enzyme passes through the periplasmic membrane. (Note: this is a repeat of what we did in the Mass Spec/Proteomics lab exercise). How does this molecular weight compare with the SDS-PAGE and actual mass spectrometry data. Also determine the pI for the mature protein – this is related to the choice of the ion exchange column used to purify the enzyme.
2. Search the Protein Data Bank for two 1.75 Å structures (+ and – inorganic phosphate) of alkaline phosphatase published by Stec et. al. in 2000, that will be examined in this exercise. In order to visualize the protein AND answer the following questions, it will be necessary to use one of the following programs: RasMol, First Glance (uses Chime), Protein Explorer (uses Chime), or DeepView (Swiss PDB Viewer). Use whatever program you are most comfortable with and has been downloaded onto your computer.
3. Describe the overall shape of the protein in the homo-dimer (sphere, oblate spheroid, square bi-pyramidal, etc.) Rotate the molecule so you can locate both active sites, and describe the geometry of the surface(s) of the molecule in the region surrounding the active sites. (Hint: it might be best to switch to a CPK representation of the AP in order to visualize this).
4. Locate the metal atoms at the active site and describe their relative degrees of solvent accessibility. Again, a CPK representation is best to use here. Describe the binding of inorganic phosphate to the active site metals – To which metal is it bound? Is inorganic phosphate buried in the interior of the protein, or exposed to the solvent?
5. Taking into account #3 and #4, discuss how the structure of the enzyme is related to the specificity (or lack thereof) that the enzyme has for the “R” group of phosphorylated substrates (ie. ROPO_3^{2-}) as described in the

- Garen and Levinthal and Coleman papers. Justify your discussion based on the specific data presented in those papers.
6. Calculate the distance between the three metals at the active site of AP in the presence and absence of inorganic phosphate. If the distances change with phosphate binding (or does not change) what does this tell you about changes (or lack thereof) in the conformation of the enzyme upon binding of the substrate.
 7. Display the side chain of Ser102 in both the $-P_i$ and $+P_i$ structures. In the $-P_i$ structure, the side chain is shown in a single conformation, whereas in the $+P_i$ structure, the side chain is shown in two orientations. Postulate an explanation for the differences in the orientation of the Ser102 side chain in these two structures. Locate the Ser102 side chain O atom (listed as the gamma O or OG in PDB nomenclature) in the $-P_i$ structure. How is it oriented with respect to the two Zn and the Mg atoms? Determine the distance from the Ser102 OG and the Mg. How is the relatively large distance between the Ser102 OG and the Mg accounted for in the postulated involvement of the Mg in the catalytic mechanism suggested by Stec. et. al.?
 8. The reason why enzymes catalyze reactions faster than the non-catalyzed reaction is because the enzyme can stabilize the transition state as the substrate is converted to product. As Ser 102 attacked the P atom, an important amino acid is critically located to assist in transition state stabilization. What is that amino acid and what precise role does it play?
 9. To answer the following question, it is best to switch to a ribbon (cartoon in Chime) representation of the enzyme. The majority of the enzyme consists of alpha helices and beta strands arranged in a very specific folding motif. Describe the motif. The result of this folding motif is the formation of a beta-sheet that runs through the interior of the protein. Are the beta strands parallel or anti-parallel? How is the orientation of the strands related to the folding motif? Based on the location of the beta sheet, what would be the chemical nature of most of the amino acid side chains comprising this secondary structure?
 10. When you performed the native PAGE gel experiment, you ran two enzymes on the same gel, beta-galactosidase and AP. Students that assayed for beta-galactosidase first using a buffer that contained BME, saw no activity for AP when they assayed for that enzyme. BME is known to reduce disulfide bonds within the enzymes. Are there any disulfide bonds within AP and how might their reduction by BME be related to the loss of enzymatic activity by AP?