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AUTOMATED HIGH-PERFORMANCE IMMUNOSORBENT ASSAY FOR RECOMBINANT LEUKOCYTE A INTERFERON

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SUMMARY

A rapid assay for recombinant leukocyte A interferon has been developed that includes a small immunosorbent column (Amicon glass column *ca.* 10 × 6 mm; *i.e.*, *ca.* 0.5 ml), containing monoclonal antibody, immobilized on Nugel-polyhydroxy phase silica, 500 Å, 200–400 mesh (Diagnostic Specialties, Metuchen, NJ, U.S.A.). The column has been automated so that the operator need only inject sample (0.25 ml) every 18 min (or one can use an automatic sample injector) and initiate the program cycle of the microprocessor. A hard-copy result from an integrator is available in less than 20 min. Routine analyses were performed at a flow-rate of 4 ml/min in the concentration range 0.02–0.3 mg/ml. Reproducibility of the assay was checked by assaying the same crude extract seven times in succession. Standard deviation was 3.96% and correlation coefficient was 0.9996.

The advantages of this technique include rapid analysis time and relative simplicity, compared to the enzyme immunoassay.

INTRODUCTION

We have described previously[1] the use of monoclonal antibody bonded Nugel-polyhydroxy phase silica as a support in the purification of recombinant leukocyte A interferon.

In this paper we describe the use of same bonded support as a rapid assay procedure for recombinant leukocyte A interferon.

EXPERIMENTAL

Preparation of immunosorbent gel

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 previously[1,2]. To 2 g of Nugel GP-500 silica were added 4 ml of 1% sodium meta 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 4 ml of coupling buffer (0.1 *M* sodium phosphate, 0.1 *M* sodium chloride, pH 7.0), containing 44 mg LI-8 mono-

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clonal antibody[3] 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 column material.

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.

Assay procedure

Volumes of 0.5 ml of the coupled gel were packed in a jacketed glass column (10 × 6 mm, Amicon, Danvers, MA, U.S.A.). Column temperature was maintained at 4°C by a refrigerated recirculating bath (Neslab, Portsmouth, NH, U.S.A.). All other parts of the system were at room temperature.

Sample at pH 7.0 was loaded onto the column, which was equilibrated with buffer A (0.286 *M* guanidine–HCl, 0.02 *M* sodium dihydrogen phosphate, 0.2 *M* sodium chloride, 0.025% Tween 20, pH 7.0).

The column was then flushed with the same buffer to remove unadsorbed material. The adsorbed material was eluted with buffer B (0.286 *M* guanidine–HCl, 0.2 *M* acetic acid, 0.1 *M* sodium chloride, 0.025% Tween-20). Following elution, the column was flushed with buffer C (2 *M* guanidine–HCl, 1 *M* sodium chloride) and then re-equilibrated with buffer A. Total time required for the above analysis is 18 min, *i.e.*, the time from injection of the sample until a quantitative hard-copy result is available from the integrator. The numerical result is a peak area, which must then be matched to a standard curve to determine the amount of interferon present in the sample. Automation of the immunosorbent Ab column included interfacing with a time-based microprocessor and selector valves, essentially as described by Tarnowski and Liptak[4]. A typical integrator profile of an *Escherichia coli* crude extract is shown in Fig. 1.

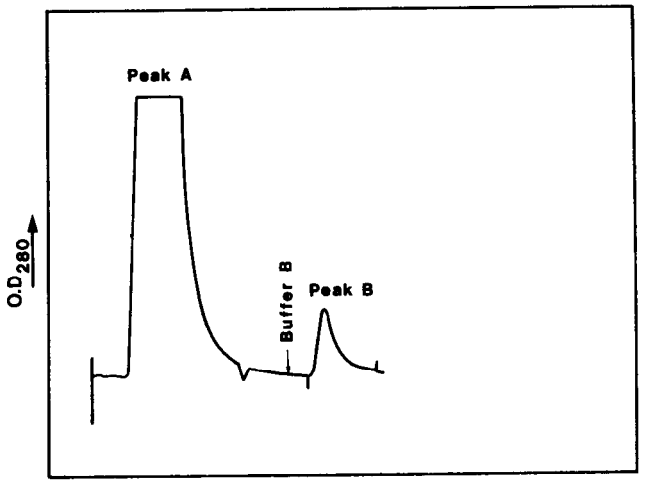

Fig. 1. Typical integrator profile showing analysis of an *E. coli* crude extract. The initial peak ($O.D._{280}$) represents unbound protein which we call flowthrough. The second peak is interferon eluted with buffer B. The integrated areas of the second peak in arbitrary units could be matched to a standard curve for determination of interferon concentration.

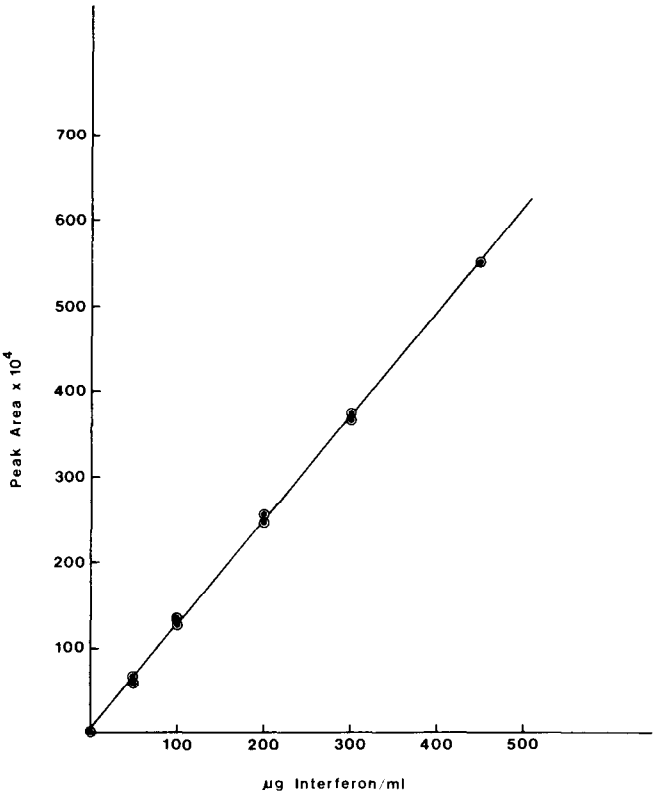

Fig. 2. Standard curve for leukocyte A interferon peak area as a function of concentration in the sample load. Flow-rate, 4 ml/min; column, 0.5 ml; sample size, 0.25 ml sample loop.

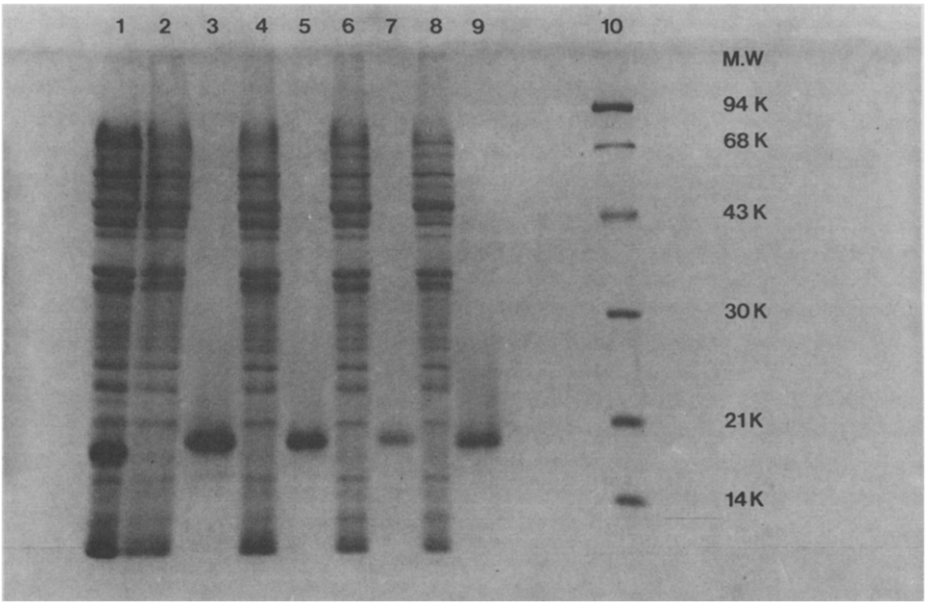

Fig. 3. High capture efficiency of immunosorbent column assay, as demonstrated by SDS–PAGE under reducing conditions. Tracks 2, 4, 6 and 8 are unbound peaks at 4 ml/min. Tracks 3, 5, 7 and 9 are the corresponding eluates. Track 1 is the column load. Track 10 is the molecular weight standard.

RESULTS AND DISCUSSION

A standard curve, prepared from known amounts of pure interferon, is shown in Fig. 2. We verified that all the interferon from crude *E. coli* extract was binding to the column by analyzing unbound peaks and elution peaks in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)[5] and by enzyme immunoassay[6]. There was no detectable interferon in the unbound peaks but homogeneous interferon bands present in the elution is shown in Fig. 3. Routine analyses were performed at a flow-rate of 4 ml/min for a total analysis time of 18 min. From the standard curve, we could determine the amount of interferon present in the sample. The useful concentration range is roughly 30–300 μg/ml. Reproducibility of the assay was checked by assaying the same crude extract seven times in succession. The standard deviation was 3.96. The small column described here has been cycled through more than 400 assays.

Advantages

The advantages of this technique include rapid analysis time and relative simplicity compared to enzyme immunoassay.

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