PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 486-489 (1976)

Affinity of Human Fibroblast Interferon for Blue Dextran (39574)

T. C. CESARIO, PATRICIA SCHRYER, ADRIAN MANDEL, AND J. G. TILLES

Department of Medicine, University of California, Irvine, California 92668; and National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California 94035

Recently, several investigators have reported the ability of interferon to bind to various substances (1-3) and have indicated that such methods are of value in the purification process. Furthermore, information relative to such interactions is useful in defining the characteristics of the interferon molecule. When estimating the molecular weight of crude interferon, we first realized that human fibroblast interferon binds to blue dextran (BD), the marker used to estimate void volume in gel chromatography. (BD is a sulfonated polyaromatic dye bound to dextran, a cross-linked complex of dextrose polymers). We have studied the affinity of BD for human interferon and find that the binding can be reversed through the addition to KCl. In this communication, we will report our studies on the interaction between blue dextran and human interferon.

Methods. Blue Dextran Crystalline, Pharmacia Chemicals (Piscataway, N. J.), was dissolved in distilled water at a concentration of 1 mg/ml, passed through a 300-nm Millipore filter, and stored at 4° in 50-ml aliquots.

Interferon preparations. Interferon was induced with a complex of polyriboinosinic acid-polyribocytidylic acid (IC) and diethylaminoethyl dextran (DEAE-D) in human muscle skin fibroblasts (MSF) subsequently maintained in Liebovitz L-15 media supplemented with 5% fetal calf serum (FCS) and glutamine (30 μg/ml), arginine (90 μg/ml), glucose (1 mg/ml), potassium penicillin G (150 U/ml), and streptomycin sulfate (250 μg/ml). This method has already been reported in detail (4, 5).

Interferon assay. Interferon was assayed by the microtiter technique employing MSF cells and vesicular stomatitis virus as already described (4).

Gel chromatography. Molecular weights were determined on Sepharose 6B and Sephadex G-200 superfine gels in 1.6 by 20-cm columns calibrated with known molecular weight markers. The eluant was 0.05 *M* phosphate-buffered saline (PBS); the fraction volume, 1 ml; and the running temperature, 6°. At the conclusion of the experiment, each fraction was assayed for interferon.

Differential ultracentrifugation. Five-milliliter quantities of blue dextran solution were centrifuged at 50,000 rpm for 4 hr in the Beckman L2-65B ultracentrifuge with a SW65 rotor. The supernatant was removed and the pellet was suspended in 0.5 ml of distilled water. The process of Millipore filtration and preliminary centrifugation necessary to sterilize the BD and to eliminate smaller molecules was found to eliminate 50% of the BD originally present. Then 1 ml of an interferon preparation, either at pH 7.0 or acidified to pH 2.8 with citric acid, was added and the mixture was allowed to incubate overnight. The following day, sufficient quantities of either PBS, KCl, nicotinamide adenine dinucleotide (NAD) or adenosine 5′-triphosphate (ATP) solutions, for interferon at pH 7.0, or 0.1 *M* citric acid, for acidified interferon, were added to fill the respective ultracentrifuge tubes. After agitation to achieve even distribution of the contents, centrifugation was performed for a further 4 hr at 50,000 rpm. At the completion of this procedure, the upper 4.5 ml of supernatant was harvested and the pellet was resuspended in the lowest 0.5 ml of the solution. Pellet and supernatant either were dialyzed against PBS overnight (citric acid or KCl solutions) and then assayed for interferon activity, or were assayed directly (PBS solutions). To calculate the amount of interferon in the unsuspended pellet, the quantity of interferon in 0.5 ml of supernatant was subtracted from the total activity of the pellet solution.

Protein determinations. Protein concen-

trations were determined by the Lowry method (6).

Results. It can be seen in Fig. 1 that chromatography of interferon on Sephadex G-200 in the presence of blue dextran resulted in most of the recoverable bioactivity appearing in the void volume along with the BD. Similar results occurred on Sepharose 6B gels indicating a molecular weight of greater than 4×10^6 for both interferon and BD. In contrast, in the absence of blue dextran the recoverable interferon bioactivity peaked beyond the void volume on both gels and yielded an estimated molecular weight for MSF-IC interferon of 300,000.

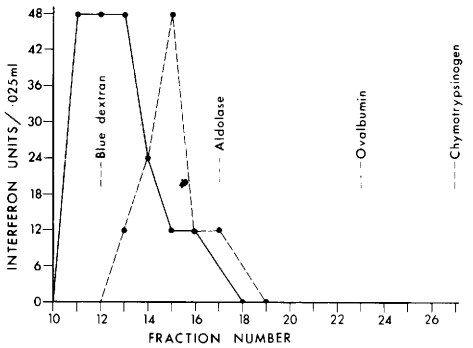

FIG. 1. Chromatography of MSF-IC on G-200 superfine gel at 6° with and without blue dextran. Column is calibrated with known molecular weight markers as indicated. One-half of the sample is applied to the column and counting is begun. Fraction size is 1 ml. Repeat testing gave similar results. (——) With blue dextran, 20,000 units applied, 30% recovery, (-----) without blue dextran, 30,000 units applied, 15% recovery.

Upon ultracentrifugation of MSF-IC interferon for 4 hr at 50,000 rpm, the interferon bioactivity remained in the supernatant (Table I). On the other hand, when the sedimentation was carried out in the presence of blue dextran (0.5 mg/ml), all of the BD and most of the interferon bioactivity appeared in the pellet (Table I). Thus, it appears that interferon binds to BD under the given conditions. Furthermore, with the quantity of BD and the volume constant, the amount of interferon bound varied linearly with the amount of interferon added. Somewhat similar results occurred at pH 2.8 with previously acidified interferon (Table I), indicating that the low molecular weight form of MSF-IC we have demonstrated at acid pH also binds to BD.

An attempt was made to free interferon bound to BD by increasing the ionic strength of the solution. The results when interferon bound to BD was exposed to

TABLE I. DISTRIBUTION OF INTERFERON ACTIVITY FOLLOWING ULTRACENTRIFUGATION WITH AND WITHOUT BLUE DEXTRAN.

	Interferon diluent	Interferon units added	Interferon units recovered		units bound mg blue dextran[a]
			Pellet	Supernatant	
A. Blue dextran absent					
pH 7.0	0.15 *M* PBS	2,400	120	2,400	–
	0.15 *M* PBS	19,200	60	10,080	–
	0.15 *M* PBS	42,240	60	42,240	–
	0.15 *M* PBS	80,000	60	80,000	–
B. Blue dextran[b] present					
pH 7.0	0.15 *M* PBS	2,400	1,920	600	768
	0.15 *M* PBS	3,840	3,840	600	1,536
	0.15 *M* PBS	9,600	9,540	660	3,816
	0.15 *M* PBS	19,200	19,800	1,320	7,920
	0.15 *M* PBS	42,240	30,480	2,640	12,192
	0.15 *M* PBS	76,800	75,600	1,320	30,240
	0.01 *M* NAD[c]	76,800	75,600	1,320	30,240
	0.01 *M* ATP[c]	76,800	76,200	660	30,480
	L-15 Media	9,600	7,440	2,640	2,976
pH 2.8	0.1 *M* Citric acid	9,600	3,600	2,640	1,440

[a] Calculated using 2.5 mg of BD, the amount remaining after preliminary centrifugation.

[b] For samples diluted in PBS, the difference in the proportion of interferon found in the pellet is highly significant when BD is present compared to when it is absent. $P < 0.01$ by Student's t test.

[c] In 0.15 *M* PBS.

varying concentrations of KCl immediately prior to and during sedimentation are recorded in Table II. It can be seen that interferon was maximally released at 2 *M* concentrations. In contrast, interferon was not released by solutions of ATP or NAD.

It was found that the attachment of interferon to blue dextran with subsequent release in 3 *M* KCl results in 3.5-fold purification (Table III).

Discussion. The present work describes the binding and release of human IC-induced fibroblast interferon to a sulfonated polyaromatic dye. The ability of interferon to bind to blue dextran at pH 7 is clearly shown both by gel chromatography and by ultracentrifugation. In both cases the addition of blue dextran changes the location of the interferon so that antiviral activity is associated with the blue dye either in the void volume or the pellet, respectively. Crude MSF-IC interferon probably binds to other proteins at pH 7.0 to give the large molecular weight form. We have previously shown that at pH 2.8, MSF-IC interferon has a molecular weight of 14,000 and therefore is presumed to have dissociated from other proteins (7). It is assumed that both the large and small molecular weight forms bind to BD, since even at this lower pH the majority (58%) of the interferon activity recovered is found associated with the blue dextran. Additionally, we have found that MSF-IC interferon partially purified by gel chromatography also attaches to blue dextran. We have not attempted to investigate the kinetics of the binding reaction rigorously but have detected similar binding when the incubation of BD and interferon prior to molecular separation has been omitted.

The binding reaction is clearly dependent on the ionic strength of the solution. By centrifugation of the interferon in the presence of blue dextran and treatment of the

TABLE II. THE RELATIONSHIP OF KCl CONCENTRATION TO THE RELEASE OF MSF-IC INTERFERON FROM BLUE DEXTRAN.

	Total recoverable interferon[a]	
KCl conc (molarity)	% Attached	% Released
0.01	100	0
0.01	100	0
0.50	63	37
1.00	27	73
2.00	8	92
2.50	8	92
3.00	8	92

[a] The interferon concentration used in these experiments varied from $10^{4.8}$ to $10^{5.2}$ units/ml.

TABLE III. PARTIAL PURIFICATION OF INTERFERON BY ATTACHMENT TO BLUE DEXTRAN AND ELUTION WITH 3 *M* KCl.

	Interferon		Protein total (mg)	Specific activity (units/mg)	Purification	% Recovered
	Total (units)	Concn (units/ml)				
Experiment A						
A. Crude interferon	9600	9600	4.2	2286	1.0 ×	–
B. Centrifugation of interferon plus BD						
Supernatant	2400	480	2.6	923	0.4 ×	25%
Pellet[a]	NT	–	NT	–	–	–
C. Centrifugation of pellet resuspended in 3 *M* KCl						
Supernatant	4800	960	0.6	8000	3.5 ×	50%
Pellet	960	1920	NT	–	–	10%
Experiment B						
A. Crude Interferon	9600	9600	5	1920	1 ×	–
B. Centrifugation of interferon plus BD						
Supernatant	<1200	–	4	–	–	–
Pellet	9600	19200	NT	–	–	100%
C. Centrifugation of pellet resuspended in KCl						
Supernatant	4800	960	0.6	8000	4.2 ×	50%
Pellet	<800	–	NT	–	–	–

[a] The supernatant was totally removed from this pellet.

pellet with 3 *M* KCl, a 3.5-fold increase in specific activity can be attained.

Thompson *et al.* (8) have recently suggested that binding of proteins to blue dextran may be either to a specific dinucleotide-fold contained within the molecule or via an ionic mechanism. Proteins containing the dinucleotide-fold should be eluted from blue dextran after exposure to low concentrations (0.01 *M*) of nucleotide ligands (8) such as ATP and NAD. It is clear from our studies that the NAD and ATP solutions do not release interferon from blue dextran, suggesting that the binding is ionic in nature.

Summary. Human fibroblast interferon has been shown to bind to blue dextran with binding that is reversible on the addition of 3 *M* KCl. Attachment of human fibroblast interferon to blue dextran and subsequent release results in a 3.5-fold purification with 50% recovery.

This investigation was supported, in part, by Grant No. 05-041-012 of the National Aeronautics and Space Administration, and by Public Health Service Grant No. T)1-AI 00466 from the National Institutes of Health.

1. Besancon, F., and Bourgeade, M., J. Immunol. **113,** 1061 (1974).
2. Davey, M., Huang, J. Sulkowski, E., and Carter, W., J. Biol. Chem. **249,** 6354 (1974).
3. Huang, J. W., Davey, M. W., Hejna, C., von Muenchhausen, W., Sulkowski, E., and Carter, W., J. Biol. Chem. **249,** 4665 (1974).
4. Cesario, T. C., and Tilles, J. G., J. Infect. Dis. **127,** 311 (1973).
5. Tilles, J. G., Proc. Soc. Exp. Biol. Med. **133,** 1334 (1970).
6. Lowry O. H., Rosebrough, N., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 256 (1951).
7. Cesario, T. C., Schryer, P. J., and Tilles, J. G., Clin. Res. **24,** 112 (1975).
8. Thompson, S., Cass, K., and Stellwagen, E., Proc. Natl. Acad. Sci. USA **72,** 669 (1975).

Received June 16, 1976. P.S.E.B.M. 1976, Vol. 153.