Production of HLA-B27

Cell Line:

619F200 is the name Malini gave to the B27 CHO cell line. These cells grow in αMEM, 5% DFCS, 200μM MSX, and penicillin/streptomycin. I typically thaw one vial of frozen cells, grow them to confluency, and split them several times to a total of 50 plates (10 ml of media/plate). Plates are harvested every other day. For some strange reason, this cell line produced higher quantities on plates as opposed to the Cell Pharm. To the harvest I add azide to a final concentration of 0.05% azide and EDTA to 1mM final concentration, and then filter the harvest through a 0.45 μm nalgene filter. Harvests are stored in the coldroom until they can be purified.

Purification:

The harvests are run over the W6/32 columns located in the coldroom. Currently, there are two such columns made by Malini. The columns should be run in series, and 1.5 L of harvest should be run at a time. After the harvest is run over the columns, the columns are washed with 5-10 column volumes 50 mM Tris pH 7.4, 0.05% azide, 1 mM EDTA. The columns are then eluted with 50 mM diethylamine, and 1 ml fractions are collected. Each tube used for collecting fractions should contain 100 μl of 1 M Tris pH 7.4 to neutralize the diethylamine as it comes out of the column. This prevents degradation of the protein due to high base. After elution, the pH of the columns is neutralized with 5-10 column volumes of 50 mM Tris pH 7.4, 0.05% azide, and 1 mM EDTA. The columns are then ready to have another harvest run over them. Typically, healthy plates of cells will produce 1-2 mg/L quantities of protein.

Concentration and Characterization:

The best way to concentrate the protein is to use the vacuum dialysis apparatus with a 25 kDa MWCO bag. The fractions containing protein (these are determined by reading the A_{280} of the individual fractions) are put into the apparatus, vacuum is applied until the volume left in the bag is around 1 ml, the buffer is poured out and replaced with fresh buffer (50 mM Tris pH 7.4, 0.05% NaN_3, and 1 mM EDTA [150 mM NaCl can also be helpful if you wish to put it in]), and the sample is allowed to dialyze overnight. The next day vacuum is again applied if necessary to obtain a final volume around 0.5 ml (I feel that this is the lower limit of recovery from the apparatus). Remove the protein and rinse the bag with 0.5 ml buffer to wash off any protein sticking to the membrane. Determine the con-

centration by BCA assay, and run the protein on a 13% SDS gel. Store protein at 4ºC.

Denaturing and Renaturing Protein

We attempted to denature and renature this protein in the same manner as $K^d/c\beta_2m$. When equilibrium dialysis experiments were run with the denatured/renatured B27, it was apparent that the process did not work as in the case of $K^d/c\beta_2m$. [See my "Inhibition Data" notebook for the results of the experiments]. Malini developed a different protocol for determining K_D's with the HLA-B27 protein.