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Biochemistry

Characterization of an interferon receptor on human lymphoblastoid cells

(receptor on plasma membranes/solubilized receptor)

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ABSTRACT **A cell-free assay was developed to measure the binding of iodinated human interferon-α2 to membranes prepared from lymphoblastoid Daudi cells. The kinetics of binding were similar at 0°C and 30°C, with 1.3-fold more interferon bound at the higher temperature. Membrane preparations treated with Triton X-100 proved to be a convenient source of solubilized receptor. An assay was developed to measure the binding of ^{125}I-labeled interferon (^{125}I-interferon) to solubilized receptors, based on the precipitation of interferon–receptor complexes with polyethylene glycol. Optimal binding with this assay was obtained at 0°C. The solubilized receptor was analyzed by zonal sedimentation centrifugation and gel filtration. Sedimentation analysis in H_2O and ^2H_2O gradients provided the sedimentation coefficient and the partial specific volume of the receptor–Triton X-100 complex. Gel filtration chromatography provided the Stokes radius of this complex. From these data we calculated several physical parameters, including M_r = 95,000 for the protein portion of the complex. The receptor is a highly asymmetric and hydrophobic membrane protein. ^{125}I-Interferon could be crosslinked to receptors of intact Daudi cells or of isolated membranes by use of disuccinimidyl suberate. The covalently linked ^{125}I-interferon–receptor complexes were analyzed by gel electrophoresis. A single band with M_r = 140,000 was detected in gel autoradiographs. If one molecule of interferon is present in this complex, the M_r of the receptor is close to 120,000. Possible reasons for the different M_r values obtained with the two analytical procedures used are discussed.**

An understanding of the mechanism of action of interferon (IFN) depends upon a detailed knowledge of the events that elicit specific cellular responses. The initial interaction of IFN with cells involves its binding to high-affinity receptors (1–4). Early studies had suggested that binding of IFN to the cell surface is required to initiate the antiviral response (see ref. 5 for references), but only recently the specific binding of ^{125}I-labeled IFN (^{125}I-IFN) to receptors on the surface of different mammalian cells was demonstrated (1–4, 6–9) and correlated with biological responses to IFN (6, 9, 10). These studies were carried out with intact cells, and the receptors were defined in terms of their affinity for IFN (1–4) or of their specificity for different IFN moieties (2, 3, 11). Treatment with proteolytic enzymes destroys the IFN-binding activity of mouse cells; this indicates that the receptor is a protein (9).

An understanding of the role of the receptors in the cellular response to IFN requires some precise information about their molecular properties. The purpose of the present work is the physical characterization of an IFN receptor of human cells. Because studies with intact cells provide limited information on receptors and are complicated by events that follow the receptor–IFN interaction, such as the internalization and degradation of cell-bound IFN (10, 12), we have developed an assay for IFN receptors that uses isolated plasma membranes. The membrane preparations retain high-affinity binding sites for IFN and provide a convenient material from which to obtain receptors solubilized with detergent. We have developed an assay for solubilized IFN receptors and have determined several of their physical properties. These studies show that the IFN receptor is a highly hydrophobic and asymmetric membrane protein. In addition, ^{125}I-IFN was crosslinked to cellular receptors. When the covalently linked IFN–receptor complex was analyzed by gel electrophoresis, a single band was found in the autoradiographs.

MATERIALS AND METHODS

Cells and IFN. Daudi and HeLa cells were cultured as reported (2). Purified human IFN-α2 (HuIFN-α2) produced in *Escherichia coli* (13) was a kind gift of Charles Weissmann and had an antiviral activity of 2×10^8 reference units per mg of protein. For iodination, 10 μg of IFN in 20 μl of 0.1 M sodium borate (pH 8.5) was allowed to react for 2 hr at 4°C with 1 mCi of Bolton–Hunter ^{125}I reagent (New England Nuclear; 2,000 Ci/mmol; 1 Ci = 3.7×10^{10} Bq). After addition of 0.5 ml of 0.2 M glycine/0.1 M sodium borate, pH 8.5, the reaction mixture was chromatographed on a Sephadex G-75 column (0.7 × 26 cm) equilibrated with 0.25% gelatin in phosphate-buffered saline (P_iNaCl) at pH 7. Fractions containing ^{125}I-IFN were pooled as described (2). The initial specific activity of the ^{125}I-IFN was 740 Ci/mmol; recovery of antiviral activity was >30%. For use in binding assays the ^{125}I-IFN was diluted 1:10 with 20 mM Hepes KOH, pH 7.4/0.1% bovine serum albumin (Hepes-alb.) and filtered through a Millex-GV filter (Millipore).

Binding of ^{125}I-IFN to Plasma Membranes. Plasma membranes were prepared from Daudi cells by the hypoosmotic borate method of Thom *et al.* (14). The purified membranes were suspended at 10 mg/ml in 10 mM Hepes buffer (pH 7.4) and stored at −70°C. For a standard binding assay, 1–3 μl of membrane suspension was incubated for 30 min at 30°C with 0.5 ng of ^{125}I-IFN and Hepes-alb. in 50-μl reaction mixtures. The incubations were diluted with 0.2 ml of cold Hepes-alb. and filtered through GVWP Durapore filters (Millipore). The filters were washed with 15 ml of Hepes-alb. and counted in a γ-counter. An incubation without membranes was used as a blank; this corresponded to <2% of the input radioactivity. Nonspecific binding was measured in the presence of a 50-fold excess of unlabeled HuIFN-α2 at different time points and ^{125}I-IFN concentrations. This nonspecific binding was consistently <15% of the total binding and was subtracted from the data reported.

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Abbreviations: IFN, interferon; HuIFN, human IFN; P_i/NaCl, phosphate-buffered saline; PEG, polyethylene glycol.

Binding of ^{125}I-IFN to Solubilized Receptors. Membrane suspensions were diluted to 2 mg/ml and solubilized with 1% Triton X-100/10% (vol/vol) glycerol/Hepes buffer, pH 7.4. After 40 min at room temperature, unsolubilized material was removed by centrifugation for 1 hr at 100,000 × *g*. In standard 0.1-ml assays, 10 μl of the supernatant fraction was incubated for 1 hr at 4°C with 0.5 ng of ^{125}I-IFN and Hepes-alb. The IFN–receptor complexes were precipitated by a modification of the procedure of Cohen *et al.* (15). To each assay were added 0.5 ml of 0.1% rabbit gamma globulin in 0.1 M sodium phosphate at pH 7.4 and polyethylene glycol (PEG; M_r 6,000–7,500) to a final concentration of 10%. After 10 min at 0°C the precipitate was collected on GVWP filters and washed with 6 ml of 8.5% (wt/vol) PEG/0.1 M sodium phosphate at pH 7.4. A reaction without solubilized receptor was used as a blank; this represented 3–4% of the input radioactivity. Nonspecific binding was determined at different time points and ^{125}I-IFN concentrations in the presence of a 75-fold excess of unlabeled HuIFN-α2. This nonspecific binding was about 30% of the total binding and was subtracted from the data reported.

For physical studies, following procedures described by Costrini *et al.* (16), the solubilized receptor preparations were dialyzed for 40 hr against buffer A (1 mM dithiothreitol/4 mM Hepes, pH 7.4, in Hanks balanced salt solution) containing 0.1% Triton X-100, centrifuged for 1 hr at 30,000 × *g*, and filtered through a 0.45-μm HAVP filter (Millipore). For zonal centrifugation studies, 0.4 ml of this preparation was centrifuged at 4°C and 48,000 rpm in a SW 50.1 rotor on 5–20% sucrose (wt/wt) gradients in buffer A with 0.05% Triton X-100 in either H_2O or ^2H_2O for 10 or 16 hr, respectively; 0.25-ml fractions were collected. For gel filtration chromatography, 0.7 ml of receptor preparation was fractionated at 4°C on a Sepharose 4B column (1 × 60 cm) equilibrated with buffer A containing 0.05% Triton X-100 and 10% sucrose. The 0.95-ml fractions were collected at a flow rate of 4 ml/hr. Enzyme markers were included in the preparations analyzed. The activities of malate dehydrogenase, catalase, and aldolase were determined as described (17); β-galactosidase was assayed according to Hestrin *et al.* (18).

Crosslinking of ^{125}I-IFN to Receptors. Daudi and HeLa cells were collected by centrifugation, washed with P_i/NaCl, and resuspended at 2 × 10^7/ml in P_i/NaCl containing bovine serum albumin at 1 mg/ml; 0.25 ml of cell suspension was incubated for 45 min at 37°C with 1.5 ng of ^{125}I-IFN. The incubation mixtures were cooled to 0°C and 50 mM disuccinimidyl suberate in dimethyl sulfoxide was added to a final concentration of 1 mM. After 15 min the cells were centrifuged for 1 min at 10,000 × *g* through a 1-ml layer of 10% sucrose in P_i/NaCl. The cells were resuspended in 0.1 ml of P_i/NaCl containing 1 mM phenylmethylsulfonyl fluoride, and Triton X-100 was added to 1% final concentration. After 5 min, a soluble fraction was obtained by centrifugation for 5 min at 10,000 × *g* and was analyzed by gel electrophoresis (19) followed by autoradiography. Binding of ^{125}I-IFN to receptors of isolated plasma membranes was carried out in 0.1-ml incubation mixtures containing 0.25 mg of membrane protein. The crosslinking reaction was carried out as described above, and the membranes were centrifuged through a layer of 2.5% sucrose and immediately dissolved in sample buffer for gel electrophoresis.

RESULTS

In preliminary experiments, we treated Daudi cells with 1% Triton X-100 to solubilize the IFN receptor. The post-nuclear supernatant fraction obtained was allowed to react with ^{125}I-IFN, and increasing amounts of PEG were added to precipitate the interferon–receptor complexes. Little free ^{125}I-IFN was precipitated by 10% PEG, but the interferon–receptor complexes were apparently precipitated and were collected on filters. However, the nonspecific binding determined in the presence of an excess of unlabeled IFN was quite high, representing >50% of the total binding. In addition, these preparations lost most of their binding activity upon freezing and thawing. Therefore, in view of the satisfactory results obtained with plasma membrane preparations in the study of hormone receptors (20, 21), we used Daudi cell membranes as the starting material to characterize the solubilized IFN receptor.

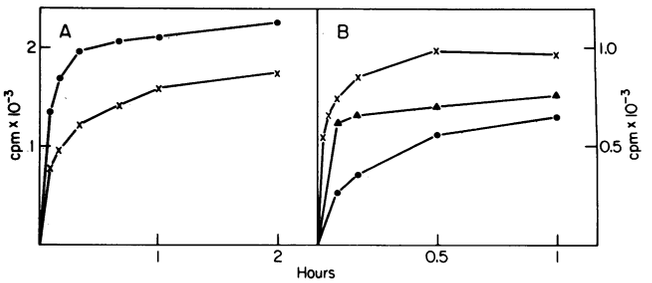

FIG. 1. Time course of the binding of ^{125}I-IFN to plasma membranes (*A*) and solubilized receptors (*B*). For each time point, 3 μl of membrane suspension or 10 μl of solubilized receptor preparation was incubated at 30°C (●), 21°C (▲), or 0°C (×) with 0.5 ng of ^{125}I-IFN.

The binding of ^{125}I-IFN to plasma membranes prepared as described by Thom *et al.* (14) was measured at 0°C and 30°C to find optimal conditions for binding assays. The binding reached apparent equilibrium after about 1 hr at both temperatures; 30% more ^{125}I-IFN was bound at the higher temperature (Fig. 1*A*). When increasing amounts of ^{125}I-IFN were added to binding assays, the bound radioactivity leveled off (Fig. 2). These data were represented in a Scatchard plot (Fig. 2 *Inset*) which was used to calculate a dissociation constant of 1 × 10^{-11} M. These results indicated that the membrane preparations retained the saturable high-affinity IFN binding sites present in intact Daudi cells (2). They also retained the binding specificity previously described (2) because addition of a 50-fold excess of unlabeled IFN-α or IFN-β decreased the binding of ^{125}I-IFN by 90%, whereas the addition of an equivalent amount of in-

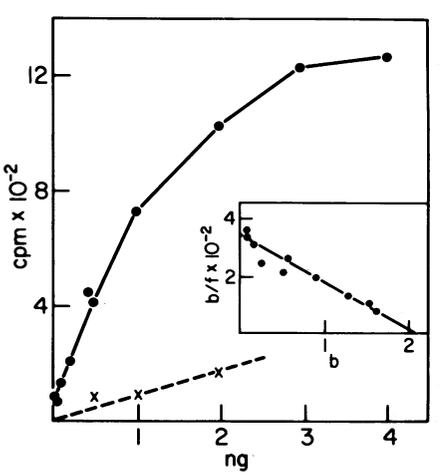

FIG. 2. Saturation of ^{125}I-IFN binding sites on plasma membranes. For each point, 1 μl of membrane suspension was incubated for 30 min at 30°C with the amount of ^{125}I-IFN indicated in the abscissa to measure total binding or with a 50-fold excess of unlabeled HuIFN-α2 to measure nonspecific binding (×). The specific binding (●) was calculated by subtracting nonspecific from total binding. (*Inset*) Scatchard plot of the specific binding data; *b* and *f* are the fmol of bound and free IFN, respectively.

terferon-γ decreased the binding by 18% (data not shown). The binding at 0°C of ^{125}I-IFN per mg of protein was about 3-fold greater than the binding obtained with intact cells; the recovery of binding activity in fractionated membranes was about 15% of that originally present in Daudi cells (data not shown). Membrane preparations could be stored at −70°C without apparent loss of ^{125}I-IFN binding activity and provided a convenient starting material for solubilizing and characterizing the IFN receptor.

The membranes were treated with Triton X-100 and the solubilized receptors were assayed for ^{125}I-IFN binding. The binding of ^{125}I-IFN was measured at 0°C, 21°C, and 30°C (Fig. 1*B*). The binding reached apparent equilibrium after 30 min, with greater binding at 0°C than at the higher temperatures. A possible explanation for this finding was protease activity, but addition of 2,000 kallikrein inhibitory units of aprotinin per ml and of 5 mM phenylmethylsulfonyl fluoride did not increase the binding obtained at 21°C or 30°C (data not shown). Comparison of the binding at 0°C to solubilized receptors and membranes indicated that the IFN-binding activity was quantitatively recovered in the experiment shown in Fig. 1. In repeated experiments, >75% of the activity was recovered and the specific binding per mg of protein increased about 2-fold over that obtained with membranes.

Characterization of the Solubilized IFN Receptor. Solubilized membrane proteins form complexes with detergent. To calculate the M_r of the IFN receptor it was necessary to determine the sedimentation coefficient ($s^0_{20,w}$), Stokes radius, partial specific volume, and detergent content of the Triton X-100–receptor complex. A sedimentation pattern of this complex representative of three independent experiments is shown in Fig. 3. Calibrating enzymes were included in the sucrose gradients; the major peak of IFN-binding activity sedimented somewhat slower than malate dehydrogenase. Other peaks of IFN-binding activity with higher sedimentation coefficients were variable in amount; because their relative proportion increased with the time of sedimentation (data not shown), they may represent aggregated forms of the Triton X-100–receptor complex.

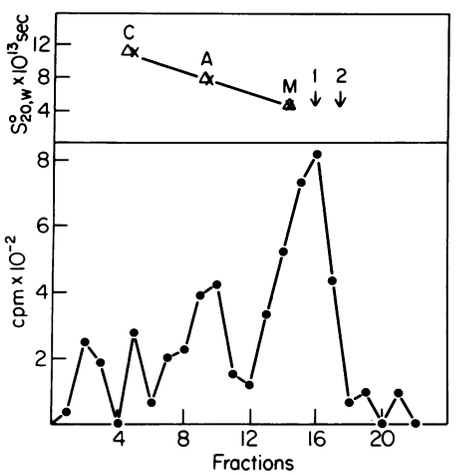

FIG. 3. Zonal centrifugation of solubilized IFN receptor. (*Lower*) ^{125}I-IFN binding activity of fractions obtained after centrifugation for 10 hr at 48,000 rpm (SW 50.1 rotor) on a 5–20% sucrose gradient in H_2O. From each fraction, 0.1 ml was incubated for 1 hr at 4°C with 0.5 ng of ^{125}I-IFN and the binding was measured. (*Upper*) Position, in the gradient, of the calibrating enzymes catalase (C), aldolase (A), and malate dehydrogenase (M) plotted against their $s^0_{20,w}$ (△). The position of these markers in a 5–20% sucrose gradient in ^2H_2O centrifuged for 16 hr at 48,000 rpm is also shown (×). The arrows indicate the average position of the major peak of ^{125}I-IFN binding activity at the top of H_2O (arrow 1) and ^2H_2O gradients (arrow 2).

The partial specific volume ($\bar{v}$) of this complex was determined by comparing its sedimentation behavior in sucrose gradients prepared in ^2H_2O with that in gradients prepared in H_2O relative to enzymes with known physical properties. If the receptor–detergent complex has a $\bar{v}$ different from that of the calibrating enzymes, its relative sedimentation coefficient will differ between the gradients in ^2H_2O and those in H_2O. This method was developed by Edelstein and Schachman (22) and Meunier *et al.* (23) and has been utilized in the study of membrane proteins (16, 24, 25). The sedimentation pattern of the IFN-binding activity in ^2H_2O gradients was similar to that in H_2O, with several peaks present. However, the major peak of binding activity near the top of the ^2H_2O gradient was shifted, relative to malate dehydrogenase, to a lower apparent sedimentation coefficient than in the H_2O gradient (Fig. 3). Therefore, the $\bar{v}$ of the receptor–detergent complex in this peak differed from that of the calibrating enzymes and had a value of 0.83 cm^3/g when calculated as described in Table 1. This value of $\bar{v}$ was greater than that of the calibrating enzymes ($\bar{v}$ = 0.74), possibly because of the presence of large amounts of Triton X-100 ($\bar{v}$ = 0.94) bound to the receptor. Assuming that equal amounts of this detergent are bound in H_2O and ^2H_2O and using an equation developed by Clarke (25), we calculated that the receptor–detergent complex had 0.82 mg of Triton X-100 bound per mg of protein (Table 1).

The Stokes radius of this complex was determined by gel filtration on Sepharose 4B (Fig. 4). A major peak of IFN-binding activity eluted close to the β-galactosidase enzyme marker. The distribution coefficient (K_D) was calculated for each enzyme marker and plotted against known Stokes radii (Fig. 4 *Inset*). The K_D for the IFN-binding activity was calculated and the Stokes radius for the receptor–detergent complex was determined graphically to be 72 Å. The physical properties of the solubilized interferon receptor are summarized in Table 1.

Crosslinking of ^{125}I-IFN to Cell Surface Receptors. The ^{125}I-IFN was incubated with intact HeLa or Daudi cells which were then treated with the crosslinking reagent disuccinimidyl suberate. Triton X-100 was then added and the soluble fraction

Table 1. Physical characteristics of IFN receptor–Triton X-100 complex

Parameter	Value
Partial specific volume, cm^3/g	0.83 ± 0.04
Standard sedimentation coefficient, S	3.5 ± 0.2
Stokes radius, Å	72 ± 4
M_r of receptor–Triton X-100 complex	170,000
Frictional ratio (f/f_0)	1.8
Triton X-100 bound, mg/mg of protein	0.82
M_r of protein portion of complex	95,000

The parameters were calculated as described (16, 24–26). Briefly, the partial specific volume ($\bar{v}$) was calculated by the method of Clarke (25) according to his equation 14 which relates $\bar{v}$ to the apparent sedimentation coefficients (s), viscosity (η), and density (ρ) determined in H_2O and ^2H_2O gradients. The values for s, η, and ρ were calculated from the data in Fig. 3 as described by Clarke (25). The standard sedimentation coefficient was calculated according to equation 13 of Clarke (25). The Stokes radius was determined graphically from the data in Fig. 4 in which the K_D or K_{ave} for each enzyme was calculated as described by Laurent and Killander (26). The M_r of the receptor–Triton X-100 complex was calculated according to equation 3 of Costrini *et al.* (16). The frictional ratio, a measure of the asymmetry of the molecule, was calculated according to equation 4 of Costrini *et al.* (16), with the solvation factor δ taken to be 0.2 g of solvent per g of protein. The Triton X-100 bound per mg of protein was calculated according to equation 4 of Clarke (25) by using values of 0.94 cm^3/g and 0.74 cm^3/g for the partial specific volumes of Triton X-100 and of the protein portion, respectively. The M_r of the protein portion was calculated according to equation 3 of ref. 24.

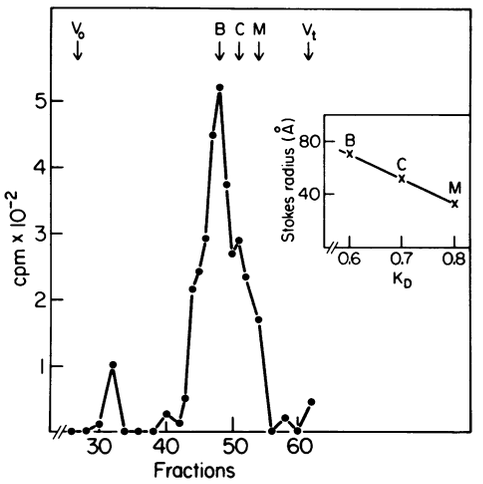

FIG. 4. Gel filtration of solubilized IFN receptor. Aliquots (0.2 ml) of the fractions obtained by gel filtration on Sepharose 4B were incubated for 2 hr at 4°C with 0.5 ng of ^{125}I-IFN and the binding was measured. The calibrating enzymes β-galactosidase (B), catalase (C), and malate dehydrogenase (M) were included; their elution positions are shown by the arrows. Void volume (V_0) and total volume (V_t) are also shown. (*Inset*) Linear relationship between the Stokes radius and the distribution coefficient (K_D) of the calibrating enzymes.

was analyzed by gel electrophoresis and autoradiography (Fig. 5). The autoradiograms showed ^{125}I-IFN migrating at the bottom of the gel and a broad band of M_r = 140,000 ± 20,000. A band of identical M_r was obtained with HeLa and Daudi cells, although it was much more pronounced in the latter cells which were previously shown to have many more IFN receptors than HeLa cells (2). The radioactive band disappeared when a 25-fold excess of unlabeled competitor IFN was included in the binding assay (Fig. 5). When increasing amounts of ^{125}I-IFN were allowed to react with Daudi cells and crosslinked, the band of M_r = 140,000 increased proportionately to the IFN concentration (data not shown). A band of similar M_r was obtained by incubating membranes prepared from Daudi cells with ^{125}I-IFN and crosslinking with disuccinimidyl suberate (Fig. 5). Some radioactive material did not enter the 10% polyacrylamide gels used in the analysis of receptors of intact cells or the 7.5% gel

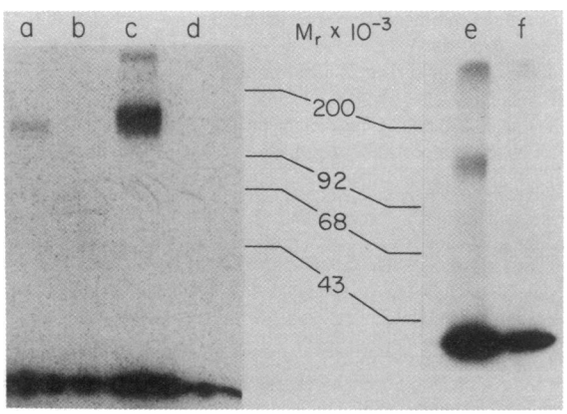

FIG. 5. Gel electrophoresis of ^{125}I-IFN covalently linked to receptors of intact HeLa (lanes a and b) or Daudi (lanes c and d) cells and to fractionated plasma membranes prepared from Daudi cells (lanes e and f). The incubation mixtures represented in lanes b, d, and f contained 750 reference units of unlabeled HuIFN-α2 as competitor. The fraction solubilized from cells with Triton X-100 was analyzed on a 10% polyacrylamide gel; that from membranes was analyzed on a 7.5% gel. The positions of M_r markers run in parallel are indicated.

used in the analysis of membrane receptors. The relative proportion of this material increased when greater concentrations of the crosslinking reagent were used (data not shown). This suggested that the reagent caused formation of aggregates that could not enter the gels.

DISCUSSION

The aim of the present studies was to determine the physical properties, including M_r, of the IFN receptor. Initially we developed a cell-free binding assay with plasma membranes prepared from Daudi cells. These membranes retained high-affinity ^{125}I-IFN binding sites. The dissociation constant of IFN–receptor complexes was about 1/6th that previously reported for intact Daudi cells (2) and was close to values reported by other investigators for mouse and human cells (1, 3). It should be pointed out that in the present studies the IFN was iodinated with the Bolton–Hunter reagent, whereas previously it had been iodinated in a reaction catalyzed by lactoperoxidase (2). The binding of ^{125}I-IFN to membranes at 30°C was 1.3-fold greater than that at 0°C (Fig. 1*A*), whereas with intact cells the binding at 30°C was about 5-fold greater than that at 0°C (10). The internalization of IFN–receptor complexes may be responsible for the increased binding at 30–37°C observed with intact cells (10).

The membrane preparations were solubilized with detergent and an assay was developed for the quantitation of IFN–receptor complexes. With this assay, we determined that the IFN-binding activity of plasma membranes was quantitatively recovered after solubilization. When the binding was compared at different temperatures, the best activity was obtained at 0°C (Fig. 1*B*). The binding could not be increased by adding serine protease inhibitors to the assays, but we could not rule out that other proteases are responsible for this effect of temperature on the recovery of IFN–receptor complexes. The formation of such complexes appeared to be highly specific because it could be inhibited competitively by the addition of unlabeled IFN.

Several properties of the solubilized receptor were determined by zonal centrifugation and gel filtration (Figs. 3 and 4). The partial specific volume $\bar{v}$ was determined from the difference in sedimentation between gradients prepared in H_2O and ^2H_2O, and the Stokes radius was determined from the gel filtration analysis. The average values obtained for the physical properties of the receptor–Triton X-100 complex (Table 1) allowed us to calculate a M_r for this complex of approximately 170,000. To estimate the M_r of the protein portion of the receptor, we had to determine the amount of Triton X-100 present in the complex. This calculation is based on the deviation of $\bar{v}$ of the receptor–detergent complex from that of a typical protein, a procedure developed by Clarke (25) to determine the M_r of unpurified membrane proteins. By subtracting the Triton X-100 content, we obtained a M_r of about 95,000 for the protein portion of the receptor. It should be pointed out that this constitutes a major correction of the M_r, subject to a large error. Moreover, the association of a significant amount of lipids, which have a high $\bar{v}$, with the solubilized receptor, might lead us to overestimate the amount of detergent in the complex and to calculate a M_r for the protein part of the receptor lower than the actual value.

In a parallel study, the M_r of the receptor was determined by covalently linking cell- or membrane-bound ^{125}I-IFN, followed by gel electrophoresis and autoradiography. A M_r of about 140,000 for an IFN–receptor complex was thus obtained. If we subtract 20,000, the M_r of one molecule of interferon (13), we obtain M_r = 120,000. We cannot exclude, however, that more than one molecule of IFN is bound per receptor or that other

membrane proteins become crosslinked to the receptor, even though we could not detect other labeled polypeptides of different M_r when the concentration of the crosslinking reagent was varied over a 20-fold range. Only one predominant broad band was observed with both Daudi and HeLa cells, although the yield of this band was much less with HeLa cells which have fewer IFN receptors (2).

The difference between the M_r obtained by physical studies and that obtained by gel electrophoresis is approximately 25%. The IFN receptor appears to be a single polypeptide of M_r 95,000–120,000, but we cannot rule out that this polypeptide may be a subunit of a larger membrane protein because the gel electrophoresis was run under reducing conditions and peaks of binding activity with sedimentation coefficients greater than the coefficient of the major peak were detected in sucrose gradients (Fig. 3). Similar peaks were observed after centrifugation of nerve growth factor receptor and were attributed to aggregation (16).

The high frictional ratio, high $\bar{v}$, and apparent binding of large amounts of detergent indicate that the IFN receptor of Daudi cells is a highly asymmetric and highly hydrophobic membrane protein. Several other membrane proteins have similar high $\bar{v}$ and bind large amounts of detergent, compared to nonmembrane proteins (25). The marked asymmetry of the IFN receptor is similar to that reported for other receptors (16, 27, 28) and integral membrane proteins (25). These studies may serve as a starting point to investigate specific changes that may occur in the receptor after binding IFN.

Note Added in Proof. After this manuscript was submitted for review, Joshi *et al.* (29) reported that the cross-linking of ^{125}I-IFN-α2 to its receptor yields a complex with an apparent M_r of $\approx$150,000.

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