Human Lymphoblastoid Interferon

LARGE SCALE PRODUCTION AND PARTIAL PURIFICATION*

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SUMMARY

Human lymphoblastoid interferon was produced on an 800-liter scale (2.6 x 10^7 units) by induction of Namalva cells with Newcastle disease virus, strain B1. The interferon was partially purified by anti-leukocyte interferon affinity chromatography, sulfopropyl Sephadex ion exchange chromatography, isoelectric focusing, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Recovery of interferon after gel electrophoresis varied from 11 to 33% based on the original crude material, with about 35,000-fold purification. The gel electrophoresis resolved the antiviral activity into two components with apparent molecular weights of 18,000 and 22,000; treatment with glycosidases resulted in complete (75 to 106%) recovery in various SDS-gel systems both before and after staining with Coomassie brilliant blue. Removal of the carbohydrate portion of the interferon molecule resulted in a single peak of activity using both isoelectric focusing and SDS-polyacrylamide gel electrophoresis.

Human lymphoblastoid interferon was produced by the cell line Namalva induced with Newcastle disease virus, strain B1 and cells were removed by centrifugation. Precipitation of proteins from the tissue culture fluid and removal of trichloroacetic acid by Sephadex G-25 resulted in approximately a 50-fold concentration of the crude interferon. The solution was applied to an anti-leukocyte interferon affinity column (Fig. 1) which bound the applied lymphoblastoid interferon. Sorensen's citrate buffer, pH 2.2, was used to elute the interferon (25 to 100%), which was purified 250- to 450-fold. We have recently found that prolonged washing of the column with NaCl/P, after application of the sample gives greater purification without loss of interferon activity.

Since the isoelectric point of interferon is approximately 6, SP-Sephadex which has a pH range 2 to 10, was chosen for ion exchange chromatography. McIlvaine's citrate/phosphate buffer was used because of its wide range of buffering capacity. When applied directly to a SP-Sephadex column equilibrated with McIlvaine's citrate/phosphate buffer, pH 4.5, only 70 to 75% of the interferon was bound. However, prior dialysis of the interferon-containing fraction against the equilibration buffer resulted in complete binding of the interferon. Since dialysis of large volumes is cumbersome, these two purification steps have been modified. After the affinity column was washed with NaCl/P, it was further washed with approximately 900 ml of McIlvaine's citrate/phosphate buffer, pH 5.5. Adjusting the pH of this buffer to 2.6 eluted the interferon, which could then be applied directly to a SP-Sephadex column equilibrated with the same buffer. Recovery of the interferon (40 to 96%) by means of a pH gradient (Fig. 2) gave a 6- to 13-fold purification but a large volume. Smaller volumes of eluate (about 5 times lower) could be achieved with less purification (5-fold) by using a single-step elution at pH 6.8. Since lymphoblastoid interferon elutes from the column at pH 5.5, it must be applied at pH 4.5 or below.

Interferon shows a heterogeneous character (and only 31% of its activity)

Our own long range aims involve isolation, sequencing, and synthesis. However, since others may have interest in the possibilities of large scale biological production, conditions for cell growth and interferon production and purification are given here in some detail. In the experiments presented, lymphoblastoid interferon has been purified greater than 35,000-fold using affinity and ion exchange chromatography and SDS-polyacrylamide gel electrophoresis, and is now being produced at batch levels of 800 liters (2.6 x 10^7 units). Full biological activity has been recovered in various SDS-gel systems both before and after staining with Coomassie brilliant blue. Removal of the carbohydrate portion of the interferon molecule resulted in a single peak of activity using both isoelectric focusing and SDS-polyacrylamide gel electrophoresis.

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recovery) when subjected to isoelectric focusing (Fig. 3a). Treatment with glycosidases and α-mannosidase (3, 12, 13) resulted in a single peak after isoelectric focusing which gave increased recovery (60%) and purification (12-fold). This procedure appears to be a suitable purification step to follow SP-Sephadex chromatography. The recovery of interferon activity from stained and unstained SDS-polyacrylamide gels is shown in Table I. The recovery, calculated as a percentage of the interferon activity of each sample applied, varied from 85 to 156% for the unstained gels and from 55 to 150% after staining.

Neither the running buffer nor the percentage of acrylamide used in forming the gel made any appreciable difference in the percentage of activity recovered (Table I). Heating the sample for 2 min at 100°C prior to application to the gel did not appear to affect the recovery. However, the addition of mercaptoethanol before heating resulted in only 18% recovery of the applied interferon. In all cases the activity was spread over a distance of about 2.5 cm.

All the experiments showed two peaks of activity (Fig. 4). The leading peak, corresponding to a protein of apparent molecular weight 18,000 (± 1000) contained the majority (>70%) of the active interferon. The smaller peak which corresponded to a protein of apparent molecular weight 22,000 (± 1000) was always present but always contained less than 30% of the total activity. Treatment with glycosidases (3) resulted in 100% recovery of interferon activity in the lower molecular weight species.

Preparative SDS-polyacrylamide slab gel electrophoresis of partially purified interferon resulted in a 20-fold purification giving a specific activity of $1.1 \times 10^7$ units/mg of protein in the lower molecular weight peak fraction. Two broad bands of activity were isolated from the gel with the apparent molecular weights 18,000 and 22,000 and no detectable loss of interferon activity. Fibroblast interferon has also been purified by preparative SDS-gel electrophoresis; however, only 60% of the interferon activity was recovered from the gel (5). Törnä and Paucker (6) and Stewart and Desmyter (17) have shown that 70 to 120% leukocyte interferon activity can be recovered from SDS gels. Two peaks of activity having apparent molecular weight of 15,000 to 16,000 and 20,000 to 21,000 were also

![Fig. 1. Purification of human lymphoblastoid interferon by anti-leukocyte interferon affinity chromatography. Crude interferon (3.1 x 10^5 units) was applied to a column (11 x 4.5 cm). Specific activity in citrate peak, 7.8 x 10^4 units/mg of protein; 446-fold purification. PBS, NaCl/Pi.]

![Fig. 2. Ion exchange chromatography of lymphoblastoid interferon (IF) on SP-Sephadex C-25. Interferon (3.4 x 10^5 units) was applied to a column (15 x 2.2 cm). A pH gradient 4.5 to 7.7 eluted the interferon (2.9 x 10^5 units, 6-fold purification).]

![Fig. 3. Isoelectric focusing of human lymphoblastoid interferon (a) before and (b) after treatment with glycosidases in 0.1 M phosphate/citrate buffer, pH 6.0 for 2 h at 37°C. Interferon (1.3 x 10^6 units in each case) was focused between pH 5 and 8 using an LKB MultiPhor.]
TABLE I  
Recovery of interferon activity from SDS-polyacrylamide gels (% of applied interferon)  

| Gel system   | Tris/glycine buffer | Phosphate buffer |
|--------------|---------------------|------------------|
| Recovery     |                     |                  |
| Unstained    | 145%                | 12.5%            |
| Stained      | 106%                | 11%              |

a % acrylamide.

b These figures are an average of the recoveries from several experiments.

found for leukocyte interferon. Staining the gels with Coomasie brilliant blue R-250 did not adversely affect the recovery of lymphoblastoid interferon from the gels. Assuming a minimum specific activity of 2 x 10^4 units/mg (5), the amount of interferon applied to each gel would correspond to less than 1 μg. It is unlikely that the interferon could be detected by Coomasie blue staining at this concentration. Thus, we are not able to conclude whether the recovery of protein activity is due to a poor or reversible reaction with the stain, or whether the regions of the molecule to which the stain binds are not essential for activity. Further purification of lymphoblastoid interferon to a level suitable for preliminary structure studies may be achieved by re-electrophoresing isolated peaks of interferon activity on analytical SDS slab gels. This procedure has been used successfully by Knight (5) in the purification of fibroblast interferon.

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