MOLECULAR WEIGHT STUDY OF HUMAN FIBROBLAST INTERFERON

Fred H. Reynolds, Jr. and Paula M. Pitha

The Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland

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SUMMARY. Human fibroblast interferon was electrophoresed on polyacrylamide gels containing sodium dodecyl sulfate; when the interferon activity was recovered from these gels, discrete multiple mol. wt. fractions were obtained. However, when 6 M urea was included in the gels, only a single peak of activity was obtained at 25,000 daltons. Coomasie blue stained gels of human fibroblast interferon contained impurities in the high mol. wt. region which were fractionated from the 25,000 dalton peak of interferon activity. These results suggest that the confusing multiple mol. wts. for human fibroblast interferon, which were previous reported, may represent aggregations with other proteins in the preparation.

A large degree of heterogenicity of the mol. wt.* of vertebrate interferon has been observed (1). These mol. wt. differences have been observed, even with interferon of an individual specie and individual cell type. For example, seemingly reliable mol. wt. estimates of human fibroblast interferon span the range from 200,000 to 12,000 daltons (2). Furthermore, interferons induced by non-viral compounds reportedly have larger mol. wts. than those induced by virus particles (3-6). We have previously reported that poly(rI)·poly(rC)-induced human fibroblast interferon has a mol. wt. of 96,000 daltons (as determined by gel filtration), which can be dissociated by low ionic strength or low pH to lower mol. wt. products of 24,000 and 12,000 daltons (7-9). These data gave support to the previous hypothesis that the largest mol. wt. form may be a discrete aggregate of similar, perhaps identical, subunits of interferon (10). However, these experiments did not exclude the possibility that the high mol. wt. material could also represent aggregation of human fibroblast interferon to other protein impurities

*Abbreviations Used: 1) mol. wt. - molecular weight
2) H-chain IgG - heavy chain of γG-immunoglobulins
3) L-chain IgG - light chain of γG-immunoglobulins
4) MEM - Eagle's minimal medium (with Earle salts)

in the preparation. The existence of an identical subunit structure of mouse interferon was recently challenged, and molecular heterogenicity of mouse interferon suggested (11). In the present study, we examined the mol. wt. of poly(rI)·poly(rC)-induced human fibroblast interferon by employing a modification of the technique of reversible denaturation and fractionation, which was recently described for mouse interferon (11).

MATERIALS AND METHODS. Interferon was induced from human fibroblast in cell culture as previously described (12,13). The preparations were centrifuged to remove cells and cellular debris, but they were not subjected to any further purification steps which might result in the loss of minor species of interferon. The preparation contained 3.0×10^4 international research reference units. The electrophoresis in sodium dodecyl sulfate gels and the treatment of the data were as described by Weber (14). Protein was quantitated by the Lowry method (15). Interferon titers were determined either colorimetrically, employing Vesicular stomatitis virus (New Jersey serotype) as challenge virus, or by the reduction in virus yield (15-17). All interferon assays were conducted in the presence of 15% fetal bovine serum to minimize the toxic effects of residual denaturant from the electrophoresis procedure. The observation of virus induced cytopathological effects proved to be an unreliable criterion of interferon titer for these studies due to alterations in cellular morphology induced by residual denaturant.

RESULTS AND DISCUSSION. Reversible denaturation and fractionation of human interferon by electrophoresis on sodium dodecyl sulfate polyacrylamide gels resulted in a major interferon peak occurring at a mol. wt. of approximately 76,000 daltons with additional multiple species of interferon at lower mol. wt. (Fig. 1). Although the high mol. wt. and 25,000 dalton peaks were reproducible, intermediate mol. wt. fractions occurred unpredictably in various experiments. For this reason, the electrophoresis conditions were changed to include 6 M urea in the gels and gel buffer, and the samples were prepared in the presence of 8 M urea. This technique resulted in a reproducible

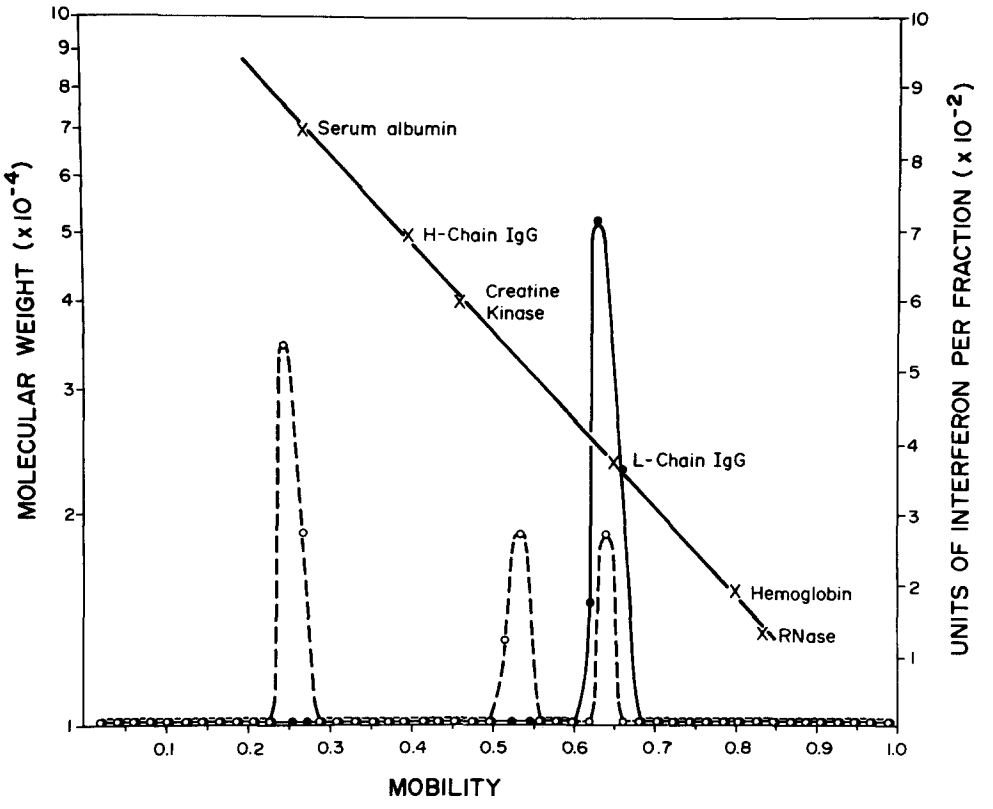

Fig. 1 - Human interferon mol wt. determination by electrophoresis in polyacrylamide gels under denaturing conditions. Interferon (1500 units) was made 1% in sodium dodecyl sulfate, and 0.1 M in 2-mercaptoethanol, heated to 95°C for 3 min., made 20% in glycerol, and applied to 6 mm diameter by 10 cm length 10% polyacrylamide gels, and electrophoresed with 8 mA per gel. Later, gel samples were electrophoresed with 8 M urea included in the sample preparation step and 6 M urea included in the gels and buffer. Gels were assayed for activity by slicing into 2 mm portions (Biorad gel slicer) squashing the gel fractions and eluding into phosphate buffered saline (0.14 M NaCl, 0.02 M sodium phosphate buffer) for one hour at 26°C, followed by the addition of fetal bovine serum to 50%. Interferon titers were determined from the sliced gel. Identical gels were fixed with 10% trichloroacetic acid and stained with Coomasie blue to locate impurities and marker proteins. Mobilities were calculated to correct for individual gel migrations and swelling as follows:

$$\text{Mobility} = \frac{\text{distance of migration}}{\text{length after destaining}} \times \frac{\text{length before staining}}{\text{distance of dye migration}}$$

Marker proteins are denoted as (x———x), sodium dodecyl sulfate gels as (o———o), and gels modified to contain urea by (•———•).

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single 25,000 mol. wt. peak of interferon (Fig. 1). This value is in good correlation with the mol. wt. of 20,000 to 25,000 daltons, which was recently

reported for human leucocyte interferon induced by Newcastle disease virus and purified by affinity chromatography, employing antibodies immobolized to an agarose support (18).

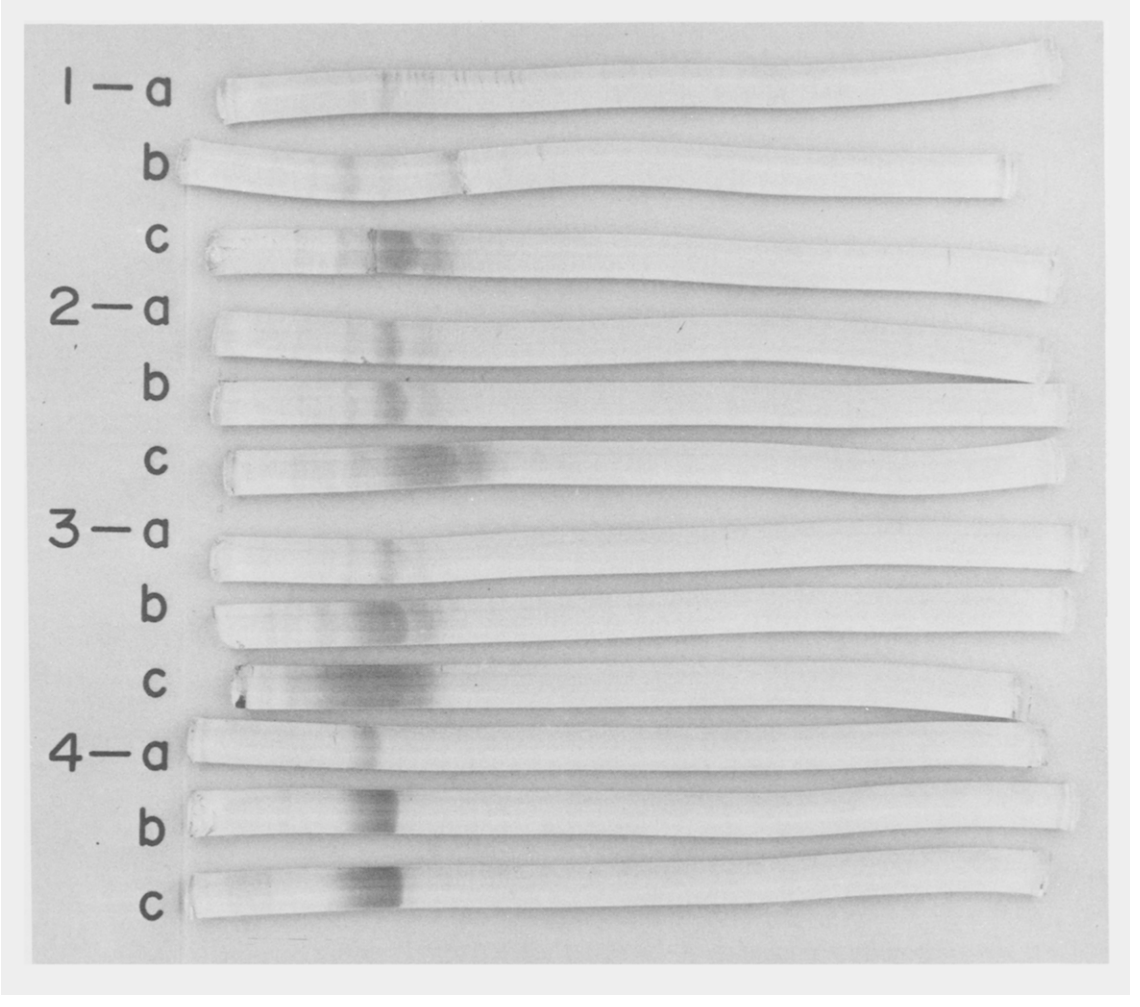

Fig. 2 - Electrophoretic separation of human fibroblast interferon from its contaminants. Electrophoresis was conducted in the presence of urea, sodium dodecyl sulfate and 2-mercaptoethanol, and the gels were stained as described in the legend to Fig. 1. Anodal migration is from left to right. Sample one contains MEM supplemented with 2% fetal calf serum. Sample two contains the same media after 24 hrs. incubation at 37°C in a humidified CO_2 incubator with normal human fibroblasts. Sample three is the same as sample two except that the cells were induced to produce interferon. Sample four contains bovine serum albumin. In all sample experiments performed, the total protein applied to the gels is (a) 10 µg, (b) 30 µg, and (c) 50 µg.

FRACTIONATION OF THE MAJOR SIALOGLYCOPEPTIDES OF THE HUMAN RED BLOOD CELL MEMBRANE

H. Furthmayr, M. Tomita, and V.T. Marchesi

Yale University School of Medicine

Department of Pathology

New Haven, Connecticut

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SUMMARY. The major sialoglycopeptides extracted from human red blood cell membranes by the LIS-phenol procedure can be separated into two distinct components by gel filtration in Ammonyx-LO. The main fraction, comprising ∿ 75% of the total, is a sialoglycopeptide containing 131 amino acids and 16 oligosaccharide chains and has been designated glycophorin A. A second glycopeptide, which can be distinguished from glycophorin A on the basis of its amino acid composition and tryptic peptide pattern, elutes in two fractions and has the capacity to form high molecular weight aggregates when analyzed by SDS gel electrophoresis.

Sialoglycopeptides isolated from human red blood cell membranes appear to be composed of at least three distinct electrophoretic species when analyzed by SDS-gel electrophoresis (1-3). These components, designated PAS-1, -2, -3 (4), have apparent molecular weights of 83000, 45000, and 25000, but their actual molecular weights are still unknown since it is now recognized that their high degree of glycosylation (60% carbohydrate in the case of PAS-1) complicates molecular weight analysis by this technique. Recently it has been reported that the largest glycopeptide (PAS-1) can be converted to the intermediate form (PAS-2) by heating red cell membranes or the isolated glycoprotein in SDS before electrophoresis (5,6). This result suggested that PAS-1 might be an aggregated form (possibly a dimer) of PAS-2. The PAS-3 form was unchanged by this treatment, and its relation to the higher molecular weight forms remained unclear.

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We have found that the total sialoglycopeptide mixture extracted from human red cells can be fractionated into two components by gel filtration in the presence of the non-ionic detergent Ammonyx-LO. Approximately 75% of the material is composed of a single sialoglycopeptide (now designated glycophorin A) whose complete amino acid sequence has been determined (7), and the remaining material seems to correspond to a second, previously unidentified glycopeptide. This second peptide is similar in gross composition to glycophorin A, but it also has distinct differences some of which are described here. The isolation and characterization of both glycopeptides has been complicated by the fact that each component is capable of forming high molecular weight aggregates which have similar mobilities when analyzed by SDS-gel electrophoresis.

METHODS. Sialoglycopeptides were isolated from RBC ghost membranes by the LIS-phenol[1] method (8) using freshly drawn blood either from individual donors or pooled samples. The protein was dissolved at 10 mg/ml in 0.1% Ammonyx-LO (Onyx Chem. Co., N.J.), 25 mM NaCl, 5 mM phosphate pH 8.0, and applied to a Biorad A 0.5 (200/400 mesh, or A 1.5) column (90 x 1.5 cm) equilibrated with the same buffer containing 0.01% sodium azide and run at room temperature. Column effluents were continuously monitored on a model 124 Perkin-Elmer spectrophotometer. Tryptophan was determined by fluorometry on a model 801 spectrofluorometer (Farrand Optical Co., Inc., N.Y.) using 290 nm for excitation and 355 nm for emission or after hydrolysis (9). Tryptic digests were prepared by incubating a 1% glycopeptide solution in trypsin [2x cryst., Worthington, Freehold, N.J., treated with L-(1-tosylamido-2-phenyl) ethyl chloromethyl ketone] at an enzyme-substrate ratio of 1:30 in 0.05 M Tris-HCl, pH 8.5, for 12 hours at 37°C. After incubation, the solution was brought to pH 4.0 with acetic acid, and the precipitate which formed was removed by centrifugation for 30 min. at 30000 x g (2,3). The supernate

[1] Abbreviations used in the text: SDS-sodium dodecylsulfate; LIS-Lithium diiodosalicylate; PAS-periodic acid-Schiff's reagent.