THE UNO Q1 WAS CHOSEN FOR THIS SEPARATION BECAUSE OF ITS UNIQUE CONTINUOUS BED MATRIX WHICH ALLOWS:

- **QUICK SCREENING FOR OPTIMUM ELUTION SCHEMES, EVEN WITH VISCOUS BUFFERS**
- **MINIMAL LOSS OF RESOLUTION AT FAST FLOW RATES, SINCE MASS TRANSFER EFFECTS ARE MINIMAL**
- **FAST ONE STEP PURIFICATION OF rIL-13 AFTER SAMPLE PREPARATION OF CELL PELLET**

Purification of rIL-13 Using an UNO™ Q1 Column

Interleukin 13 is a recently described cytokine produced by activated T cells. This protein is responsible for both positive and negative responses to regulatory molecules within the immune system. rIL-13 has been shown to modulate macrophage functions as well as play a direct role in regulation of proliferation and differentiation of hematopoietic stem cells. In addition, a recent study has indicated that rIL-13 inhibits human immunodeficiency virus type I production in blood derived macrophages. For this application, recombinant rIL-13 was overexpressed in *E. coli*. in the form of inclusion bodies. This report describes the isolation and purification of rIL-13 using an UNO Q1 (Bio-Rad catalog # 720-0001) column.

Experimental

rIL-13 was obtained from the cell pellet in a two part process, sample preparation and chromatographic separation. See Figure 1 for a flow chart of sample preparation. Sample preparation involved suspending the cell pellet in 50 mM Tris, pH 8.0, 20 mM DTT with 350 µl of bacterial protease inhibitor (Sigma P8465). The suspension was then passed through a Microfluidics cell disruptor at 12,000 psi five times. The lysed cell suspension was then centrifuged, washed, and the rIL-13 was solubilized with 8 M urea. It was found that rIL-13 will only solubilize with a high concentration of chaotrope under reducing conditions. The final solubilized preparation was then filtered through a 0.45 µm filter and applied to an UNO Q1 column.

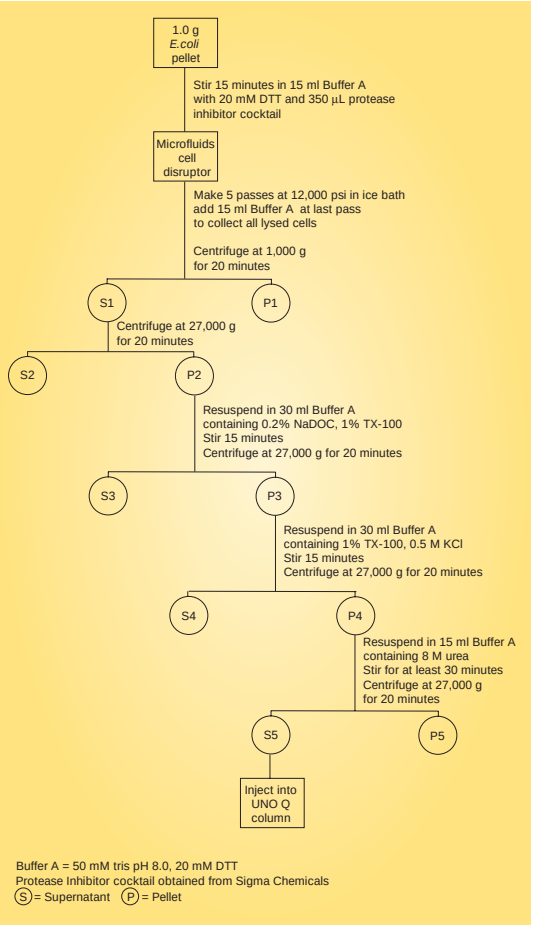

Fig. 1. Flow chart of sample preparation for rIL-13.

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The rIL-13 was eluted by a linear gradient as described in Figure 2. Due to the viscosity of the buffers used in the experiment, the UNO Q1 column was run at 3 ml/min.

Results

Figure 2 shows the elution profile from the UNO Q1 column. rIL-13 was found to elute from this column in the conductivity range of 1.75-3.55 ms/cm.

SDS-PAGE (Figure 3) analysis using a 15% Tris-HCl Ready Gel (Bio-Rad catalog #161-1157) indicated fractions 18–20 contained rIL-13 of at least 90% purity, while fraction 21 contained a small amount of impurities. A western blot of the gel was performed to identify the rIL-13 (data not shown). The apparent heterogeneity in the elution profile may arise from protein-protein interaction or folding intermediates, since by SDS-PAGE, the rIL-13 was nearly homogeneous.

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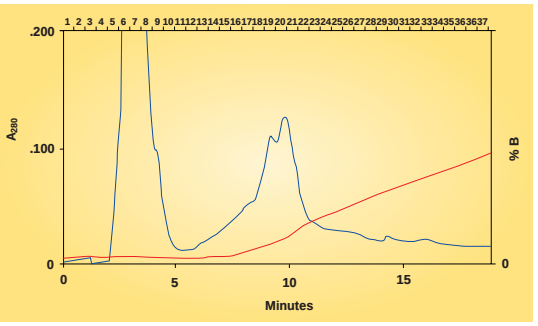

Fig. 2. Chromatogram of rIL-13 separated from fraction S5 on UNO Q1 column. Buffer A: 20 mM CHES, 8.0 M urea, pH 9.8. Buffer B: Buffer A: + 1.0 M NaCl. gradient was 0–20% B over 10 column volumes.

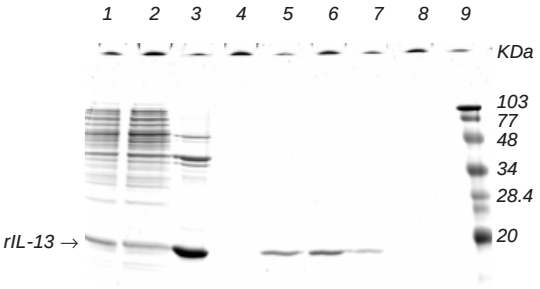

Fig. 3. SDS-PAGE of rIL-13 fractions from UNO Q1 column on 15% Tris-Glycine Ready Gel. Lane 1: cell pellet. Lane 2: lysed resuspension. Lane 3: S5 from Figure 1. Lane 4–7: fractions 18–21. Lane 8: fraction 41. Lane 9: MW standard (catalog #161-0305).

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