

Mass Spec and Proteomics Homework

The purpose of this homework is to refresh our memories about some of the websites visited and the useful information that can be obtained from these websites. Have fun with this problem set, you can point and click yourself into cyber bliss, don't feel constrained to the instructions given below! Answers will be turned in next Tuesday.

1. Using your class exercises for background information, return to the ExPASy website and search for *E. coli* alkaline phosphatase (AP) again:
 - (a) What is the gene name for this protein?
 - (b) AP exists within the cytoplasm of *E. coli* as a precursor protein that contains a signal peptide. The purpose of the signal peptide is to direct the protein to the cytoplasmic membrane where it binds to an enzyme called a peptide translocase that also carries out proteolytic cleavage of the signal peptide from the precursor protein as the protein is passed through the cytoplasmic membrane. The first amino acid in the mature translocated AP is what amino acid? Based on this identity, this translocase has a catalytic specificity similar to what common protease (consult Lehninger for proteases and which amino acids they cleave next to)?
 - (c) Calculate the molecular weight of the monomeric form of the mature protein as well as its pI. Remember that in class you were shown a MALDI-TOF spectrum of AP obtained using purified AP from Sigma Chemical and you examined the similarity of the experimentally determined mass from the mass calculated by ExPASy. How closely do these masses compare to the mass you determined for AP from the SDS-PAGE gel you ran a couple weeks ago? If there is a significant difference, offer a brief explanation for your observation (and NO, the gel apparatus was not defective!). Hint: sometimes when using broad spectrum molecular weight markers, the two highest molecular weight species do not resolve themselves.
 - (d) Later in the semester you will be purifying AP from *E. coli*. If you wanted to purify the protein in a pH 7.4 buffer and taking into account the pI from ExPASy which ion exchange resin, CMC or DEAE, would be better to use and why?
 - (e) From the information given in ExPASy, determine the number of disulfide bonds within each AP monomer, making note of the cysteine residues that are involved in these disulfide bonds. In the purification that you will carry out for AP, the second step in the procedure is called a heat denaturation step, in which many proteins, but not AP, are denatured by taking the cell lysate up to 85° C then rapidly bringing the temperature back to 4° C in an ice bath. What role do you think the disulfide bonds play in preventing irreversible thermal denaturation of AP?
 - (f) In this next exercise we will perform an *in silico* digest of AP using the FASTA amino acid sequence you can cut from ExPASy and a program available from Protein Prospector's **MS-Digest**.
 - Copy the one letter amino acid sequence for AP from ExPASy for the mature protein. Paste this sequence into **MS-Digest** in Protein Prospector. Set up a digestion using your favorite protease (remember you can set the number of missed cuts). How many peptides were obtained by this *in silico* digest? How does that compare to the maximum number of theoretical number using the protease you

Bioc 463a; Fall 2009

chosed? Each peptide should begin with the same amino acid (or type of amino acid) which is specific for the protease.

- Now using the masses of the peptides obtained from **MS-Digest**, enter them into **MS-Fit**, carefully set up the search parameters, and perform searches of the NCBI and Swiss Prot data bases. One important parameter for performing this search is specifying the organism. You can either search all organisms or you can specify E. coli for AP. There can be advantages to doing both types of searches. For your AP data, do you get the same matches by limiting your search to E. coli compared to a search of all organisms? List the top three matches for the ALL organism search and their respective MOWSE scores.

In case you wondering, will you be doing any mass spec work in this class, the answer is someone will! At the end of the purification we will determine whose sample has the highest purity and activity. That sample will be processed for mass spec analysis, the details of which are being worked out now with Dr. Brechi.