Waters
Lab Highlights
An Internal Communication of Applications and Techniques
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LITERATURE CORNER

PURIFICATION OF IMMUNOLOGICALLY ACTIVE PROTEINS USING μBONDAPAK™ C_{18}

A recent publication from the Frederick Cancer Research Institute in Frederick, Maryland, USA[1], describes the use of reverse phase high performance liquid chromatography (RP-HPLC) for the purification of Interleukin 3 (IL3), an immunologically active protein with an estimated molecular weight of 40,000 Daltons. This compound is included in the protein class known as Lymphokines. These proteins are responsible for mediating immune cell (lymphocyte) growth. Interferons are included in this group.

The isolation of Interleukin 3 from cell lines derived from bone marrow culture followed the sample preparation shown below:

Cell culture media containing IL3

↓ 80% Ammonium sulfate Precipitation

Crude Protein Mixture (PPT)

↓ DEAE Cellulose Chromatography

IL3 Active Fraction

↓ 1. Dialysis vs. 0.01 M Na_2PO_4, pH 7.0

2. Reconstitute in 0.1% TFA (trifluoroacetic acid)

RP-HPLC

Solvent A: 0.1% TFA in H_2O

Solvent B: 0.1% TFA in CH_3CN

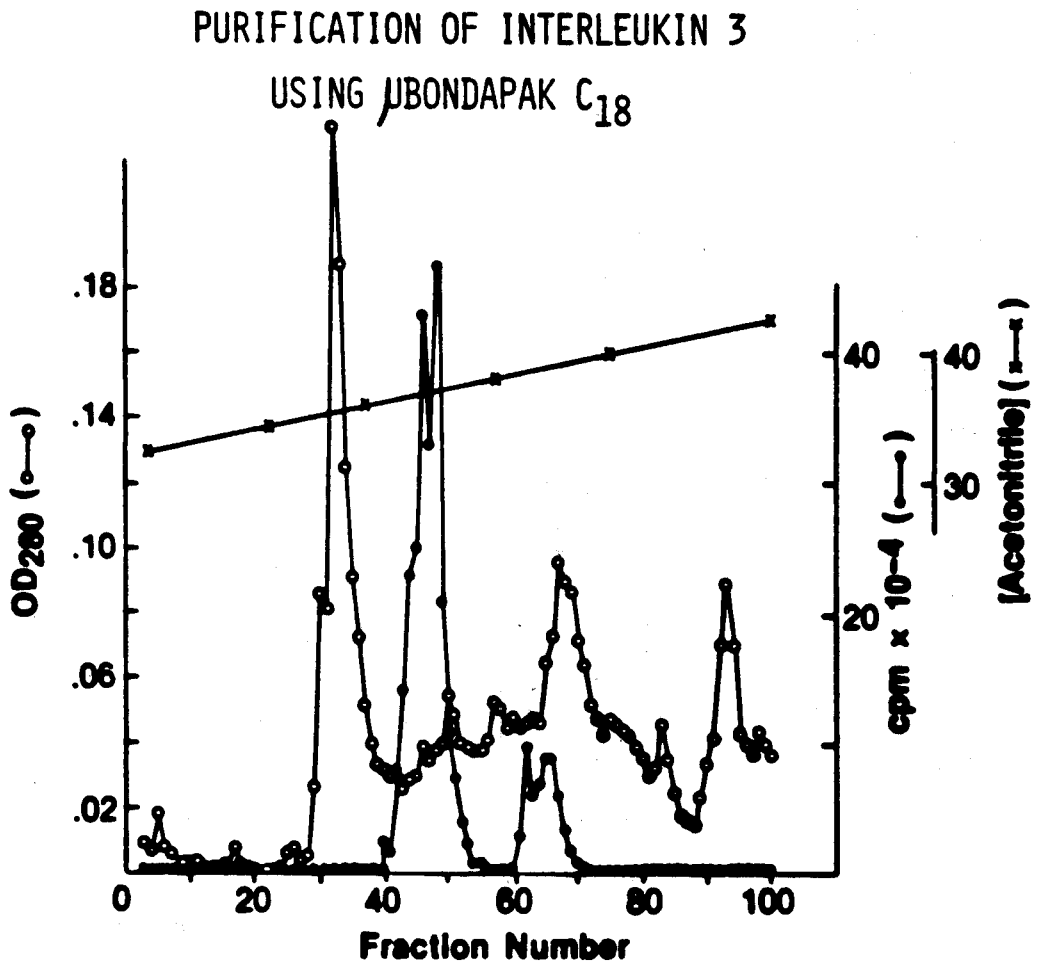
Flow Rate: 1.0 ml/min

Gradient: 0 ——> 60% B, Curve 6, 120 min.

It was significant that these workers were able to recover not only protein mass, but also protein activity. This activity was assayed by the ability of the protein fraction to induce lymphocyte proliferation (cell line FDC-P1) at a 1/2000 dilution after HPLC isolation. Note in Figure 1 that the activity peak did not coelute with the peak of maximum absorbance at 280 nm, emphasizing that one should not be mislead by the UV trace when isolating proteins.

See reverse side for figure.

Bob Pfeifer



(●——●) INDUCED PROLIFERATION OF FDC-P1 CELLS

DATA FROM J.N. IHLE, ET AL, J. IMMUNOL, 129, 4, 1377 (1982)

1. J. N. Ihle, J. Keller, J. S. Greenberger, L. Henderson, R. A. Yetter, and H. C. Morse, III, Phenotypic Characteristics of Cell Lines Requiring Interleukin 3 for Growth, J. of Immunol., 129, 4, 1377-1383, (October, 1982).