JOURNAL OF VIROLOGY, Feb. 1976, p. 439-445

Vol. 17, No. 2
Printed in U.S.A.

Purification and Characterization of Mouse Interferon with Novel Affinity Sorbents

MARY W. DAVEY, EUGENE SULKOWSKI, AND WILLIAM A. CARTER*

Roswell Park Memorial Institute, Department of Medical Viral Oncology, Buffalo, New York 14263

Received for publication 29 September 1975

Several novel selective sorbents for mouse interferon are described that exploit the hydrophobic property and glycoprotein nature of this molecule. Low-molecular-weight ligands (hydrocarbons) and high-molecular-weight ligands (bovine serum albumin) immobilized on agarose bind selectively mouse L-cell interferon. The high selectivity of binding is due primarily to a hydrophobic effect, although electrostatic forces are also apparently involved. Mouse L-cell interferon binds to immobilized serum albumin and can be completely recovered by raising the ionic strength of the eluant. The specific activity of interferon preparations can be increased 2,000-fold to a value of 3×10^8 reference units per mg of protein in a single step with full recovery of the antiviral activity. A selective adsorption, although to a lesser degree, can be also obtained on hydrocarbon-coated agarose (Affi-Gel 202), resulting in 300-fold purification on desorption. The existence of two major components of mouse interferon was revealed upon its chromatography on the following sorbents: (i) bovine serum albumin-agarose, (ii) ω-carboxypentyl-agarose; and (iii) *Bandeiraea simplicifolia* lectin-agarose. This report thus provides for the first time a means for efficient and clear-cut separation of interferon components, thus enabling their further characterization.

Mouse interferon has been successfully purified in several laboratories by affinity chromatography on solid-phase immunoadsorbents (10, 11) and by more classical physicochemical methods (9, 13). The extent of purification by a particular procedure varies from 20-fold to 2,000-fold, critically depending on the starting materials, e.g., the titer of an interferon preparation and the quality of the isolated antibody (10, 11). Specific activity of the best interferon preparations varies within a narrow range of 2×10^8 to 3.8×10^8 reference units per mg of protein, indicating a high degree of purification (4, 9, 10, 13). There is, however, good documentation that further considerable purification is required (10, 13). Recently, the homogeneity of a mouse interferon preparation, having 2.5×10^8 reference units per mg of protein, has been reported, although the experimental data presented seem rather in variance, than in support, of this claim (9).

Any further purification of mouse interferon may be critically dependent on the availability of novel sorbents that display diverse bias in their adsorption and desorption requirements. Moreover, these novel sorbents should provide for high selectivity in adsorption of mouse interferon and for its near-to-complete recovery. We now suggest one such sorbent: bovine serum albumin (BSA), immobilized on agarose. The binding of mouse interferon to this sorbent is quite selective, inasmuch as it results in a 2,000-fold purification. The recovery of interferon activity is complete (the specific activity of desorbed interferon is about 3.0×10^8 reference units per mg of protein) and, furthermore, the sorbent can be readily prepared and used.

MATERIALS AND METHODS

Interferon preparations. Mouse L_{929}-cell interferon was prepared as described previously (2).

Chemicals. BSA, a crystalline preparation, was obtained from Miles Laboratories. Sepharose 4B, CNBr activated, was purchased from Pharmacia Fine Chemicals. D-Galactose and methyl α-D-mannopyranoside were obtained from Sigma Chemical Co. Fluorescamine was obtained from Roche Diagnostics. All media and sera were obtained from Grand Island Biological Co. All other reagents were of analytical grade. Double glass-distilled water was used for all solutions.

Adsorbents. Concanavalin A-Sepharose 4B (concanavalin A-agarose) and CH-Sepharose 4B (●-$NH[CH_2]_6COOH$) were purchased from Pharmacia Fine Chemicals. Affi-Gel 202 (●-OCH_2-$CONH[CH_2]_3NH[CH_2]_3NHCO[CH_2]_2COOH$) was from Bio-Rad Laboratories. *Bandeiraea simplicifolia* lectin-agarose was a generous gift of I. J. Goldstein. BSA-agarose was prepared as described previously (6).

Interferon assay. Interferon samples were assayed

on mouse L_{929} cells by the colorimetric technique of Finter (5) with vesicular stomatitis virus as the challenge virus (multiplicity of infection, 0.125 PFU per cell). (In our assay, international reference standard G 002-904-511 assayed as 4,000 U [nominal value, 12,000 U] was used. All interferon titers in this communication are expressed as reference units.)

Protein determination. Protein concentration was measured by absorbance at 280 nm or by fluorometric assay (1), with BSA as standard.

RESULTS

Chromatography of mouse interferon on low-molecular-weight ligands (hydrocarbons) immobilized on agarose matrix. (i) Chromatography on CH-Sepharose 4B and Affi-Gel 202 at pH 7.4. Figure 1 illustrates the elution profiles of a mouse interferon preparation from CH-Sepharose 4B and Affi-Gel 202 columns. Both adsorbents were equilibrated and developed with phosphate-buffered saline. It is readily apparent that there is no significant difference in the elution profiles of bulk proteins. However, mouse interferon activity was selectively retarded on the Affi-Gel 202 column (Fig. 1B). It should be noted that the Affi-Gel 202 adsorbent carries significantly longer hydrocarbon side chains than CH-Sepharose 4B, although of lower intrinsic hydrophobicity. The overall hydrophobic character of the side chain must be influenced to a greater extent by the carboxyl head group for CH-Sepharose 4B than for Affi-Gel 202. We reasoned that a decrease in the charge of the polar head group should result in a stronger hydrophobic interaction of the hydrocarbon chain with mouse interferon. To this end, the chromatographic behavior of mouse interferon on both adsorbents was studied at pH 5.0.

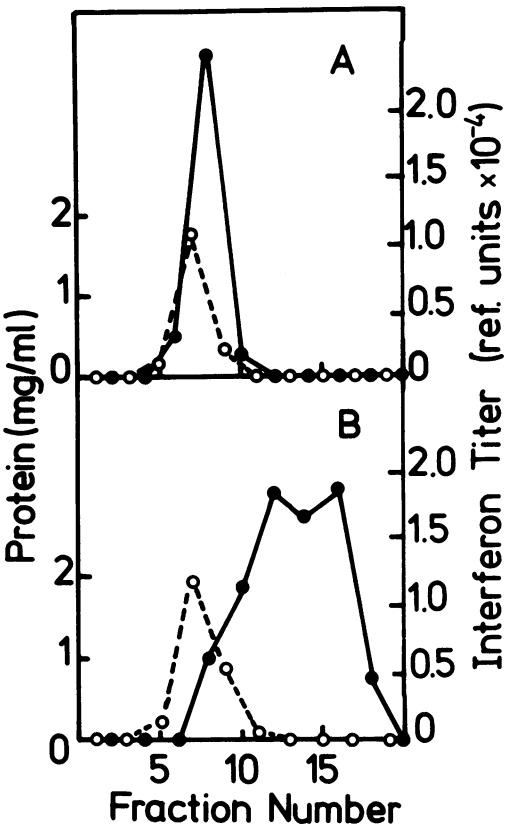

FIG. 1. *Chromatography of mouse L-cell interferon (Newcastle disease virus [NDV] induced) on CH-Sepharose 4B and Affi-Gel 202 at pH 7.4. Interferon preparations were dialyzed against 0.02 M sodium phosphate, pH 7.4, containing 0.15 M NaCl (PBS) for 16 h at 4 C. The interferon sample was applied on a column equilibrated with PBS at 4 C. Fractions (1 ml) were collected at a flow rate of 30 ml/cm² per h. The material from the even-numbered fractions were used to measure protein; the odd-numbered fractions, collected into tubes containing 0.5 ml of a 1% solution of BSA in PBS, were assayed for interferon activity. Symbols: O, protein; ●, interferon. (A) Chromatography on CH-Sepharose 4B. An interferon sample (2 ml), containing 3,600 reference units of interferon and 1.5 mg of protein per ml, was applied on a column (0.9 by 20 cm), and the column was washed with PBS. The breakthrough fraction contained 100% of protein and 100% of interferon activity. (B) Chromatography on Affi-Gel 202. An interferon sample (2 ml), containing 7,200 reference units of interferon activity and 1.5 mg of protein per ml, was applied on a column (0.9 by 20 cm), and the column was washed with PBS. The breakthrough fraction contained 100% of the protein. The interferon activity (100%) was recovered from the column by further elution with PBS.*

(ii) Chromatography on CH-Sepharose 4B by adsorption at pH 5.0 and elution at pH 7.4. Figure 2 illustrates the elution profiles of bulk proteins and interferon activity when the column was equilibrated and the interferon preparation was applied in 0.05 M sodium acetate buffer at pH 5.0 (Fig. 2A). The breakthrough fraction contained 90% of the applied protein and no interferon activity. The elution of the column with 0.02 M sodium phosphate buffer, pH 7.4, resulted in a partial recovery (60%) of interferon activity. Further elution with 0.5 M sodium chloride in phosphate buffer displaced the remainder (40%) of the activity from the column. The separation of interferon activity into two fractions could indicate the existence of two components in the preparation or simply a chromatographic artifact. To eliminate the latter possibility, a rechromatography of both fractions I and II, as shown on Fig. 2A, was undertaken. Figures 2B and C establish that both fractions preserve their chromatographic distinctness. Clearly, this observation is of con-

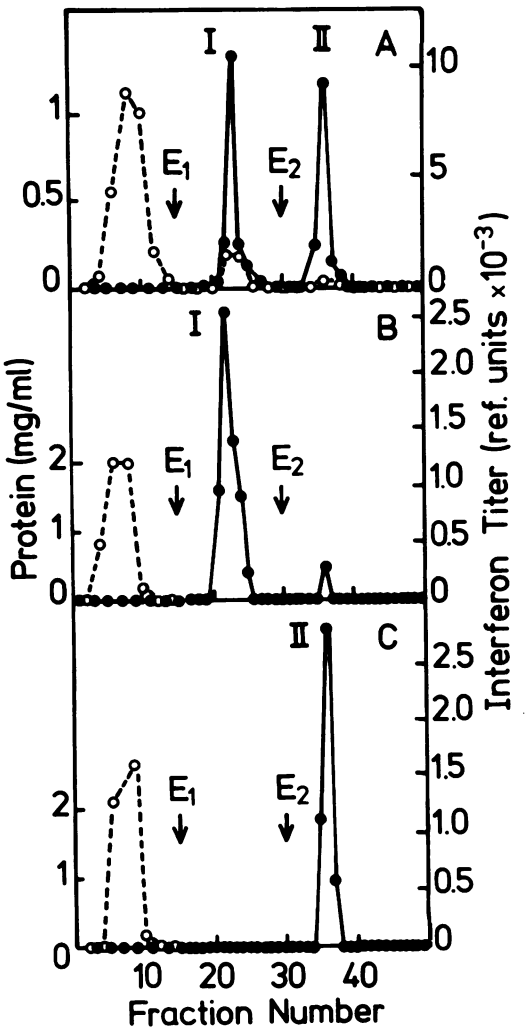

FIG. 2. *Chromatography and rechromatography of mouse L-cell interferon (Newcastle disease virus induced) on CH-Sepharose 4B. An interferon preparation was dialyzed against 0.05 M sodium acetate, pH 5.0, for 16 h at 4 C. Samples were applied on columns (0.9 by 15 cm) equilibrated with 0.05 M sodium acetate, pH 5.0, at 4 C. Columns were washed with equilibrating buffer, followed by 0.02 M sodium phosphate, pH 7.4 (E_1), and finally with E_1 containing 0.5 M NaCl (E_2). Symbols: ○, protein; ●, interferon. (A) Chromatography of interferon preparation. The interferon sample (5 ml), containing 6,000 reference units of interferon and 1.3 mg of protein per ml, was applied, and the column was washed with 0.05 M sodium acetate, pH 5.0. The breakthrough fraction contained 90% of the applied protein. The column was washed with E_1; the wash yielded about 9% of the protein and 60% of the interferon activity (I). Elution with E_2 yielded the remaining 40% of interferon activity (II) and traces of protein. (B) Rechromatography of fraction I. Fraction I, supplemented with BSA, was dialyzed against 0.05 M sodium acetate, pH 5.0, and a sample (2 ml), containing 3,000 reference units of interferon and 2.5 mg of protein per ml, was applied on a column. The breakthrough fraction contained 90% of the applied interferon activity except for traces recovered with solvent E_2. (C) Rechromatography of fraction II. Fraction II, supplemented with BSA, was dialyzed against 0.05 M sodium acetate (pH 5.0), and a sample (2 ml), containing 2,250 reference units of interferon and 2.5 mg of protein per ml, was applied on a column. The breakthrough fraction contained all of the applied protein. All interferon activity was recovered with solvent E_2.*

siderable interest, as it may provide a tool for molecular characterization of the heterogeneity in mouse interferon preparations. The selectivity of binding, especially for fraction I, is not sufficiently satisfactory for further purification purposes.

Chromatography on Affi-Gel 202 by adsorption at pH 5.0 and elution at pH 7.4. Figure 3 illustrates the results of a purification experiment in which a large volume of mouse interferon preparation was applied to a column in 0.05 M sodium acetate, pH 5.0, at 4 C. Under these conditions, there was complete retention of interferon activity. By contrast, the bulk proteins resident in this preparation were to a large extent (96%) unretained and could be further displaced (2%) from the column by a shift from 0.05 M sodium acetate, pH 5.0, to 0.02 M sodium phosphate, pH 7.4. Selectivity in desorption was achieved with a linear gradient of sodium chloride in phosphate buffer. The overall purification of interferon was about 300-fold and its recovery was 95%.

Chromatography of mouse interferon on high-molecular-weight ligand (BSA) immobilized on agarose matrix. (i) Chromatography on BSA-agarose at pH 5.0. Figure 4 illustrates the results of chromatography of mouse interferon preparations on BSA-agarose. Figure 4A depicts the binding and elution of mouse interferon induced by Newcastle disease virus, whereas Fig. 4B records an analogous experiment for poly(I)·poly(C)-induced interferon. Three chromatographic facets of the data presented deserve immediate comment. First, there is a complete and highly selective retention of interferon activity; second, the recovery of the activity is complete under mild solvent conditions; and third, two major fractions of interferon activity are present regardless of the nature of the inducer.

(ii) Chromatography on BSA-agarose by adsorption at pH 5.0 and elution at pH 7.4. Figure 5 illustrates the results of the purification experiment. A large volume (100 ml) of a

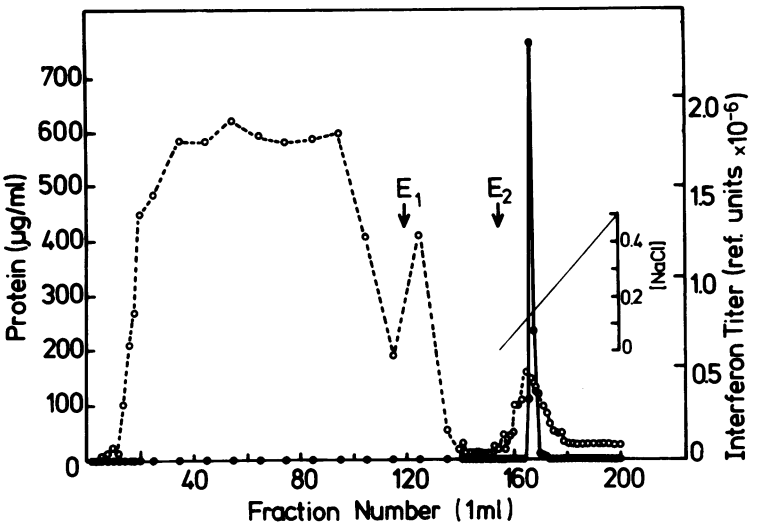

FIG. 3. *Purification of mouse L-cell interferon (Newcastle disease virus induced) on Affi-Gel 202. An interferon preparation (100 ml), containing 3.6 × 10⁴ reference units of interferon activity and 620 µg of protein per ml, was dialyzed against 0.05 M sodium acetate, pH 5.0, for 15 h at 4 C and applied on a column (0.9 by 10 cm) equilibrated with the same buffer by means of a peristaltic pump at a flow rate of 30 ml/cm² per h. The eluate from the column was divided by a stream-splitting device in a ratio of 1:9. The 10% portion of the eluate was collected into 1 ml of a 1% solution of BSA in PBS and used to assay the interferon activity. The 90% portion of the eluate was used to measure the protein concentration. Fractions from 1 to 20 and from 140 to 200 were 1 ml each; fractions from 21 to 139 were collected as 10-ml portions. The breakthrough fraction (5 to 110) contained about 95% of the applied protein and no interferon activity. Further elution of the column with 0.02 M sodium phosphate, pH 7.4 (E_1), resulted in the recovery of 4% of the interferon activity and 2% of protein. The column was then developed with a linear gradient of sodium chloride (E_2). The interferon activity (95% of the applied) was recovered in fractions 163 to 167; the specific activity of fraction 164 is 2 × 10⁷ reference units per mg of protein. Symbols: O, protein, ●, interferon; —, sodium chloride gradient.*

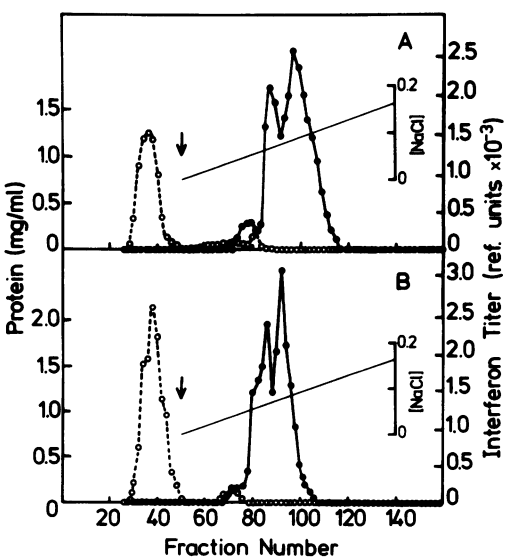

FIG. 4. *Chromatography of mouse L-cell interferon on BSA-agarose at pH 5.0. Interferon preparations were dialyzed against 0.05 M sodium acetate, pH 5.0, for 16 h at 4 C. The interferon sample was applied on a column (0.9 by 60 cm) equilibrated with 0.05 M sodium acetate, pH 5.0, at 4 C. The column was then developed with a sodium chloride gradient in the equilibrating solvent. Symbols: O, protein; ●, interferon; —, sodium chloride gradient. (A) Elution profile of Newcastle disease virus-induced interferon. An interferon sample (3 ml), containing 3,000 reference units of interferon activity and 1.5 mg of protein per ml, was applied, and the column was washed with 0.05 M sodium acetate, pH 5.0. The breakthrough fraction contained 96% of the applied protein. Elution of the column with sodium chloride resulted in displacement of the remaining protein and total recovery of interferon activity. (B) Elution profile of poly(I)·poly(C)-induced interferon. An interferon sample (10 ml), containing 1,830 reference units of interferon activity and 2 mg of protein per ml, was applied, and the column was washed with 0.05 M sodium acetate, pH 5.0. The breakthrough fraction contained about 95% of the applied protein. Elution of the column with sodium chloride resulted in recovery of the remaining protein and total recovery of interferon activity.*

mouse interferon preparation was applied on a BSA-agarose column in 0.05 M sodium acetate, pH 5.0, at 4 C. The column was washed with 0.02 M sodium phosphate, pH 7.4 (E_1), until equilibration and then developed with a linear gradient of sodium chloride. The pertinent

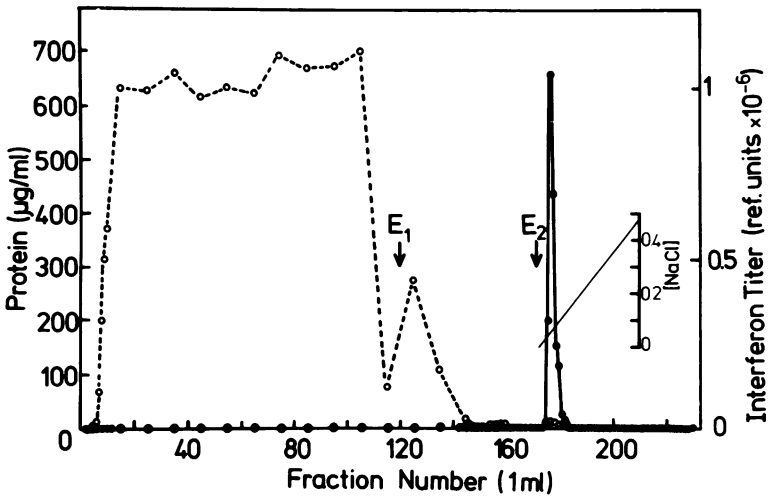

FIG. 5. *Purification of mouse L-cell interferon (Newcastle disease virus induced) on BSA-agarose. An interferon preparation (100 ml), containing 2.4 × 10⁴ reference units of interferon activity and 640 µg of protein per ml, was dialyzed against 0.05 M sodium acetate, pH 5.0, for 16 h at 4 C; it was applied on a column (0.9 by 10 cm) equilibrated with 0.05 M sodium acetate, pH 5.0, by means of a peristaltic pump, at a flow rate of 30 ml/cm² per h. The column eluate was divided by a stream-splitting device in a ratio of 1:9; the 10% portion of the eluate was collected into 1 ml of a 1% solution of BSA in PBS and used to assay interferon activity; the 90% portion of the eluate was used to measure the protein concentration. Fractions from 1 to 10 and from 170 to 230 were 1 ml each; fractions from 11 to 169 were collected as 10-ml portions. The breakthrough fraction (5 to 100) contained about 96% of the protein and less than 1% of the interferon activity. The column was then equilibrated with 0.02 M sodium phosphate, pH 7.4 (E_1). This removed some protein (about 3%) and about 1% of the interferon activity. Further elution of the column with sodium chloride resulted in complete recovery of interferon activity. Fractions 176 to 179 contained 90% of the applied interferon activity; the specific activity was 3 × 10⁸ reference units per mg of protein. Symbols: ○, protein; ●, interferon; —, linear gradient of NaCl formed by mixing two solvents: (i) 0.02 M sodium phosphate, pH 7.4, 20 ml; and (ii) 0.02 M sodium phosphate, pH 7.4, 0.5 M NaCl, 20 ml.*

details are given in the legend to Fig. 5. The equilibration of the column with phosphate buffer, at pH 7.4, resulted in displacement of the remaining 3% of contaminating protein and a negligible amount (1%) of interferon activity. The displaced interferon activity (E_2) was not subdivided into two fractions; moreover, it could be recovered in a small volume providing a significant concentration as well. The overall purification was about 2,000-fold, the recovery was 90%, and the specific activity was about 3 × 10^8 reference units per mg of protein.

Chromatography of mouse interferon on lectins immobilized on agarose matrix. (i) Chromatography on concanavalin A-agarose. When mouse interferon preparations were applied on concanavalin A-agarose columns equilibrated with PBS, partial binding of interferon activity was consistently observed. However, a large portion of applied activity was not retained on the column and was recovered in the breakthrough fraction. The activity retained on the column could be recovered with methyl α-D-mannopyranoside (0.1 M) together with other glycoproteins. The lack of complete retention and the relatively low binding selectivity of interferon made the use of concanavalin A-agarose adsorbent unattractive for further purification purposes. However, the binding behavior of mouse interferon preparations indicates a gross difference in the glycosylation of the various interferon molecules present and is thus the most plausible cause for the heterogeneity of interferon observed on the other sorbents described in this report.

(ii) Chromatography on B. simplicifolia lectin-agarose. Figure 6 illustrates the affinity chromatography of a mouse interferon preparation on *Bandeiraea* lectin. Two major fractions of interferon activity are observed: a breakthrough fraction and a fraction recovered from the lectin column with 0.1 M D-galactose (Fig. 6A). Both fractions preserve their identity upon rechromatography (Fig. 6B and C) and are therefore apparently genuine components. Chromatography on this lectin can be very useful for the purification of interferon fraction selectively adsorbed. Additionally, the selective binding of one interferon component indicates the presence and availability of D-galactose

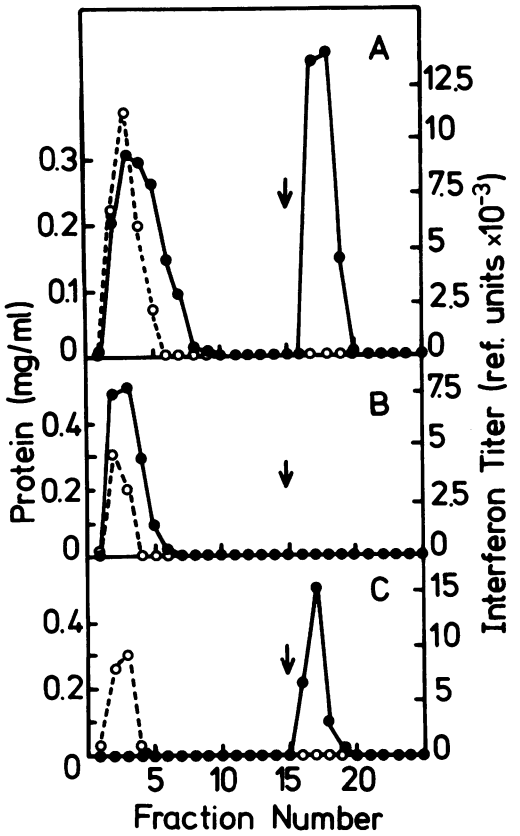

FIG. 6. *Chromatography of mouse L-cell interferon (Newcastle disease virus induced) on B. simplicifolia lectin-agarose. An interferon preparation was dialyzed against PBS, containing calcium chloride at 1 mM concentration, for 16 h at 4 C. The interferon sample was applied on a column (0.5 by 1 cm) of the lectin equilibrated with PBS (1 mM $CaCl_2$) at 4 C. Fractions (1 ml) were collected at a flow rate of 30 ml/cm² per h and assayed for interferon activity and protein. Symbols: O, protein; ●, interferon. (A) Elution profile of mouse interferon preparation. An interferon sample (5 ml), containing 1.4 × 10⁴ reference units of interferon activity and 1.5 mg of protein per ml, was applied on the column. The breakthrough fraction contained 98% of the applied protein and 54% of the interferon activity. Further elution with the equilibrating solvent, containing 0.1 M D-galactose (↓), resulted in the recovery of the remaining interferon activity. (B) Rechromatography of the breakthrough fraction of interferon activity. An interferon sample (2 ml), containing 1.1 × 10⁴ reference units of interferon activity per ml, was applied on the column. (The sample was derived from the breakthrough fraction of interferon activity depicted on part A.) The column was washed with PBS (1 mM $CaCl_2$), and all of the interferon activity was recovered again in the breakthrough fraction. (C) Rechromatography of the fraction eluted with D-galactose. The fraction of interferon activity recovered with D-galactose (A) was extensively dialyzed against PBS (1 mM $CaCl_2$), and a sample (2 ml), containing 1.2 × 10⁴ reference units of interferon activity per ml, was applied on a column. The column was washed with PBS (1 mM $CaCl_2$) and the breakthrough fraction contained no interferon. The retained interferon activity was recovered (↓) with PBS (1 mM $CaCl_2$) containing 0.1 M D-galactose.*

units (6); it does not necessarily indicate the absence of D-galactose on the other interferon component unretained on the column. It does indicate, however, the inaccessibility or different anomery of these D-galactose units, if present.

DISCUSSION

A slight retardation of the mouse interferon activity, with respect to bulk proteins in the breakthrough fractions, was previously reported for BSA-agarose (8); there was, however, no binding of interferon on ω-carboxypentyl-agarose (3). We now report that the interaction of mouse interferon with both sorbents can be significantly increased by lowering both the pH value and ionic strength of the solvent during the adsorption phase of the chromatography. At pH 5.0, BSA is close to its isoelectric point, and mouse interferon carries an overall positive charge (9, 13). An electrostatic interaction is therefore possible, although it cannot account by itself for the high selectivity in retention of mouse interferon. This selectivity is more easily explained by participation of a hydrophobic interaction. In the case of human interferon, the binding to immobilized albumin is exceptionally strong and can be reversed only by a hydrophobic solute such as ethylene glycol (8). This is not the case with mouse interferon, although the interaction of mouse interferon with serum albumin takes place at pH 5.0, a point at which the hydrophobic interactions of serum albumin are most pronounced in model systems (7). Nevertheless, the hydrophobic interaction of mouse interferon with hydrocarbon-coated agarose is probably the main force responsible for the high selectivity of binding, although electrostatic interactions at pH 5.0 may also occur. When electrostatic binding is probably the only force involved, as on carboxymethyl-Sephadex, the selectivity of retention of the mouse interferon molecule is very modest (9, 13).

It has been recognized that mouse interferon preparations contain many normal host cell components of similar molecular size and electric charge (10). These proteins apparently still contaminate highly purified mouse interferon preparations (9, 10, 13). Since the highly selec-

tive retention of mouse interferon on immobilized serum albumin and hydrocarbons undoubtedly is due to a different chromatographic bias than that in more classical chromatographic sorbents (9, 13) or immunoadsorbents (4, 10, 11), these new sorbents may be of critical importance for further purification of mouse interferon; this, however, remains to be established.

Other investigators have previously reported the phenomenon of heterogeneity in mouse interferon preparations on electrophoresis. For example, fast and slow components were reported to exist in interferon preparations induced by Newcastle disease virus (12, 13) or MM virus (9). The present study suggests several novel affinity sorbents that allow, for the first time, a structural characterization of interferon molecule by exploiting its hydrophobic property and glycoprotein nature. For example, it seems plausible that the two components of mouse interferon observed on *Bandeiraea* lectin chromatography correspond to those revealed previously by electrophoresis. The interrelations of all components, as observed during chromatography on BSA-agarose, ω-carboxypentyl-agarose, concanavalin A-agarose, and *Bandeiraea* lectin-agarose, will, however, require further study. This report provides a means for their efficient and clear-cut separation and thus enables their further characterization.

ACKNOWLEDGMENTS

We wish to express our appreciation to I. J. Goldstein for his generous gift of *B. simplicifolia* lectin-agarose, and for his kind advice in its use.

This work was supported by Public Health Service Center Grant in Viral Chemotherapy (CA 14801-01) from the National Cancer Institute.

LITERATURE CITED

1. Böhlen, P., S. Stein, W. Dairman, and S. Udenfriend. 1973. Fluorometric assay of proteins in the nanogram range. Arch. Biochem. Biophys. **155**:213-220.
2. Chadha, K. C., M. W. Davey, D. M. Byrd, and W. A. Carter. 1974. Differential production of interferon and refractoriness inducing principle in L cells. Infect. Immun. **10**:1057-1061.
3. Davey, M. W., J. W. Huang, E. Sulkowski, and W. A. Carter. 1975. Hydrophobic binding sites on human interferon. J. Biol. Chem. **250**:348-349.
4. De Maeyer, E., J. De Maeyer-Guignard, and M. Vandeputte. 1975. Inhibition by interferon of delayed-type hypersensitivity in the mouse. Proc. Natl. Acad. Sci. U.S.A. **72**:1753-1757.
5. Finter, N. B. 1969. Dye uptake methods for assessing viral cytopathogenicity and their application to interferon assays. J. Gen. Virol. **5**:419-427.
6. Hayes, C. E., and I. J. Goldstein. 1974. An α-D-galactosyl-binding lectin from Bandeiraea simplicifolia seeds. J. Biol. Chem. **249**:1904-1914.
7. Hofstee, B. H. J. 1973. Hydrophobic affinity chromatography of proteins. Anal. Biochem. **52**:430-448.
8. Huang, J. W., M. W. Davey, C. J. Hejna, W. Muenchhausen, E. Sulkowski, and W. A. Carter. 1974. Selective binding of human interferon to albumin immobilized on agarose. J. Biol. Chem. **249**:4665-4667.
9. Knight, E. 1975. Heterogeneity of purified mouse interferons. J. Biol. Chem. **250**:4139-4144.
10. Ogburn, C. A., K. Berg, and K. Paucker. 1973. Purification of mouse interferon by affinity chromatography on anti-interferon globulin-Sepharose. J. Immunol. **111**:1206-1218.
11. Sipe, J. D., J. De Maeyer-Guignard, B. Fauconnier, and E. De Maeyer. 1973. Purification of mouse interferon by affinity chromatography on a solid-phase immunoadsorbent. Proc. Natl. Acad. Sci. U.S.A. **70**:1037-1040.
12. Stewart, W. A. II. 1974. Distinct molecular species of interferons. Virology **61**:80-86.
13. Yamamoto, Y., K. Tsukui, M. Ohwaki, and Y. Kawade. 1974. Electrophoretic characterization of purified mouse L-cell interferon of high specific activity. J. Gen. Virol. **23**:23-32.