Binding of Human Interferons to Immobilized Cibacron Blue F3GA: The Nature of Molecular Interaction†

Wieslaw J. Jankowski,‡ Wittiches von Muenchhausen, Eugene Sulkowski, and William A. Carter*

ABSTRACT: Blue Dextran (Cibacron Blue F3GA-dextran) was immobilized on cyanogen bromide activated agarose and used as a ligand for human fibroblast and leukocyte interferons in a solvent of phosphate-buffered (pH 7.4), physiological saline (0.15 M NaCl). Fibroblast interferon binds completely and is not displaced from the column by an increase in ionic strength of the solvent (1.0 M NaCl); it can be, however, recovered with ethylene glycol, indicating the hydrophobic nature of interaction. Leukocyte interferon also binds to Blue Dextran–agarose but it can be recovered simply by an increase in the ionic strength of the solvent, indicating primarily the electrostatic nature of binding. Attempts to displace both interferons selectively with nucleotides and aromatic amino acids were unsuccessful. When Cibacron Blue F3GA is immobilized directly to agarose matrix or via molecular arm, the strength of binding of fibroblast interfern is significantly decreased, although ethylene glycol is still required for its displacement from the column. Leukocyte interferon, by contrast, does not bind at all under the same solvent conditions; it does bind when the pH value of the solvent is in the range 3–5, i.e., below its isoelectric point. Human fibroblast interferon binds completely to: aminobenzene, aminonaphthalene, and aminoanthracene, all immobilized on agarose, and it can be recovered with ethylene glycol. In contrast, human leukocyte interferon does not bind to benzene–agarose; it is retarded on naphthalene–agarose and completely retained on an anthracene–agarose column. All data point to a higher intrinsic hydrophobicity of human fibroblast interferon vis-á-vis human leukocyte interferon. Selective binding of human fibroblast interferon to Cibacron Blue F3GA–agarose results in a significant purification, about 800-fold.

Many proteins bind avidly to Blue Dextran, i.e., dextran substituted with Cibacron Blue F3GA: some proteins recognize blue chromophore due to its structural similarity to nucleotide cofactors, while others bind in a less specific manner (Thompson et al., 1975). The first group of proteins can be displaced from affinity columns at low concentrations of specific ligands, whereas the second group requires high salt concentrations for elution, indicating nonspecific ionic binding. The potential of the blue chromophore for hydrophobic or π–π interactions is also evident from its chemical structure. This diversity of possible interactions made Cibacron Blue F3GA an attractive potential ligand for human interferons, a subject of this report.

In order to evaluate some of the binding characteristics of this polyaromatic dye, several ligands of an increasing aromaticity—namely, aminobenzene, aminonaphthalene, and aminoanthracene—were coupled to an agarose matrix and their binding properties for human interferon were investigated. We now report that both human interferons bind to Cibacron Blue F3GA under appropriate solvent conditions and with a particular mode of its immobilization. This binding results in a significant purification of fibroblast interferon and reveals the molecular heterogeneity of human leukocyte interferon under solvent conditions of physiological saline. The differential interaction of human interferons with immobilized aromatic amines provides an additional characterization of their relative hydrophobicities first reported from this laboratory (Huang et al., 1974; Davey et al., 1975, 1976).

Experimental Procedure

Materials. Cibacron Blue F3GA was obtained from CIBA-GEIGY Co.; Blue Dextran 2000, CNBr-activated Sepharose 4B, and superfine Sephadex G-100 were purchased from Pharmacia Fine Chemicals.

Affi-Gel 10 (activated *N*-hydroxysuccinimide ester of succinylaminopropylagarose) was obtained from BioRad Laboratories. Aniline and 2-aminonaphthalene were purchased from Sigma Chemical Co. and 2-aminoanthracene from Aldrich Chemical Co. Bovine serum albumin and human serum albumin, both crystalline preparations, were obtained from Miles Laboratories. Fluorescamine was obtained from Roche Diagnostics; ethylene glycol was purchased from Baker Chemical Co., and dioxane from Fisher Scientific Co. All other reagents were of analytical grade; double-glass-distilled water was used for all solutions.

Source and Assay of Interferons. Human fibroblast interferon, induced by $rI_n \cdot rC_n$,[1] was prepared in human foreskin diploid fibroblasts, essentially according to Havell and Vilček (1972). Human leukocyte interferon induced by Newcastle disease virus was prepared essentially according to Pidot et al. (1972). Interferon activity was assayed on human fibroblasts by a modification of the colorimetric technique of Finter (1969) using vesicular stomatitis virus as the challenge virus (multiplicity of infection, 0.1) (Carter et al., 1972). In our assay, the titer of a human reference interferon (G 023-901-527) is 12 000 to 18 000 in comparison with its nominal value of 20 000 units.

Chromatographic Procedure. Interferon preparations were dialyzed against 0.02 M sodium phosphate (pH 7.4) containing

† From the Department of Medical Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York 14263. *Received May 3, 1976.* This work was supported, in part, by a Center Grant in Viral Chemotherapy (CA 14801-01) and by National Institutes of Health Research Grant No. AI 12933-01.

‡ On leave of absence from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

[1] Abbreviations used: EG, ethylene glycol; $rI_n \cdot rC_n$, poly(riboinosinic acid)·poly(ribocytidylic acid); NAD^+ and NADH, nicotinamide adenine dinucleotide and its reduced form.

0.15 M NaCl for 16 h at 4 °C. The chromatography was routinely performed on columns (0.9 × 5 cm) equilibrated with 0.02 M sodium phosphate (pH 7.4) containing 0.15 M NaCl at room temperature (unless otherwise stated). The columns were developed at a flow rate of 30 ml per cm^2 per h; fractions (1 ml) were collected. Fractions to be tested for interferon activity were collected into 0.5 ml of a 1% solution of bovine serum albumin in 0.02 M sodium phosphate (pH 7.4), 0.15 M NaCl.

Molecular Weight Determination. All determinations were done by sieving on a superfine Sephadex G-100 column (1.5 × 80 cm) equilibrated with 0.02 M sodium phosphate (pH 7.4) containing 0.15 M NaCl at 4 °C. The column was developed at a flow rate of 2.8 ml per cm^2 per h; 0.7-ml fractions were collected. The following proteins were used as molecular weight markers: bovine serum albumin (mol wt 67 000), egg albumin (mol wt 45 000), α-chymotrypsinogen A (mol wt 25 000), and bovine pancreatic ribonuclease A (13 700).

Protein Determination. Protein concentration was measured by absorbance at 280 nm or by fluorometric assay (Böhlen et al., 1973) using bovine serum albumin as a standard.

Couplings. Coupling of Blue Dextran 2000 to CNBr-activated Sepharose 4B. Blue Dextran, 500 mg, was dissolved in 25 ml of 0.4 M bicarbonate, pH 9.5. Activated agarose, 7.5 g, was washed with 1 mM HCl on a glass filter for 15 min at room temperature. The solution of Blue Dextran and the agarose cake were combined and the suspension was mixed in a screw-cap bottle on a slowly rotating wheel at 4 °C for 24 h. The coupling was terminated by an extensive wash of the cake with 0.4 M bicarbonate, pH 9.5 (500 ml), water (5000 ml), 0.02 M sodium phosphate (pH 7.4) containing 0.15 M NaCl (500 ml), and finally with 0.02 M sodium phosphate (pH 7.4), 0.15 M NaCl, containing 50% (v/v) ethylene glycol (500 ml). About 15 mg of Blue Dextran was coupled to 1 ml of bed volume of the cake (80% coupling efficiency). Blue Dextran-agarose was allowed to age for 10 days at 4 °C before use.

Coupling of Cibacron Blue F3GA to CNBr-Activated Sepharose 4B. Cibacron Blue (50 mg) was coupled to 7.5 g of activated agarose and the cake was washed as described above for coupling of Blue Dextran 2000. About 1 mg of the dye was coupled to 1 ml of bed volume of the agarose cake. Cibacron Blue F3GA-agarose was aged for 10 days before use.

Coupling of Cibacron Blue F3GA to Affi-Gel 10. A solution of dye (1 mg/ml), 25 ml, in 0.1 M sodium phosphate (pH 7.0) was added to a bottle of Affi-Gel 10 (1 g of dehydrated material) and the coupling was allowed to proceed for 24 h at 4 °C, while the bottle was secured on a rotating wheel. The washing procedure was the same as described above for preparing of Blue Dextran-agarose.

Coupling of Aniline, 2-Aminonaphthalene, and 2-Aminoanthracene to CNBr-Activated Sepharose 4B. All solutions (20 ml) of amines (0.05 M) were prepared in a solvent of dioxane (3 parts) and sodium phosphate (0.4 M), pH 7.0 (1 part). The coupling to 5 g of activated agarose was allowed to proceed for 24 h at 10 °C. The washing of the substituted agaroses was done with 75% (v/v) dioxane in 0.1 M sodium phosphate, 500 ml, and with 50% (v/v) ethylene glycol in 0.02 M sodium phosphate (pH 7.4) containing 0.15 M NaCl (500 ml). The extent of substitution was not estimated; the agarose cakes were allowed to age for 10 days at 4 °C.

Results

The chromatography of human fibroblast and leukocyte interferons on Blue Dextran-agarose columns is illustrated in Figure 1. Fibroblast interferon binds completely (Figure 1A)

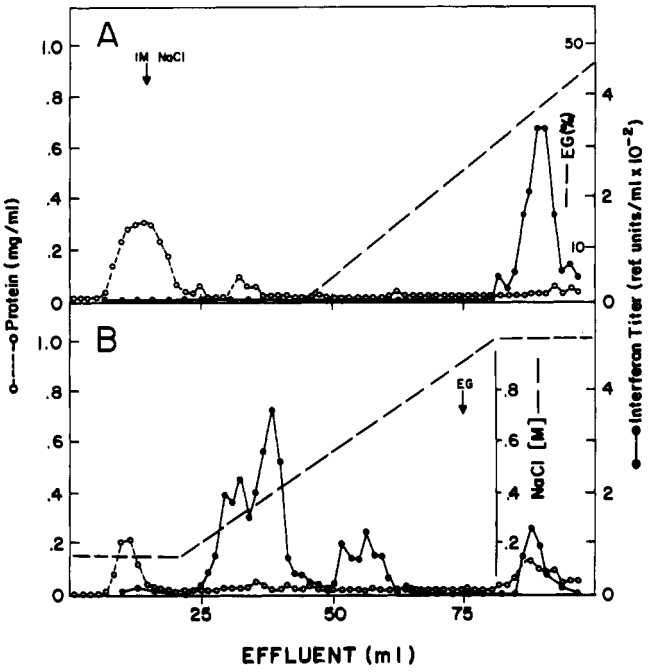

FIGURE 1: Chromatography of human fibroblast and leukocyte interferons on Blue Dextran-agarose. (A) Human fibroblast interferon (9 ml), 500 reference units per ml, was applied on a column (0.9 × 15 cm); the column was washed with 1 M NaCl in 0.02 M sodium phosphate, pH 7.4 (↓) and then a linear concentration gradient of ethylene glycol (EG) was developed by mixing 30 ml of equilibrating solvent with 30 ml of 50% (v/v) ethylene glycol in the equilibrating solvent. (B) Human leukocyte interferon (2 ml, 5400 reference units per ml) was applied on a column (0.9 × 15 cm); the column was washed with equilibrating buffer and then a linear concentration gradient of sodium chloride was developed by mixing 30 ml of 0.15 M NaCl in 0.02 M sodium phosphate, pH 7.4, and 30 ml of 1.0 M NaCl in 0.02 M sodium phosphate, pH 7.4. Finally, the column was developed with 50% (v/v) ethylene glycol in 1.0 M NaCl, 0.02 M in sodium phosphate, pH 7.4 (↓EG).

when applied in phosphate-buffered (0.02 M, pH 7.4), physiological saline (0.15 M NaCl). The binding cannot be reversed by an increase in ionic strength of the solvent (1 M NaCl) indicating the nonelectrostatic nature of interaction. However, interferon activity can be displaced with a hydrophobic solute, ethylene glycol, at about 35% concentration. Leukocyte interferon also binds to Blue Dextran-agarose under the same solvent conditions as for fibroblast interferon (Figure 1B). The development of the column with a linear concentration gradient of sodium chloride displaced practically all of the applied interferon activity. A small fraction of activity can be recovered with 50% (v/v) ethylene glycol and thus resembles fibroblast interferon in its binding behavior; this fraction of activity is present in the majority of human leukocyte interferons preparations tested. The bulk of leukocyte interferon activity is eluted, however, with a salt gradient, thus indicating principally electrostatic nature of interaction between leukocyte interferon and Blue Dextran-agarose. A resolution of two leukocyte interferon fractions, the first eluted at about 0.3 M NaCl (and designated as component I), and a second eluted at about 0.6 M NaCl (and designated as component II), was observed in all individually prepared leukocyte interferon preparations. The quantitive variability of both components in two extreme cases, out of a total of eight investigated, is illustrated in Figure 2. The rechromatography of components I and II on Blue Dextran-agarose firmly established their chromatographic identity (not illustrated).

In order to analyze further the observed heterogeneity, a

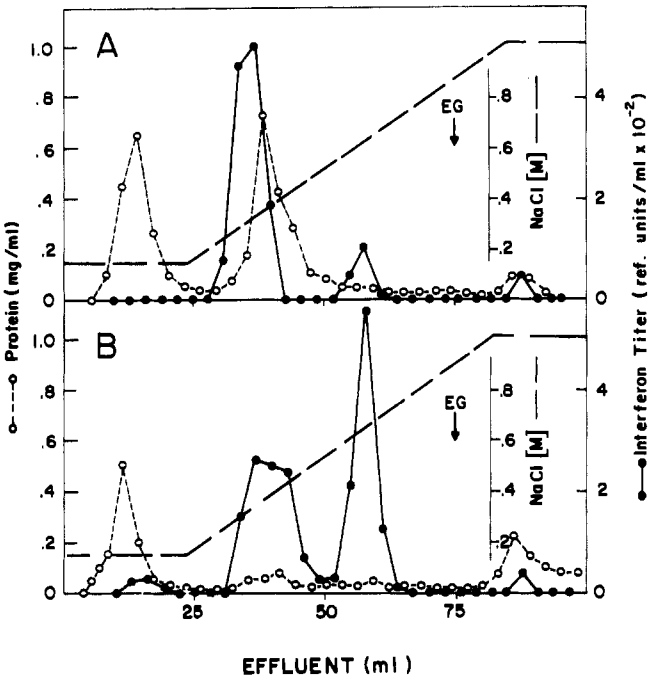

FIGURE 2: Quantitative variability of human leukocyte interferon components I and II. The chromatographic procedure on Blue Dextran-agarose was identical with that described in Figure 1B. (A) Leukocyte interferon preparation enriched in component I. (B) Leukocyte interferon preparation enriched in component II.

crude leukocyte interferon preparation, as well as components I and II (obtained upon chromatography on Blue Dextran-agarose), were sieved on a Sephadex G-100 column (not illustrated). In three individual runs, the apparent molecular weight of leukocyte interferon (crude preparation) is about 26 500, in an excellent agreement with values reported by others (Bose et al., 1976). The molecular weights of components I (24 000) and II (26 500) are so close that it is difficult to ascertain if the differences are statistically valid; it appears most likely that their molecular weights are identical. Therefore, differences in molecular weight do not seem to underlie the molecular heterogeneity of leukocyte interferon as displayed on Blue Dextran-agarose.

An attempt was also made (not illustrated) to elute selectively one or both components with NAD^+ (1-10 mM), NADH (1-10 mM), L-tryptophan (10 mM), and L-tryptophyl-L-tryptophan (10 mM). Upon prolonged elution of the column with NAD^+ (10 mM) or NADH (10 mM), component I was displaced; control experiments established, however, that component I could be eluted simply with the equilibrating solvent and prolonged washing. Component II always required an increase in sodium chloride concentration to about 0.6 M for displacement from the column. These experiments thus failed to establish a specific form of affinity interaction of leukocyte interferon which might have been revealed by selective elution with any of the nucleotides or aromatic amino acids tested. In parallel experiments, human fibroblast interferon could not either be displaced from Blue Dextran-agarose

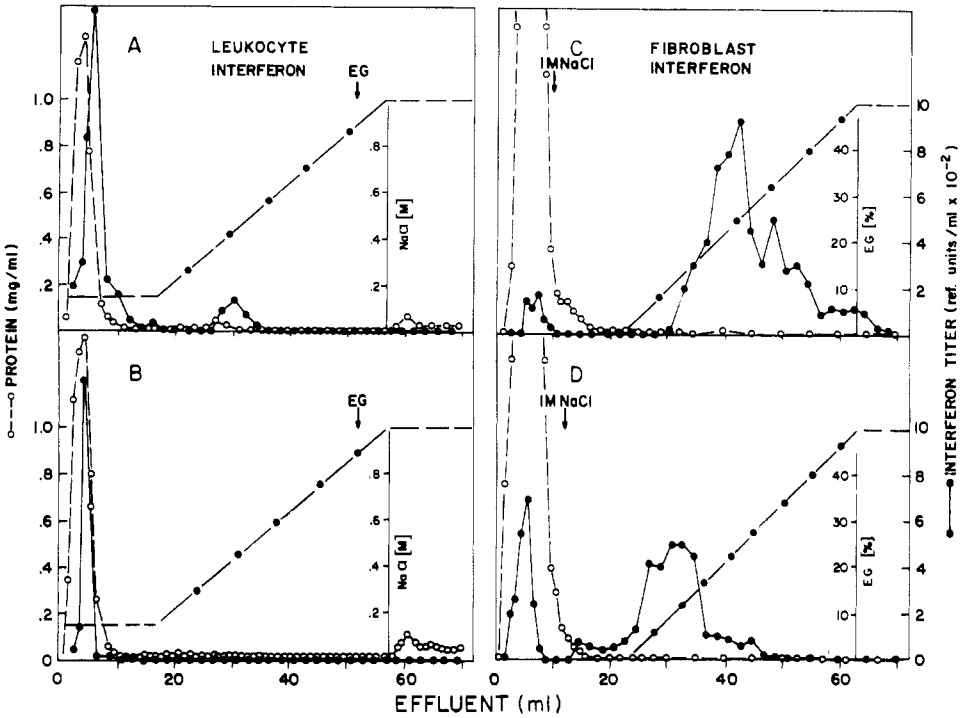

FIGURE 3: Chromatography of human leukocyte and fibroblast interferons on Cibacron Blue F3GA immobilized directly or via a molecular arm to an agarose matrix. (A) Elution of leukocyte interferon from Cibacron F3GA-agarose. An interferon preparation, 3000 units per ml, 3 ml, was applied and the column (0.9 × 5 cm) was washed with 0.15 M NaCl, 0.02 M sodium phosphate, pH 7.4. A linear concentration gradient of sodium chloride was then developed by mixing 0.15 M NaCl, 0.02 M sodium phosphate, pH 7.4 (20 ml), and 1.0 M NaCl, 0.02 M sodium phosphate, pH 7.4 (20 ml). Finally, the column was developed with 50% (v/v) ethylene glycol in 1 M NaCl, 0.02 M sodium phosphate, pH 7.4. (B) Elution of leukocyte interferon from Cibacron Blue F3GA-Affi-Gel 10. An interferon preparation, 1300 units per ml, 3 ml, was applied and the column (0.9 × 5 cm) developed as described above. (C) Elution of fibroblast interferon from Cibacron Blue F3GA-agarose. An interferon preparation, 3600 units per ml, 5 ml, was applied and the column (0.9 × 5 cm) was washed with 0.15 M NaCl, 0.02 M sodium phosphate, pH 7.4. The column was then equilibrated with 1 M NaCl (↓) in 0.02 M sodium phosphate, pH 7.4. A linear concentration gradient of ethylene glycol was then developed by mixing 1 M NaCl, 0.02 M sodium phosphate, pH 7.4 (20 ml), and 50% (v/v) ethylene glycol in 1 M NaCl, 0.02 M sodium phosphate, pH 7.4 (20 ml). Finally, the column was washed with 50% (v/v) ethylene glycol in 1 M NaCl, 0.02 M sodium phosphate, pH 7.4. (D) Elution of fibroblast interferon from Cibacron Blue F3GA-Affi-Gel 10. An interferon preparation, 2700 units per ml, 5 ml, was applied and the column (0.9 × 5 cm) was developed as described above for Figure 3C.

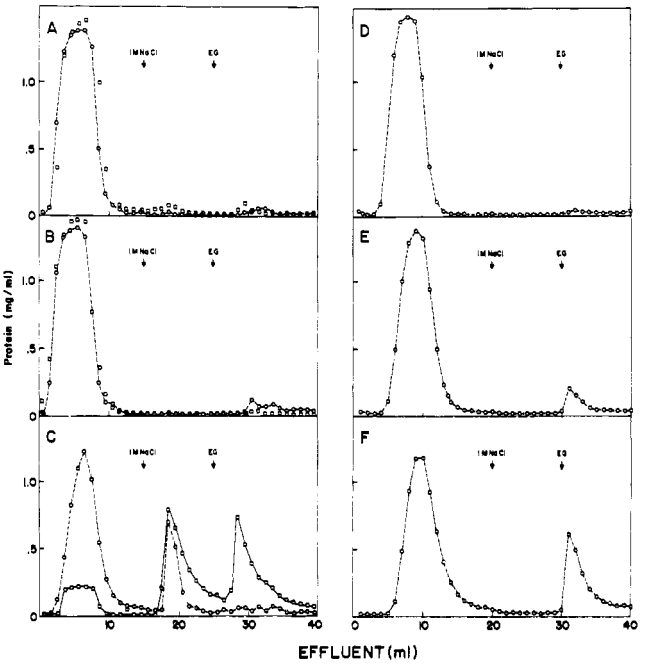

FIGURE 4: Chromatography of bovine serum albumin and human serum albumin on Cibacron Blue F3GA-agarose (A), on Cibacron Blue F3GA-Affi-Gel 10 (B), on Blue Dextran-agarose (C), and of bovine serum albumin on benzene-agarose (D), naphthalene-agarose (E), and anthracene-agarose (F). Solutions (0.05%) of both albumins, 5 ml, were applied on columns (0.9 × 5 cm); the columns were washed with an equilibrating buffer and developed with 1 M NaCl (0.02 M sodium phosphate, pH 7.4) and 50% (v/v) ethylene glycol in 1 M NaCl (0.02 M sodium phosphate, pH 7.4) as indicated by arrows. (O---O) Bovine serum albumin; (□---□) human serum albumin.

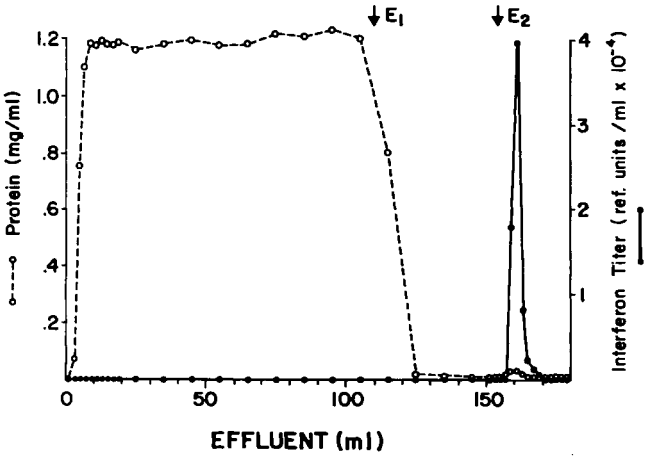

FIGURE 5: Purification of human fibroblast interferon on Cibacron Blue F3GA-agarose. An interferon preparation, 100 ml, containing 1650 units and 1.25 mg of protein per ml, was dialyzed against 0.02 M sodium phosphate, pH 7.4, 0.15 M NaCl overnight at 4 °C. The prepartion was applied on the column at 4 °C by means of a peristaltic pump at a flow rate of 60 ml per cm^2 per h. The eluent from the column (0.9 × 9 cm) was divided by a stream-splitting device in a ratio of 1 to 9. The 10% portion of the eluent was collected into 1 ml of a 1% solution of bovine serum albumin and used for assay of the interferon activity. The 90% portion of the eluent was used to measure the protein concentration. Fractions from 0 to 20 ml and from 150 to 180 ml had 2 ml each; fractions from 20 to 150 ml were collected as 10-ml portions. The break-through fractions from 6 to 120 ml contained about 99% of the applied protein and about 1% of the applied interferon activity. Further elution of the column with I M NaCl in 0.02 M sodium phosphate, pH 7.4, E_1, did not release any substantial amount of protein or interferon activity. The column was then washed with E_1 containing ethylene glycol, 50% (v/v) and denoted as E_2. Recovery of interferon activity with E_2 was 96%. Peak fraction 160–163 ml contained 7.9×10^4 units of interferon activity and 70 μg of protein; the specific activity is thus 1.1×10^6 units per mg of protein.

by an identical set of nucleotides, aromatic amino acids, or dipeptides.

Figure 3 illustrates the chromatographic behavior of human leukocyte and fibroblast interferons on Cibacron Blue F3GA-agarose and Cibacron Blue F3GA-Affi-Gel 10. The blue dye is coupled in both cases through an amino group with the subsequent formation of an isourea bond (Cibacron Blue F3GA-agarose) or a peptide bond (Cibacron Blue F3GA-Affi-Gel 10).[2] Immediately, it is evident that leukocyte interferon does not interact with either (Figures 3A,B) under the solvent conditions employed (phosphate-buffered, physiological saline). The binding of leukocyte interferon to Cibacron Blue F3GA-agarose can be, however, observed between pH 5 and 3, presumably because the interferon molecule, then below its isoelectric point (Bose et al., 1976), is electrostatically attracted by the negatively charged sulfonyl groups of the chromophore. Human fibroblast interferon binds to both Cibacron Blue F3GA-agarose and Cibacron Blue F3GA-Affi-Gel 10, although both adsorbents (Figure 3C,D) are somewhat "leaky" and the activity is displaced at 25 and 10% concentrations of ethylene glycol, respectively, i.e., significantly lower than that on Blue Dextran-agarose (Figure 1A).

Fibroblast interferon preparations usually contain fetal calf serum and, during purification, interferon fractions are frequently collected into solutions of bovine serum albumin or human serum albumin. Therefore, the chromatographic behavior of both bovine serum albumin and human serum albumin on Cibacron Blue F3GA immobilized in diverse ways may be of critical importance in the choice of an appropriately immobilized ligand for purification purposes. To this end, both albumins were chromatographed on Cibacron Blue F3GA-agarose (Figure 4A), Cibacron Blue F3GA-Affi-Gel 10 (Figure 4B), and Blue Dextran-agarose (Figure 4C). Bovine serum albumin binds considerably to Blue Dextran-agarose, although it can be displaced at high ionic strength of the solvent. This is not the case, however, for human serum albumin, since a significant amount remains bound at high ionic strength and would, therefore, be displaced with fibroblast interferon upon elution of the column with ethylene glycol (Figure 1A). In view of this observation, Cibacron Blue F3GA-agarose was our adsorbent of choice for a purification step of fibroblast interferon. Figure 5 illustrates purification of fibroblast interferon from a crude preparation: in a single step, an 800-fold purification was achieved with a 95% recovery of activity. The purification of human fibroblast interferon on Cibacron Blue F3GA is thus as efficient as that on concanavalin A-agarose (Davey et al., 1976) and on L-tryptophan-agarose (Sulkowski et al., 1976) in spite of the diversity of ligands utilized. It seems plausible that the occurrence of a hydrophobic interaction between fibroblast interferon and all of these ligands is the primary cause of their similar purification efficiency.

The binding of fibroblast and leukocyte interferons to the polyaromatic blue dye raises a question as to whether any of these interferons will bind to structures more simple than Cibacron Blue F3GA but sharing with it some physicochemical properties. To this end, we immobilized aromatic amines on cyanogen bromide activated agarose, and they were studied as potential ligands for human interferons. The results are illustrated in Figure 6. The interaction of human leukocyte in-

[2] Blue Dextran coupling to CNBr-activated Sepharose 4B occurs only via a small portion of the chromophore molecules available on dextran and thus the remainder of them carries free amino groups.

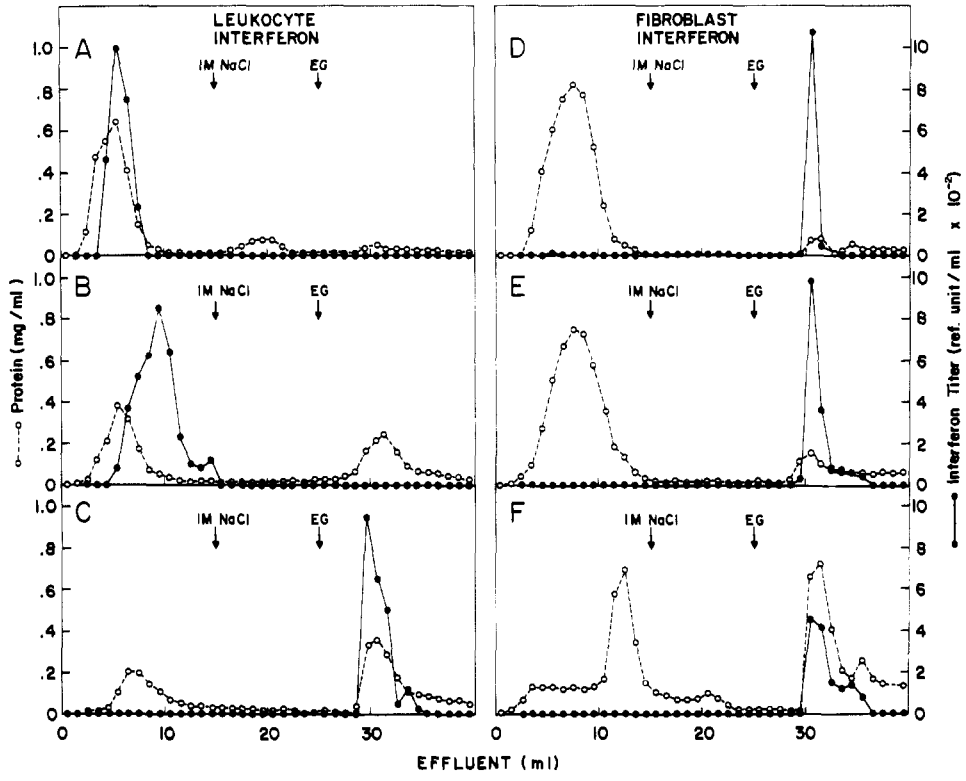

FIGURE 6: Chromatography of human interferons on aniline, aminonaphthalene, and aminoanthracene all immobilized on agarose. Chromatography was performed as described in Experimental Procedure. All columns were developed with 1 M NaCl (0.02 M sodium phosphate, pH 7.4) and with 50% (v/v) of ethylene glycol in 1 M NaCl (0.02 M sodium phosphate, pH 7.4) as indicated by arrows (↓). Human leukocyte interferon: (A) benzene-agarose; (B) naphthalene-agarose; (C) anthracene-agarose. A sample of leukocyte interferon (1.5 ml) was applied; the samples contained 1500 units per ml (A), 2500 units per ml (B), and 1850 units per ml (C). Human fibroblast interferon: (D) Benzene-agarose; (E) naphthalene-agarose; (F) anthracene-agarose. A sample of fibroblast interferon (3.5 ml) was applied; the samples contained 500 units per ml (D), 800 units per ml (E), and 740 units per ml (F).

terferon with benzene-agarose is negligible (Figure 6A); it becomes, however, pronounced with naphthalene-agarose, resulting in partial separation from the break-through proteins (Figure 6B). Moreover, a significant portion of proteins, resident in crude preparations of leukocyte interferon, interacts very strongly with the naphthalene ligand and can be displaced from the column only with ethylene glycol, 50% (v/v). When anthracene-agarose is used as a sorbent (Figure 6C), leukocyte interferon is retained and can be displaced only with ethylene glycol, 50% (v/v). The binding of fibroblast interferon is already complete with benzene-agarose (Figure 6D) in sharp contrast to leukocyte interferon (Figure 6A). Predictably, the binding of fibroblast interferon on naphthalene-agarose (Figure 6E) and anthracene-agarose (Figure 6F) is complete. The amount of other protein(s) bound to these sorbents, and subsequently displaced with ethylene glycol, increases with the molecular size of the aromatic ligands (Figure 6D–F).

Figure 4 illustrates the binding behavior of bovine serum albumin to benzene-agarose (Figure 4D), naphthalene-agarose (Figure 4E), and anthracene-agarose (Figure 4F). Immediately, it is clear that the amount of bound albumin depends on the surface area of the immobilized ligand, and that the interaction between albumin and the ligand cannot be readily reversed by an increase in ionic strength of the solvent. A comparison of Figures 4 and 6 strongly suggests that the protein primarily retained from both leukocyte and fibroblast interferon preparations, and then displaced by ethylene glycol, is bovine serum albumin. Furthermore, it is clear that the binding of leukocyte interferon and serum albumin can be independent: namely, naphthalene-agarose binds significant amount of albumin but not leukocyte interferon.

Discussion

Both human fibroblast and leukocyte interferons bind to immobilized Cibacron Blue F3GA. The binding of leukocyte interferon is apparently primarily electrostatic in nature and, therefore, not highly selective. The immobilized dye must have a free amino group for the binding to take place: leukocyte interferon binds to Blue Dextran-agarose but not to Cibacron Blue F3GA-agarose or Cibacron Blue F3GA-Affi-Gel 10. The requirement for a free amino group is not so stringent for fibroblast interferon, although the binding to Blue Dextran-agarose is still much stronger than to Cibacron Blue F3GA-agarose and Cibacron Blue F3GA-Affi-Gel 10 (chromophores coupled via amino groups).

The involvement of a hydrophobic interaction in the binding of leukocyte interferon to the blue chromophore may be induced by a decrease in the overall polarity of the latter, by lowering the pH value and thereby reversing the extent of dissociation of its sulfonyl groups. In initial experiments, we have indeed observed that leukocyte interferon binds to Cibacron Blue F3GA-agarose at pH 5.0–3.0. Further lowering of the pH value was not feasible due to the instability of the agarose matrix.[3]

In contrast, the hydrophobic interaction of fibroblast interferon with the blue chromophore is quite strong regardless of the mode of coupling of the latter. The binding of fibroblast interferon to benzene-agarose may indicate the minimal size of the interacting surface between fibroblast interferon and Cibacron Blue F3GA. The conclusion that the binding sites

on human fibroblast interferon for ligands of aromatic character are distinct fro those on leukocyte interferon is strongly indicated by the data of this report. The fine topography of these binding sites is still a matter of speculation, although it seems most reasonable to assume the involvement of aromatic side chains of amino acid residues on the interferon molecule. Recent findings from this laboratory have indicated that fibroblast interferon binds to immobilized L-tryptophan while leukocyte interferon requires a dipeptide (L-tryptophyl-L-tryptophan) for binding to occur (Sulkowski et al., 1976); these results reinforce our current conclusion that the interaction with blue chromophore may be to a significant extent hydrophobic for both interferons. The binding of leukocyte interferon, although apparently largely electrostatic in nature, may also have a hydrophobic component. The interaction of leukocyte interferon with immobilized aromatic amines is certainly supportive of this contention.

[3] This observation is being further pursued at present as the bue dye immobilized to a stable matrix may provide a very useful ligand at pH 2–3 for concentrations of leukocyte interferon which are routinely adjusted to these low pH values in order to inactivate the Newcastle disease virus used as an inducer.

References

Böhlen, P., Stein, S., Dairman, W., and Udenfriend, S. (1973), *Arch. Biochem. Biophys. 155,* 213.

Bose, S., Gurari-Rotman, D., Ruegg, U. T., Corley, L., and Anfinsen, C. B. (1976), *J. Biol. Chem. 251,* 1659.

Carter, W. A., Pitha, P. M., Marshall, L. W., Tazawa, I., Tazawa, S., and Ts'o, P. O. P. (1972), *J. Mol. Biol. 70,* 567.

Davey, M. W., Huang, J. W., Sulkowski, E., and Carter, W. A. (1975), *J. Biol. Chem. 250,* 348.

Davey, M. W., Sulkowski, E., and Carter, W. A. (1976), *Biochemistry 15,* 704.

Finter, N. B. (1969), *J. Gen. Virol. 5,* 419.

Havell, E. A., and Vilček, J. (1972), *Antimicrob. Agents Chemother. 2,* 476.

Huang, J. W., Davey, M. W., Hejna, C. I., Muenchhausen, W., Sulkowski, E., and Carter, W. A. (1974), *J. Biol. Chem. 249,* 4665.

Pidot, A. L. R., O'Keefe III, G., McManus, M., and McIntyre, O. R. (1972) , *Proc. Soc. Exp. Biol. Med. 140,* 1263.

Sulkowski, E., Davey, M. W., and Carter, W. A. (1976), *J. Biol. Chem. 251,* 5381.

Thompson, S. T., Cass, K. H., and Stellwagen, E. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 669.