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Note

High-performance immunosorbent purification of recombinant leukocyte A interferon

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In the first published example of bio-affinity stationary phases being used in high-performance liquid chromatography (HPLC), the advantages of inorganic supports such as silica were demonstrated[1]. Such supports, if properly bonded to prevent non-specific adsorption, could withstand the high pressures of HPLC. Many papers have appeared since but little attention has been given to scale-up features.

In this paper we describe the use of silica as a support in the immunosorbent purification of recombinant leukocyte A interferon. The scale-up advantages of silica compared to the agarose gel that was used in the original work of Staehelin[2] are outlined.

EXPERIMENTAL

Materials

Polyhydroxy silica support (Nugel P, GP-500, 500 Å porosity 200–400 mesh) was obtained from Diagnostic Specialties (Metuchen, NJ, U.S.A.). The procedure for immobilization of monoclonal antibody to the silica support was essentially as described by Ohlson *et al.*[1]. To 4 g of Nugel GP-500 silica were added 8 ml of 1% sodium *m*-periodate (Sigma, St. Louis, MO, U.S.A.). The suspension was agitated for 30 min at room temperature and then quickly collected and washed on a coarse sintered glass funnel with cold distilled water.

The resulting activated gel was suspended in 8 ml of coupling buffer (0.1 *M* sodium phosphate, 0.1 *M* sodium chloride, pH 7.0) containing 88 mg LI-8 monoclonal antibody[2] and 2 mg cyanoborohydride (Aldrich, Milwaukee, WI, U.S.A.) and agitated overnight at 4°C. The gel was collected on a filter and washed thoroughly with cold coupling buffer. Pooled filtrates were checked for unbound antibody by the Lowry protein method. Binding density was about 20 mg/g support.

Uncoupled activated sites were blocked by suspending the gel in 10 ml of 1 *M* ethanolamine (Fisher, Springfield, NJ, U.S.A) at pH 8.0. Cyanoborohydride (2 mg) was added to the suspension which was then agitated at 4°C for 24 h.

Column apparatus

The automated HPLC system (Waters Assoc., Milford, MA, U.S.A.) consisted

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of three M6000A pumps, an M720 system controller, an M730 data module, and a Model 408 gamma-max variable-wavelength spectrophotometer. A WISP 710 auto sampler including a 0–2-ml sample injector was used for sample injection. A Gilson Model FC-80 minifraction collector was used to collect fractions.

In preparation for packing, the antibody-coupled gel was suspended in 50% glycerol, 0.1 *M* sodium dihydrogen phosphate pH 7.4 and sonicated (benchtop sonication bath) for 10 min to remove air bubbles and make a uniform dispersion.

A Chemco slurry packing apparatus model 124-A (Alltech Assoc., Deerfield, IL, U.S.A.) was used in the upward packing mode (procedure supplied by Vendor) to pack a small stainless-steel column (5 cm × 4.1 mm I.D., volume 0.66 ml) at 1000 p.s.i.

The column was maintained at about 4°C by an Alltech column water jacket (36 × 4 cm O.D.). Otherwise, all equipment was at room temperature.

RESULTS AND DISCUSSION

Column capture efficiency

In our preliminary comparison of column media (Table I) it was clear that siliceous materials provided the best mechanical rigidity and the high flow-rates we desired. Controlled pore glass (CPG) had the disadvantage of being more than double the cost of silica. Beaded cellulose also had high flow-rates but the beads disintegrated at low pH ($\leqslant 3$). Silica would allow us to operate the most economically, under high pressure and with high expected performance.

Once flow-rate was not limiting we wanted to find how fast the column could be operated before binding or capture became limiting. A simple protocol was followed to determine the relationship between column capture efficiency and flow-rate. Purified recombinant leukocyte A interferon in 0.025 *M* ammonium acetate, pH 7.2 was loaded at various flow-rates on the column which had been equilibrated with buffer at pH 7.2. Elution was via a concave gradient (Waters Curve No. 5) from pH 7.2 to pH 2.8 at the same flow-rate as loading. Amounts loaded were determined by

TABLE I

FLOW-RATES THROUGH VARIOUS COLUMN MEDIA AT CONSTANT PRESSURE

Column media	*Flow-rate* (ml/min)*	*Flux* (cm/min)*
Silica, 200–400 mesh, 200 Å pore size	25	32
Silica, 200–400 mesh, 500 Å pore size	21	27
Sepharose 4B 200, 40–190 μm, swollen beads	2.0	2.6
Sepharose CL6B-200, 40–210 μm, swollen beads	6.2	8.1
Fractogel TSK HW-55(F), 32–63 μm	4.2	5.4
Beaded cellulose C-521	22	29
Glycophase 500, CPG, 200–400 mesh, 595 Å pore size	20	26
Celite 535 (Manville)	11	14
CM-52 Cellulose	2.2	2.8

* Average of two independent runs in Pharmacia K 10 × 5 column. Values are for distilled water at room temperature and constant pressure (10 p.s.i.g.).

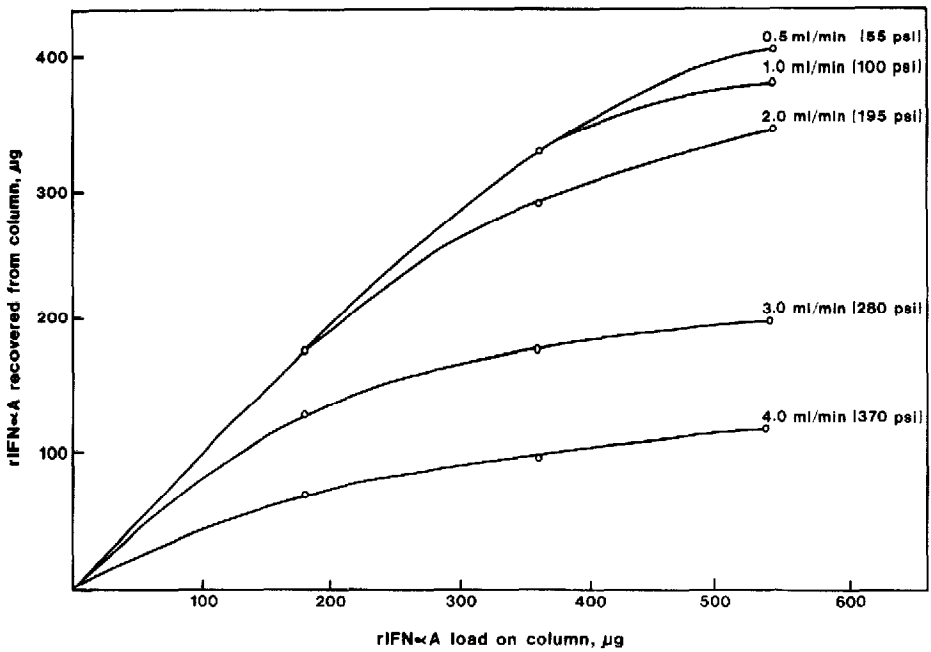

Fig. 1. Effect of flow-rate on column capture efficiency. Purified recombinant leukocyte A interferon in 0.025 *M* ammonium acetate, pH 7.2 was loaded at various flow-rates on the column (50 × 4.66 mm I.D., volume 0.85 ml) which had been equilibrated with buffer at pH 7.2. Elution was via a concave gradient (Waters Curve No. 5) from pH 7.2 to pH 2.8 at the same flow-rate as the loading. Amounts loaded were determined by the Lowry method. Recoveries were determined from area integrations of the elution profiles.

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The results in Fig. 1 show an expected trend of decreasing capture with increasing flow-rate.

Purification

At a conservative flow-rate of 1 ml/min we loaded the column with crude *Escherichia coli* extract containing recombinant leukocyte A interferon at pH 7.0. The washing and eluting protocol was modified from Staehelin[2] but the purity of the eluted rIFN-αA was the same by polyacrylamide gel electrophoresis.

Scale-up features

Water flux (linear velocity) through a small 1-cm Dia column of agarose at 10 p.s.i.g. was 2–8 cm/min (Table I). In large-scale purification of rIFN-αA in a 10-cm I.D. column of agarose the maximum flux was only 0.64 cm/min[3] because of compressibility of the gel. To maintain a flux of 0.64 cm/min in a 14-cm Dia column we added a 10 cm × 10 cm I.D. (11 cm O.D.) cylindrical, polycarbonate baffle. An example of baffing to prevent radial compression in large-scale immunosorbent columns was reported previously[4].

The data reported here suggest that the most practical way to overcome prob-

lems of compression in scale-up is to use polyhydroxy silica as a support. Water flux in a silica column was approximately ten-fold greater than in a Sepharose 4B (agarose) column.

In the capture efficiency study the 5 cm × 0.466 mm I.D. column was packed under pressure at 1000 p.s.i. Consequently the flux (27 cm/min) achieved in a gravity packed column would have required a pressure of >370 p.s.i. in the high-pressure-packed column. In practice we would not want to operate at less than 50% capacity, or 300 μg as shown in Fig. 1. To capture more than 80% of the load we would have to pump at 2 ml/min or a flux of approximately 12 cm/min. This low flux was easily achieved in a gravity packed column operated at low pressure.

Since the mechanism of separation in our column is simple adsorption–desorption we avoid the term "chromatography". There is no evidence of partitioning in an immunosorbent column. Packing under high pressure was not helpful because the concept of theoretical plates does not apply. Nevertheless, by using silica as a support we should be able at once to overcome the scale-up problems associated with compressible gels and achieve high performance.

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