

BIOC 463A 2007-2008

Alkaline Phosphatase Mass Spectrometry Data

A. Mass Spectrometry Results.

The following is a description of the analysis of the mass spectrometry data and questions that you should consider in discussing this data in your lab report. This will take some time, but hopefully it will be an enriching intellectual process that I know you will all enjoy.

The data obtained from the Mass Spectrometry Facility for analysis of the Stage 4 enzyme will be handed out in class. The mass spectrometry spectra, as well as a discussion of its significance is to be included in your write-up as part of the biophysical characterization of the enzyme that you have purified.

The technique that was used to obtain these spectra was HPLC-ESI mass spectrometry. In other words, the sample was first run through an HPLC column that separates the proteins according to size (this corresponds to the top left hand box in the figure). The numbers in this chromatograph correspond to the time required for the fraction to come off the column. Each fraction is directly fed into the ESI (electro-spray ionization) mass spectrometer, where the mass of the protein is determined. The figure in the bottom right corresponds to the mass spectrum of the sample emitting from the HPLC column at 18.17 minutes.

There are two obvious peaks in the mass spectrum. Calculate the mass difference between the two peaks. This mass difference may correspond to two forms of the enzyme, in which there has either been an amino acid substitution, a deletion, or an insertion in one of the proteins, relative to the other. A table of Mutation Mass Shifts (with the reference for the website address) has also been included that should be helpful in determining the cause of the difference in masses.

Once you have determined what is the most likely cause of the mass difference, we want to examine if there is some literature verification for the two forms of protein being present in the Stage 4 enzyme solution. In order to determine this, go to the ExPASy website (you can access this through the Mass Spec.html page), and enter Alkaline Phosphatase E. coli. As you have done in the past, recalculate the mass of the mature protein (minus the signal peptide). Check through all of the listings for different isomers of alkaline phosphatase that might account for this mass difference. If you find an isomeric form of the enzyme, determine which amino acid has been mutated, deleted, or inserted. Based on the physical location of this difference in the proteins, would you expect to see a difference in the enzyme activity of the two forms of AP?). In other words, would this mutation be expected to have any effect on enzyme activity (i.e., is this residue involved in either coordination of the metals in AP or is involved in the catalytic mechanism)? (This question might be easier to answer once you have read the Stec et al. (2000) manuscript and answered the Structure homework). A recent paper by Kajava et al. (2002, J. Biological Chemistry, **277**, 50396-50402) discusses the amino acid sequence specificity for the enzyme that AP binds to in the cell membrane, signal peptidase (SPase), and

subsequently carries out two functions. This enzyme, like most prokaryotic and eukaryotic protein translocases, carries out two functions. Transport of the precursor protein across the cell membrane with a concomitant hydrolysis of signal peptide, yielding the mature protein that diffuses into the periplasmic space. One very important step in this entire process involves initial interaction of the signal peptide with the cell membrane. Most phospholipids on the inner envelope of the membrane (i.e. cytoplasmic side) contain negatively charged head groups. Assuming the interaction of the precursor protein with the membrane is electrostatic, what type of amino acids would you expect to find in abundance in the signal peptide to facilitate this interaction? Based on the sequence given for AP in ExPASy, is your hypothesis correct? Finally, based on the mass spectrometry data, briefly discuss the "fidelity" of cleavage of the signal peptide by SPase (i.e. why do you see two forms of AP in the periplasmic space).

Also, in your write-up, you obviously will want to compare the molecular mass or weight determinations from mass spectrometry with that obtained from the SDS-PAGE gel you ran as well as the mass for the dimeric enzyme given in Garen and Levinthal.

Remember, as this material is included in your write-up, the appropriate literature citations need to be made!!!