

Bioc 463A
2010-2011

Cytochrome c and Hb ExPASy Assignment

In order to prepare for Tuesday's experiments, it will be necessary to visit a very informative proteomics-based website, ExPASy (ca.expasy.org) an acronym for Expert Protein Analysis System. This website, which contains a wealth of information, was originally designed to be utilized by the proteomics community, but is of tremendous use to the general protein biochemistry audience as well. In this exercise, we will search ExPASy to obtain three useful pieces of information (molecular weight, pI, and the extinction coefficients at 280 nm) for two proteins, bovine hemoglobin (Hb) and bovine cytochrome c (cyt c), that we will be purifying by size exclusion (Sephadex G75) and ion exchange chromatography (CMC). The former two parameters should provide information that will enable us to predict differential behavior of these proteins on these two forms of column chromatography. The extinction coefficient at 280 nm, along with the general visible spectral properties of the two proteins which you saw in the last experiment, will help us identify the proteins as they elute off

Using the above link, go to the ExPASy website. The first thing to do is **choose a data base** that you will search in this exercise. Protein databases contain the amino acid sequences (and genetic information) for huge numbers of proteins. The database we will search is **UniProtKB** (also known as Swiss-Prot and TrEMBL). In the query box type "**bovine cytochrome c**", this will open a second page containing a list of proteins with "cytochrome c" in their names. Cruise through the list until you find the entry for **bovine cytochrome c**. Click on the box and then click on the accession number **P62894**. On the next page, there is a lot of general information about the protein. You can scroll down through this until you get to the "**Sequences**" data. In the **Tools** box, choose "**Prot Param**" which takes you to a page where you must specify which sequence of amino acids you want to assess. Choose "**chain 2 – 105**". This will open the **ProtParam** page that will give you the amino acid sequence, composition, molecular weight for that peptide, and the calculated pI. Scrolling down, you will also see the amino acid composition from which has been determined the molar and 0.1% extinction (i.e. the mg/mL) coefficients for cytochrome c. Scrolling further down you will discover the algorithm used to calculate these values. Look familiar?

Repeat this process for bovine hemoglobin, however now you must choose individually the alpha (**P01966**) and beta (**P02070**) chains. Remembering that hemoglobin is a tetrameric protein (2 alpha chains, 2 beta chains), you should be

able to accurately calculate the tetrameric molecular weight, and reasonably determine a pI for the tetramer. Also make note of the extinction coefficients for both peptides. One point that needs to be addressed is conventions for proteins containing more than one subunit. Typically, extinction coefficients are expressed on a **per peptide** (A_{280} data) or a **per heme** basis (for the heme spectral properties). **Rarely**, if ever, are these parameters expressed on a per tetramer (in the case of hemoglobin) basis!

Armed with this information, you should now be able to reasonably predict which protein should elute sooner from the Sephadex G75 size exclusion column we will be using initially as well as the behavior to the two proteins on the CMC ion exchange column that will be necessary to further purify the two proteins. Specifically, should both proteins bind to the CMC column at pH 6.0 in 10 mM phosphate buffer? In order to answer this question, determine the NET charge on both hemoglobin and cytochrome c at pH 6.0. Which protein should dissociate faster from the ion exchange column if the pH of the buffer is increased by a linear gradient from pH 6 to pH 8?

As a final exercise, return to ExPASy and obtain the same information for bovine serum albumin (BSA), the protein standard we used in colorimetric reactions. Are the molar and 0.1% extinction coefficients similar to the ones listed in Table 5 of Pace et. al.? Make note of the molecular weight and pI of BSA as well. This might come in handy in interpretation of the column chromatography data! For the sake of argument, if this protein was loaded onto the same CMC column at pH 6.0, would you expect BSA to bind to the resin or simply pass through?