The Biochemical Characterization of Detergent-solubilized Insulin-like Growth Factor II Receptors from Rat Placenta*

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A membrane preparation, the $R_s$, obtained by differential centrifugation of rat placental homogenates is enriched in receptors that bind insulin-like growth factor II (IGF-II) preferentially and with avidity (Daughaday, W. H., Martz, I. K., and Trivedi, R. (1981) J. Clin. Endocrinol. Metab. 53, 282–288). When this preparation was incubated with 2% (w/v) octyl-$\beta$-D-glucopyranoside for 60 min at 0–4 °C, 60% of the membrane protein was solubilized without loss of binding activity. The $^{125}$I-IGF-II binding properties of the detergent-solubilized receptors were found to be similar to those of the membrane-associated receptor. The rate constants for association, $k_a$, and dissociation, $k_d$, and equilibrium dissociation constant, $K_D$, were 8.5 x 10$^{-8}$ M$^{-1}$ min$^{-1}$, 7.5 x 10$^{-3}$ min$^{-1}$, and 1.3 nM for the detergent-solubilized receptors and 5.3 x 10$^{-8}$ M$^{-1}$ min$^{-1}$, 4.2 x 10$^{-3}$ min$^{-1}$, and 0.6 nM for the membrane receptors.

Gel chromatography on Sephacryl S-300 concentrated the solubilized receptors into a major peak of binding activity with a Stokes radius of 7.2 nm; a second peak of less specific binding had a Stokes radius of 4.3 nm. The receptors in the major peak bound $^{125}$I-IGF-II with a $K_D$ of 0.6 nm; the total binding capacity, $R_s$, was 21.6 pmol of protein$^{-1}$ for the membrane-associated receptor. Centrifugation of the receptors on 5–20% (w/v) gradients of sucrose in $H_2O$ or $D_2O$ disclosed a heterogeneous pattern of receptor distribution. When they were labeled with $^{125}$I-IGF-II prior to centrifugation, a major form of the receptor with a sedimentation constant, $s_{20,w}$, of 9.9 x 10$^{-13}$ s and other, possibly smaller, forms of the receptor were observed. However, only the 9.9 $s_{20,w}$ form of the receptor was observed if it was labeled with $^{125}$I-IGF-II subsequent to centrifugation. Based on these hydrodynamic measurements and a partial specific volume of 0.72 cm$^3$g$^{-1}$, the IGF-II receptor was calculated to have a $M_s$ of 290,000 and a frictional ratio of 1.6. This value for the $M_s$ is similar to the mass of 220,000 or 250,000 Dal.

determined by cross-linking $^{125}$I-IGF-II to the membrane- or detergent-solubilized receptors with disuccinimidyl suberate and separating the complex by electrophoresis in sodium dodecyl sulfate-containing polyacrylamide gels in the absence or presence of dithiothreitol, respectively.

Purification of serum Sm$^1$ and NSILA from human serum have established two classes of growth factors (1) with similar molecular weights (2), i.e. 7,500 and biological properties (3–5) but with different amino acid sequences and pi values. One class of somatomedins has alkaline pi values and includes the peptide IGF-I whose structure has been established. Somatomedin C (6) and basic somatomedin (7) appear to be similar if not identical in structure. The rat also contains an analogous basic somatomedin of very similar structure (8).

The abbreviations used are: NSILA, nonsuppressible insulin-like activity; Sm, somatomedins; IGF, insulin-like growth factors; MSA, multiplication stimulating activity; ILAs, insulin-like activity; DSS, disucinimidyl suberate; RRA, radioreceptor assay; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MES, 4-morpholineethanesulfonic acid; Bicine, N,N'-bis(2-hydroxyethyl)glycine; DATD, N$^1$N$^2$-diallyltartardiamide; CMC, critical micellar concentration; NIKKOL-BL-BSY, octaethyleneglycol-mono-o-c-dodecyl ether.

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PAGE and radioautography in experiments employing isolated receptors for insulin (14) and basic Sm/IGF (15) and membrane-associated receptors that are cross-linked with iodine-125-Iodinated IGF-II and L-phenylalanine-5-sulfonic acid in the presence of 100 mM acetic acid as previously described (22). Specific activities of 40-100 mCi/μg were obtained for the various ligands.

**Membrane and Detergent-solubilized Radioiodinated IGF-II Receptors** — The methods employed to measure 125I-IGF-II binding to the placental membranes, i.e. Rs, Rz, or RB, or to receptors in detergent-solubilized extracts of Rs were essentially as described by Li and Perdue (24). In brief, 50-350 μg of membrane protein was incubated for 0-180 min (in most experiments it was 60 min) at 22°C in 350 μl of 50 mM Na2HPO4, 1 mM PMSF, pH 7.4, containing 1 mg/ml of bovine serum albumin, 0.1-0.4 ng of 125I-IGF-II, and 14,000-70,000 cpm of labeled IGF-II (22). The Rz or RB receptors were essentially as described by Daughaday et al. (22) that it is enriched in receptors which bind IGF-II, and deficient of those which bind IGF-I. In this paper, we present our initial results on the characterization of detergent-solubilized IGF-II receptors.

**Materials and Methods**

Aldolase, catalase, ferritin, thyroglobulin, and Sephacryl S-300 were purchased from Pharmacia; the "Good Buffers" including Bis-tris, HEPES, and MOPS, bovine serum albumin, myosin, ovalbumin, PMSF, Tris, and D2O were from Sigma; β-galactosidase, dehydrogenase, and phosphorylase a were from Boehringer-Mannheim; fibrin was from Transformation Research, Farmington, MA; sodium dodecyl sulfate and the chemicals and equipment used for electrophoresis was from Bo-Rad; n-octylglucoside was from Calbiochem-Behring; DSS was from Pierce Chemical Co.; Cr60 x-ray film was from DuPont Chemical Co.; [125I]NaI was from Amersham; and other chemicals were from local sources. Highly purified IGF-I (16, 15-78, 25) and insulin were purchased from Bachem and Amicon ultrasonic apparatus. The general picture that is emerging indicates that the insulin and basic Sm/IGF receptors are immunologically and structurally related. Antibodies to the insulin receptors recognize determinants on the receptor that bind IGF-I (17) and both are formed from 140- and possibly 95- and 45-kDa subunits that are disulfide-linked to form proteoglycans of 300-400 kDa (18-21). As predicted from the results of competitive binding studies (3-5), the receptors which bind the neutral-acidic Sm/IGFs are structurally very different from the basic Sm/IGF receptor. They appear to be single chained polypeptides of about 220 kDa that are maintained by internal disulfide bonds (18, 19). This was concluded from cross-linking studies with DSS, but to date no one has reported on the structural composition of the isolated receptor. Therefore, we have undertaken this task and have chosen as our tissue source the rat placenta since it has been shown by Daughaday et al. (22) that it is enriched in receptors which bind IGF-II, and deficient of those which bind IGF-I. In this paper, we present our initial results on the characterization of detergent-solubilized IGF-II receptors.

Preparation of Rat Placental Membrane — Rat placental membranes contained 36 milligrams/mg of protein and was stated to contain 44 μg of IGF-I and 82 μg of IGF-II/mg of protein. It and a partially purified preparation of MI were used as the competing ligands for certain of the experiments. The MI was purified by chromatography on a DEAE-Sephadex A-25 column (2.5 cm X 100 cm) containing Sephadex G-75. The peak of IGF-II binding was collected and dialyzed against 50 mM Na2HPO4, 1 mM PMSF, pH 7.4, containing 2 mg/ml of bovine serum albumin. The iodinated IGF-II and labeled INS were added to a final concentration of 18% (w/v) to precipitate the insulin receptor complex. After standing for 10 min at 0-4°C, bound ligand was separated from free by sedimenting three 100-μl aliquots of the mixture on the Beckman Airfuge, unseparated material was removed by suction, and that portion of the nitrocellulose tube containing the pellets was cut and counted for radioactivity on a Nuclear Chicago γ-counter at 40% efficiency. Detergent-solubilized protein (10-100 μg) was incubated with 125I-IGF-II ± unlabeled IGF-II or MSA in a final volume of 224 μl of buffer as described above. Following incubation, 126 μl of ice-cold 50% polyethylene glycol was added to a final concentration of 18% (w/v) to precipitate the IGF-I receptor complex. After standing for 10 min at 0-4°C, bound ligand was separated from free by sedimenting three 100-μl aliquots of the mixture on the Beckman Airfuge. The data are expressed as the specific binding, i.e. the difference between the 125I-labeled ligand binding to membranes or soluble receptor in the absence of tracer or an excess of unlabeled ligand divided by the quantity of ligand bound divided by the quantity of ligand bound in the presence of competition.

Cross-linking of 125I-IGF-II to Receptors — A sodium dodecyl sulfate-polyacrylamide gel electrophoresis —Cross-linking of the receptors with 125I-IGF-I or II was achieved by incubating 100 μg of membrane protein with radioactively labeled ± unlabeled ligands, i.e. impure IGF or MSA, as described above, resuspending the sedimented pellets in 20 μl of 50 mM Na2HPO4, pH 7.4, and incubating them for 5 min at 0-4°C in 25 μl of 125I-IGF-II (126, 25) and labeled INS (26). After quenching the reaction with 11 μl of 1 M Tris, 0.2 EDTA, the membranes were dissolved and heated in SDS-containing sample buffer with or without 100 mM DTT. Polyacrylamide slab gels 1.5-mm thick were prepared in the buffer system of Laemmli (26). The running gel was formed from 2.3% acrylamide, 0.3% DAST and 12.5% of 15% acrylamide, 7% DAST. The stacking gel contained 3% acrylamide, 0.02% bisacrylamide. Standard M, markers of filamin (250,000), β-galactosidase (116,000), phosphorylase a (95,000), bovine serum albumin (66,200), and ovalbumin (45,000) were run with each series. Certain of these bands were radioactively labeled and varying concentrations of these were fixed and stained with Coomassie blue, destained in 7.5% acetic acid, dried, and the regions containing radioactivity identified by autoradiography at -70°C using Cronex x-ray film and Cronex Lightning Plus intensifying screens.

Gel Filtration — Detergent-solubilized placental proteins were equilibrated with 125I-IGF-II and chromatographed at 0-4°C in buffer containing 0.2% n-octylglucoside. 100 mM NaCl, 50 mM Na2HPO4, pH 7.4, on a column (1.5 X 72 cm) of Sephacryl S-300 (Superfine). The Stokes radii of the 125I-IGF-II binding components were calculated from their partition coefficients (Kφ) and those of standard proteins by the Langevin and Killander correlation as proposed by Siegel and Monty (27). The proteins with known Stokes radii used for column calibration were thyroglobulin (85 nm), ferritin (61 nm), catalase (5.2 nm), aldolase (4.8 nm), bovine serum albumin (3.6 nm), and ovalbumin (3.1 nm).

**Sucrose Density Gradient Ultracentrifugation — Detergent-solubilized IGF-II binding proteins resolved during gel chromatography under "Results." The procedure currently in use is the following. Rs at a protein concentration of 5 mg/ml was stirred for 2 h at 0-4°C in 50 mM Na2HPO4, 1 mM PMSF, pH 7.4, containing 20 mg/ml of n-octylglucoside. Following incubation, the detergent-treated suspension was centrifuged for 6 X 106 X g·min and the supernatant decanted and dialyzed for 4 h against 10 volumes of buffered 0.2% (w/v) n-octylglucoside. Following dialysis, the clarified extract was divided into aliquots and stored frozen at -70°C or evaluated for IGFI binding.

**Iodination of Insulin-like Growth Factors — IGF-I and IGF-II were iodinated by the lactoperoxidase procedure and free iodine separated from proteins by Sephadex G-50 gel filtration in 100 mM acetic acid (22). Specific activities of 40-100 mCi/μg were obtained for the various ligands.**
were concentrated against Carbocar and either stored on ice or incubated with 125I-IGF-II in the absence or presence of unlabeled MSA. The protein, in a final volume of 0.2 ml, was layered on 4.7 ml of 5–20% (w/v) linear sucrose gradients containing 106 mM NaCl, 50 mM Na2HPO4, pH 7.4, 0.2% n-octylglucoside prepared in either H2O or 0.1% D2O. The samples were centrifuged (500 g) or 25 (D2O) h at 4 °C in a Beckman SW 50.1 rotor at 42,000 rpm according to the procedure of Martin and Ames (28). After centrifugation, approxi-
mately forty-seven 0.1-ml fractions were collected and their refractive index and distribution of calibrating proteins and 125I-IGF-II-binding activities determined. The Stokes radius, rSt, and partial specific volume of the calibrating proteins are: for catalase, 52.2 Å, 11.3 s, and 0.73 ml/g; for lactate dehydrogenase, 49.2 Å, 7.3 s, and 0.75 mg/g; for fumerase, 52.9 Å, 8.9 s, and 0.74 mg/g; and for transferrin, 40 Å, 5.5 s and 0.73 mg/g. Catalase activity was determined by following the decrease in absorbance at 240 nm (29), lactate dehydrogenase by the decrease in absorbance at 340 nm of 0.24 nm NADH in the presence of 0.7 mM pyruvate (28), and fumerase by an increase in absorbance at 240 nm in the presence of 50 mM L-malate (30).

Calculations—The molecular weight of the detergent-solubilized IGF-II receptor was determined by the formula (27)

\[ M_r = \frac{6 \times 10^5 \times (\eta_{20, s} - \eta_{20, p})}{1 - \frac{\eta_{20, p}}{\rho_{20, p}}} \]  

where \( N \) is Avogadro’s number, \( \eta_{20, s} \) and \( \rho_{20, p} \) are the viscosity and density of water at 20 °C, \( \eta \) is the Stokes radius, \( \eta_{20, s} \), and \( \rho \) is the partial specific volume of the calibrating protein, and \( \eta \) is the partial specific volume. The results are expressed as the mean ± S.E. of protein content, B/T, or specific binding with the number of experi-
ments indicated in parentheses.

**Characterization of IGF-II Binding**

Although one of us (22) had previously reported that the IGF receptors on placental membrane preferentially bind 125I-IGF-II and with high affinity, these observations were repeated and extended because (a) the methods employed to prepare the membranes and quanitate ligand binding used previously were different from those we use now; and (b) it was essential that the membrane-associated receptor be characterized more completely so that valid comparisons can be made between it and the detergent-solubilized receptors.

**RESULTS**

**Distribution of Protein and Receptors that Bind IGF-II in Differentially Centrifuged Homogenates of Rat Placenta**

The R1 fraction, obtained by centrifuging the homogenate of Rat Placenta at 9600 × g min, is made up of partially dissociated tissue and intact cells (data not presented). Over 50% of the homog-
enate’s protein is sedimented within this fraction (Table I). The R1 fraction contains broken cells, nuclei, mitochondria, and membrane fragments of varying sizes while the R3 has large fragments of smooth membrane, some mitochondria, ribo-
some-free and ribosome-bearing vesicles of varying size, free ribosomes, and cellular debris. Eighteen to 23% of the 125I-IGF-II available for binding became associated with the protein in all of these fractions. Approximately 68–77% of this binding could be displaced with an excess of unlabeled ligand (Table I). Although the R1 and R2 fractions bound near equal amounts of ligand and with almost the same degree of speci-
ficity, we have employed the R2 fraction exclusively in the further characterization and identification of the membrane-
associated IGF-II receptor since the presence of large tissue aggregates in the R1 resulted in greater variation among triplicates. The R3 is also the source of the detergent-solubil-
ized receptors.

**Table I**

| Fraction designation | Protein distribution | \( 125^1 \)-IGF-II binding properties | Specific binding |
|----------------------|----------------------|---------------------------------------|-----------------|
|                      | %                    | B/T (mol/mg)                          | %               |
| R1 (0.0096 × 10^6 × g·min) | 54.3 (2)             | 0.22 ± 0.03 (3)                        | 72 ± 3 (3)      |
| R2 (0.48 × 10^6 × g·min)  | 14.1 (1)             | 0.18 ± 0.02 (4)                        | 68 ± 7 (4)      |
| R3 (6 × 10^6 × g·min)    | 4.9 (1)              | 0.23 ± 0.01 (8)                        | 77 ± 6 (8)      |
| n-Octylglucoside extract of R3 | 61.6 ± 2.5 (6) | 0.55 ± 0.04 (5)                        | 77 ± 9 (5)      |
| Dialyzed extract        | 95.0 ± 1.8 (4)       | 0.57 ± 0.02 (5)                        | 82 ± 6 (5)      |
| Residue                 | 28.3 ± 2.5 (6)       | 0.26 ± 0.02 (4)                        | 70 ± 7 (4)      |
were evaluated for their ability to extract the IGF-I receptor. As little as 0.02% Triton X-100, which solubilizes insulin (14) and basic Sm/IGF-I (15) receptors of human placenta at concentrations of 0.5–1%, reduced the binding of $^{125}$I-IGF-II to rat placental membrane from a control value of 35–23%; nonspecific binding increased from 26 to 64%. At a detergent concentration of 0.5%, little specific binding could be demonstrated. The inhibition of binding was not due to the presence of oxidizing impurities (33). Triton X-100 that had been treated with NaHSO$_3$ and repurified still inhibited IGF-II binding to the placenta. Similar adverse effects on $^{125}$I-IGF-II binding were observed with 0.02–0.1% NIKKOL-BL-8SY and the zwitterionic detergent, Zwittergent (3–12). n-Octylglucoside at a concentration of 0.1% (w/v) had no effect on either total or specific IGF-II binding and, thus, became the detergent of choice to attempt to solubilize the receptors in the R$_p$. Incubation of these membranes for 60 min at 0–4°C with 2% (w/v) n-octylglucoside at a protein:detergent weight ratio of 1:4 solubilized 60% of the protein (Table I). These were the optimum conditions since slightly less protein was solubilized when 1% detergent was used and incubations at room temperature resulted in the preparation of extracts that bound greater quantities of $^{125}$I-IGF-II nonspecifically (data not presented). Extraction of the receptor from the R$_p$ was incomplete as evidenced by $B_0/T$ and specific binding values of 0.26 and 70%, respectively, for $^{125}$I-IGF-II binding to the residual (Table I).

Dialysis of the extract for 0.5 or 4 h against 0.2% n-octylglucoside resulted in only a small loss in protein, i.e. about 5%, with no effect on $^{125}$I-IGF-II binding. It has previously been established that 4 h of dialysis adequately decreases the level of detergent from above its critical micellar concentration of 25 to 1.5 mM (34). Thus, under conditions of assay, gel chromatography, and sucrose gradient centrifugation, the soluble receptor exists in equilibrium with the nonmicellar form of n-octylglucoside.

Polyethylene glycol combined with centrifugation was used initially to evaluate ligand-soluble receptor interactions. Based on our earlier study with the insulin receptor (24), we used a final concentration of this reagent of 6% (w/v). On re-examining the concentration dependency for precipitating the $^{125}$I-IGF-II-soluble receptor complex, we observed that twice as much radiolabeled ligand was associated specifically with the receptor protein when 18% polyethylene glycol was used compared with that obtained using 6% (Fig. 3). At higher concentrations of polyethylene glycol, nonspecific binding of $^{125}$I-IGF-II increased. A concentration of 18% was used in subsequent work.

**Comparison of IGF-II Binding to Detergent-solubilized and Membrane Receptors**

$^{125}$I-IGF-II binding to the placental membrane and detergent solubilized extract at 22°C reached equilibrium by 60–90 min and was constant to 180 min (Fig. 4A). For the purposes of kinetic analysis, we have assumed the bound IGF-II is intact, that the binding is reversible, and that there is no cooperativity between receptors. Under these conditions, the formation of the receptor-ligand complex (Rf) with time (t) can be described by the second order differential equation

$$\frac{d[R(t)]}{dt} = k_d[I_0 - R(t)] [R_0 - R(t)] - k_s R(t)$$  

(1a)

where $R(t)$ is the concentration of hormone-receptor complex at time (t), $I_0$ and $R_0$ are the total hormone and receptor concentrations, and $k_d$ and $k_s$ the association and dissociation rate constants, respectively. Differentiation of Equation 1a less the dissociation term (it was assumed the initial binding is
Characterization of Soluble IGF-II Receptors

Polyethylene glycol concentration (%)

FIG. 3. The precipitation of 125I-IGF-II-soluble receptor complex by varying concentrations of polyethylene glycol. Dialyzed extract (29 pg) was incubated with 3.8 fmol of 125I-IGF-II ± 50 pg of MSA in 50 mM PO4, 1 mM PMSF, pH 7.4, 0.1% bovine serum albumin for 60 min at 22 °C as described under "Materials and Methods." Incubations were terminated by adding 50% (w/v) polyethylene glycol to a final concentration of 6 to 24% (w/v). Following 10 min of incubation at 0-4 °C, bound ligand was separated from free ligand by sedimentation at 100,000 × g for 8 min on the Airfuge. The results are expressed as the mean ± S.E. of total (O) and nonspecific (●) 125I-IGF-II binding.

Irreversible) results in the following formula:

\[ \frac{dRI(t)}{dt} = -k_d R(t) \]

In Equation 1a. One-half dissociation rates \( k_d \) of the RI complex were determined by plotting the logarithm of bound radioactivity as a function of time following removal of free 125I-IGF-II with dextran-coated charcoal and the addition of MSA (Fig. 5). The rate constant for dissociation, \( k_d \), for the soluble receptor was 7.5 × 10⁻³ min⁻¹ based on a one-half rate of dissociation of 93 min. The \( k_d \) for the membrane receptor was 4.2 × 10⁻³ min⁻¹.

As documented in Table I and previous figures, approximately 25 and 60% of the 3.8 fmol of 125I-IGF-II available for binding became associated with the 29 pg of membrane or detergent-soluble protein, respectively (Fig. 6). This binding was inhibited by unlabeled IGF-II and IGF-II-like peptide (11) present in MSA in proportion to their concentrations. Ten ng of unlabeled IGF-II inhibited 125I-IGF-II binding to the membrane by 50% and 40 ng inhibited binding to the soluble receptors by the same extent. The curves of MSA inhibition of 125I-IGF-II binding were parallel with those obtained with IGF-II but the latter was one hundredth as potent as the latter. Nevertheless, because of its ease of preparation and availability, it has been employed extensively in this and in our current studies to measure nonspecific 125I-IGF-II binding.

The data from the competition by unlabeled IGF-II were analyzed by the method of Scatchard after correcting for nonspecific binding (Fig. 7). As evidenced by the linear slope

A, 600 ng of membrane or soluble extract protein were incubated with 3.82 fmol of 125I-IGF-II ± 14.3 pg of MSA for 0.5 to 180 min and bound ligand separated from unbound as described under "Materials and Methods." Total binding (O) and nonspecific binding (●) for the membrane and extract were presented as the mean of 3-9 determinations. B, the second order rate constant of association, \( k_a \), was determined for IGF-II binding to the membrane and extract by plotting time against log₁₀ (free IGF-II concentration/free receptor concentration). It was assumed the latter was equal to the quantity of 125I-IGF-II bound specifically to receptor, i.e., 0.26 fmol for the membrane and 0.94 fmol for the extract. The \( k_a \) for the IGF-II receptor in the membrane and extract was 5.3 and 8.5 × 10⁻³ M⁻¹ min⁻¹, respectively.

Specifically bound ligands dissociate from their receptors with first order kinetics as indicated by the second term, i.e.

\[ \frac{dRI(t)}{dt} = -k_d R(t) \]

By plotting the left term of this expression as a function of time, a straight line was obtained with a slope of \( k_a \). The \( k_a \) for the membranes and soluble receptors were 5.3 and 8.5 × 10⁻³ M⁻¹ min⁻¹, respectively (Fig. 4B).
of the regression line, IGF-II binds to a homogeneous class of membrane receptors with an equilibrium dissociation constant, \(K_d\), of 0.6 nM. For each mg of protein, 1.6 pmol of IGF-II were bound (the correlation coefficient of this analysis was 0.978). Extraction of the membrane with \(n\)-octylglucoside also apparently solubilized a single class of receptors. However, they bind the ligand with less affinity, i.e. \(K_d\) of 1.3 nm was obtained and the total binding capacity of the extract was 6-fold greater than was observed for the membrane.

Hydrodynamic Properties of the IGF-II Receptor

Gel Chromatography—\(n\)-Octylglucoside-solubilized IGF-II binding proteins can be resolved into two components on Sephacryl S-300 (Fig. 8). Those eluting between fractions 42 and 50, and designated peak 1, bind the greatest quantity of labeled ligand. This binding was also the most specific. The Stokes radius of the peak 1 receptors was determined to be 7.2 nm (Fig. 9). Proteins eluting between fractions 55 and 60 also bind IGF-II but with less specificity than the receptors in peak 1. Proteins present in peaks 1 and 2 were concentrated 4- to 5-fold with Carbowax (Union Carbide), and \(^{125}\)I-IGF-II binding, as competed for by an impure preparation of unla
erged IGF-II, was determined and the data analyzed by the method of Scatchard. As illustrated in Fig. 7, the soluble receptor in peak 1 binds IGF-II with the same affinity as that associated with the intact membrane, i.e. they both have \(K_d\) values of 0.6 nm. The chromatographed preparation was also enriched 2-fold in specific IGF-II binding capacity when compared with the extract, e.g. the \(R_o\) of the former was 21.8 fmol/mg of protein; for the latter it was 10.4. Peak 2 bound about one-fourth as much \(^{125}\)I-IGF-II as peak I and also with a lower affinity. It is possible that this binding is not to a membrane receptor; rather, it may be to a somatomedin binding protein that was either present in the placenta or introduced as a contaminant with bovine serum albumin during the labeling of the receptors with \(^{125}\)I-IGF-II. These possibilities must be considered since the Stokes radii of peak 2 proteins (Fig. 9)
and one of the somatomedin c binding proteins of human serum are both 4.3 nm (35). We evaluated these, in part, by
(a) washing the R<sub>5</sub> membrane preparation two additional times prior to detergent solubilization; and (b) leaving out
bovine serum albumin that might be contaminated with binding protein during the labeling procedure. Neither of these
 treatments had any effect on the magnitude of <sup>125</sup>I-IGF-II labeling of peak 2 proteins (data not presented).

**Sucrose Gradient Centrifugation**—The calculation of 7.2 nm for the Stokes radius of the Sm<sub>1</sub>IGF receptor in peak 1
provided no information on its molecular size and shape. To order to assign values to these parameters, we determined the
sedimentation coefficient of the <sup>125</sup>I-IGF-II receptor complex on linear gradients of sucrose prepared in H<sub>2</sub>O or D<sub>2</sub>O. The
latter medium was employed since the determination of the positions of the IGF-II binding components in this gradient
and in gradients prepared in H<sub>2</sub>O when compared to the position of standard proteins allowed the calculation of the
receptor’s partial specific volume, V<sub>p</sub> (30). Specific <sup>125</sup>I-IGF-II binding was maximum in fractions 36.5 ± 1.5 (S.E.) or 37.5 ±
0.2 when peak 1 was incubated with <sup>125</sup>I-IGF-II prior to centrifugation in the H<sub>2</sub>O- or D<sub>2</sub>O-containing gradients,
respectively (Fig. 11, A and B). If the distribution of the receptors in the two gradients was determined by measuring <sup>125</sup>I-
IGF-II binding to eluted fractions, then it was maximum in fraction 39 ± 0.6. A small quantity of specific IGF-II binding
was found consistently in fractions 24–30 if the proteins had been prelabeled with <sup>125</sup>I-IGF-II prior to centrifugation, but
specific binding could not be detected when comparable fractions were incubated with <sup>125</sup>I-IGF-II after centrifugation. As
evidenced by its rightward displacement in sucrose gradients prepared in D<sub>2</sub>O relative to that of catalase, e.g. the enzyme
activity was maximum in fractions 41.4 ± 0.2 and 39.7 ± 0.4 from the H<sub>2</sub>O- and D<sub>2</sub>O-containing gradients, respectively,

![Figure 9](http://www.jbc.org/)

**Figure 9.** Determination of the Stokes radii of the IGF-II receptors. The partition coefficient, K<sub>av</sub>, for the IGF-II receptors and
proteins employed to standardize the Sephacryl S-300 column were calculated from the respective elution volume, V<sub>e</sub>, based on the
relationship K<sub>av</sub> = V<sub>R</sub> – V<sub>e</sub>/V<sub>R</sub> – V<sub>e</sub>. These values were plotted as a function of their known Stokes radius using the correlation
of Laurent and Killander (27) to derive values of 7.2 and 4.3 nm for the Stokes radii of the receptors in peak 1 and 2, respectively.

### Table II

Physical properties of the n-octylglucoside-solubilized rat placenta IGF-II receptor

| Parameter               | Value          |
|-------------------------|----------------|
| Stokes radius           | 7.2 nm         |
| Sedimentation coefficient| 9.9 x 10<sup>13</sup> s |
| Partial specific volume | 0.72 cm<sup>3</sup>/g |
| Molecular weight        | 290,000        |
| Frictional ratio<sub>0/f<sub>0</sub></sub> | 1.6 |

The φ of the IGF-II receptor and enzyme must be similar. Using the formulation of Clarke (30) to estimate viscosity, a
φ of 0.72 cm<sup>3</sup>/g was calculated for the IGF-II receptor (Table II). Since this value is within the range of values reported
for the standard proteins, we plotted their experimentally determined and known s<sub>20,W</sub> values versus one another, and from
this relationship (Fig. 11), derived a s<sub>20,W</sub> value of 9.9 s for the receptor. From this value, a Stokes radius of 7.2 nm and φ of
9.72 cm<sup>3</sup>/g, an M<sub>0</sub> of 290,000 and a frictional ratio of 1.6 were calculated for the IGF-II receptor (Table II).
centrifugation in H2O-containing sucrose gradients were plotted as a function of their s20,w values and a s value of 9.9 × 10^13 s calculated for the IGF-II receptor.

![Graph](image)

**FIG. 11.** Sedimentation coefficients of the IGF-II receptor and standard proteins on linear sucrose gradients. The experimental s values of standard proteins and peak I proteins that were either prelabeled (●) or postlabeled (○) with 125I-IGF-II prior to centrifugation in H2O-containing sucrose gradients were plotted as a function of their s20,w values and a s value of 9.9 × 10^13 s calculated for the IGF-II receptor.

**Table 1.** Characterization of Soluble IGF-II Receptors

| MW (kDa) | MSA | DTT |
|----------|-----|-----|
| 43       | -   | +   |
| 200      | +   | +   |
| 250      | -   | -   |

One hundred pg of R3 membrane protein was incubated in 50 mM phosphate, 1 mM PMSF, pH 7.4, containing 15,000 cpm of 125I-IGF-II in the absence or presence of 50 μg of unlabeled MSA for 60 min at 24 °C and unbound radioactivity separated from bound by centrifugation for 800,000 × g min. The resuspended and washed membrane was incubated at 0-4 °C for 15 min with 0.1 mM DSS, solubilized in SDS-containing sample buffer ± 100 mM DTT, and the proteins resolved by electrophoresis on 5-15% gradient gels of acrylamide as described under "Materials and Methods." The polypeptides were stained with Coomassie blue, photographed, and dried, and the species cross-linked with radioactive ligands were identified by autoradiography. MW, proteins of standard molecular weight including filamin (250,000), myosin (200,000), β-galactosidase (119,000), phosphorylase b (95,000), bovine serum albumin (69,000), and ovalbumin (43,000). Samples A and B were run in the presence of 100 mM DTT, and C and D in its absence. The autoradiographs of the membrane proteins in lanes A, B, etc. are identified A', B', etc. A, A'; R3 + 125I-IGF-II. B, B': as in A + 50 μg of MSA. C, C'; unreduced R3 + 125I-IGF-II. D, D': as in C + 50 μg of MSA.

**FIG. 12.** Identification of the rat placental Sm/IGF receptors. One hundred pg of R3 membrane protein was incubated in 50 mM phosphate, 1 mM PMSF, pH 7.4, containing 15,000 cpm of 125I-IGF-II in the absence or presence of 50 μg of unlabeled MSA for 60 min at 24 °C and unbound radioactivity separated from bound by centrifugation for 800,000 × g min. The resuspended and washed membrane was incubated at 0-4 °C for 15 min with 0.1 mM DSS, solubilized in SDS-containing sample buffer ± 100 mM DTT, and the proteins resolved by electrophoresis on 5-15% gradient gels of acrylamide as described under "Materials and Methods." The polypeptides were stained with Coomassie blue, photographed, and dried, and the species cross-linked with radioactive ligands were identified by autoradiography. MW, proteins of standard molecular weight including filamin (250,000), myosin (200,000), β-galactosidase (119,000), phosphorylase b (95,000), bovine serum albumin (69,000), and ovalbumin (43,000). Samples A and B were run in the presence of 100 mM DTT, and C and D in its absence. The autoradiographs of the membrane proteins in lanes A, B, etc. are identified A', B', etc. A, A'; R3 + 125I-IGF-II. B, B': as in A + 50 μg of MSA. C, C'; unreduced R3 + 125I-IGF-II. D, D': as in C + 50 μg of MSA.

**FIG. 13.** Identification of the Sm/IGF receptors in detergent-soluble extracts of R3 membrane. An n-octylglucoside extract of the membrane, an extract dialyzed for 4 h, and the insoluble residue were incubated as described in the legend to Fig. 12 with 15,000 cpm 125I-IGF-II ± 50 μg of unlabeled MSA and ligand bound to the soluble receptor separated from free IGF-II by polyethylene glycol precipitation and sedimentation. 125I-IGF-II that bound to the residue and precipitated proteins were cross-linked with 0.1 mM DSS, dissolved in sample buffer containing 100 mM DTT, and electrophoresed and processed for autoradiography as described in Fig. 12. MW, molecular weight standards. A, R3 membrane; B, B', C, C': detergent-solubilized extract ± MSA. D, D', E, E': dialyzed extract ± MSA. F, F', G, G': residue ± MSA. Note the absence of a cross-linked polypeptide in the region of 250 kDa in E' but the presence of a significant quantity of 125I-IGF-II in the gel front.

**Identification of IGF-II Receptors**

Receptors in the placental membranes which were cross-linked to 125I-IGF-II subsequent to binding have M, values of ~250,000 when SDS-polyacrylamide gel electrophoresis was carried out in the presence of 100 mM DTT, and 200,000-220,000 in its absence (Fig. 12, A, A', C, and C'). The binding of the 125I-labeled ligand to these polypeptides was specific since unlabeled MSA blocked it completely (Fig. 12, B, B', D, and D'). Under comparable conditions of labeling, cross-linking, period of autoradiography, etc., 125I-IGF-I could not be cross-linked to the 250-kDa receptor or to other polypeptides (data not presented).

Treatment of the R3 with 2% (w/v) n-octylglucoside, followed by centrifugation, selectively enriched the extracts and residue fraction in specific polypeptides (Fig. 13). Although all three fractions bound 125I-IGF-II specifically (Table I), cross-linked polypeptides were only identified in the extract and dialyzed extract. The Mw of the soluble receptor was 250,000 in the presence of 100 mM DTT (Fig. 13, B, B', and D'). We have no explanation for our inability to cross-link 125I-IGF-II to the presumed receptors in the residue (Fig. 13, F and F'). As evidenced by counting the fractions prior to solubilization and electrophoresis and the presence of a differential in the number of silver grains in the region of the dye front, we believe 125I-IGF-II binds to proteins in the residue, and this binding was specific. This anomaly is being evaluated further.

As described in the previous section, Sephacryl S-300 gel chromatography separated the detergent-solubilized Sm/IGF...
FIG. 14. Identification of the Sm/IGF receptor in peak 1 and peak 2 fractions following Sephacryl S-300 gel chromatography of the dialyzed extract. One hundred μg of protein from peak 1 were incubated with 15,000 cpm 125I-IGF-II ± 50 μg of MSA and the ligand-receptor complex identified by autoradiography following cross-linking and SDS-PAGE as described in the legends to Figs. 12 and 13. M.W., molecular weight standards. A, A', B, B': dialyzed extract ± MSA. C, C', D, D': peak 1 ± MSA. E, E', F, F': peak 2 ± MSA. The autoradiograph in this figure was overexposed to demonstrate the labeling of a polypeptide of ~70 kDa (note arrow).

DISCUSSION

Studies by Daughaday et al. (22) have shown that human placental membranes bind Sm/IGF through a minimum of two receptor systems. IGF-I binds to a low capacity, high affinity system with a Kd of 1.6 nM while IGF-II binds to both high affinity, e.g., 1.8 nM, low capacity and low affinity, e.g., 20 nM, high capacity receptors. By contrast, rat placental membranes have a single clearly demonstrable Sm/IGF receptor system that binds up to 5 pmol of 125I-IGF-II/mg of protein with a Kd of 3.5 nM (22). Although a small quantity of IGF-I was also bound, it was more easily displaced by unlabeled IGF-II than it was by IGF-I (22). We have, therefore, chosen the rat placenta as our source of tissue to attempt to isolate the Sm/IGF receptors.

Differential centrifugation of placental homogenates or centrifugation of the membranes in the R1 fraction on 5–30% (w/v) discontinuous gradients of dextran did not enrich any fraction in specific IGF-II binding activity (Table I). A similar finding was obtained by Guyda et al. (37) for differentially centrifuged homogenates of human placenta from early pregnancies. Although these results are based on a limited number of observations and with fractions that have been incompletely characterized with respect to organelle composition, we must entertain the possibility that the 125I-IGF-II is binding to receptors on intracellular membranes as well as those associated with the plasma membrane. Hence, because of two possible sources of receptors, some variation in their properties, e.g. the degree of glycosylation, might be anticipated.

n-Octylglucoside became the detergent of choice to solubilize the receptors that bound 125I-IGF-II. Of the detergents evaluated, e.g. Triton X-100, NIKKOL-8L-85Y, and Zwittergent (3–12), it was the only one that did not affect total or specific ligand binding to the intact membrane when present at concentrations up to 0.1% (w/v). These results are consistent with previous studies on the effect of different detergents on the solubilization and chemical and enzymatic properties of ATPase and NADH dehydrogenase from Streptococcus faecalis (34), and rhodopsin from bovine retina (38). In addition to its mild effect on protein structure, this compound has several properties which made its use advantageous in our attempts to isolate the Sm/IGF receptor including (a) a definable structure, (b) minimal absorption in the ultraviolet, (c) a very high CMC of 20–25 mM, and (d) a Φ of 0.8197 (36).

Approximately 60% of the placenta’s protein was solubilized with 2% (w/v), i.e. 67.9 mM, detergent during 60 min of incubation at 0–4 °C. The unextracted residue bound 125I-IGF-II and the binding was inhibited by an excess of unlabeled MSA (Table I). However, because of our inability to identify the binding unit by cross-linking it with iodinated growth factor, we cannot state with certainty what the nature of this binding material is. Dialysis of the extract for 4 h against 0.2% (w/v) (16.8 mM) detergent did not precipitate protein or alter the magnitude or specificity of 125I-IGF-II binding (Table I and Fig. 6). The result is consistent with the observation of Gould et al. (39) that 125I-insulin binding to solubilized turkey erythrocyte membrane receptors was unaffected by n-octylglucoside below its CMC of 0.63% (w/v); at concentrations greater than the CMC, the total binding capacity decreased.

The solubilized 125I-IGF-II-receptor complex identified by cross-linking studies had a M, of 250,000, when reduced with DTT (Fig. 13) that was identical to that observed in the intact membrane. The receptor also bound the ligand with rate constants of association and dissociation that were similar to those determined for the membrane-associated receptor, e.g. the k, and k0 for the extract were 8.5 × 107 M⁻¹ min⁻¹ and 7.5 × 10⁻³ min⁻¹, while those for the membrane were 5.3 × 10³ M⁻¹ min⁻¹ and 4.2 × 10⁻² min⁻¹, respectively. The equilibrium dissociation constants, Kd, for the membrane and extract differed by a factor of two, e.g. 0.6 and 1.3 nM, respectively; the partially enriched receptor present in peak 1 following chromatography of the extract on Sephacryl S-300, however, had a Kd of 0.6 nM. These results are consistent with the premise that detergent-solubilization does not modify the binding properties of the IGF-II receptor. However, because of a discrepancy between the determination of Kd from measurements of the rate constants of association and dissociation, i.e. Kd = k0/k, = 8 pM, and the binding of 125I-IGF-II at equilibrium in the presence of competing concentrations of unlabeled ligand, e.g. the Kd = 600 pM, it is probable that the value for k0 may be erroneously high. Alternatively, the interaction of IGF-II with its receptor may be more complicated than the reaction R + H ↔ RH implies.

The Kd of 0.6 nM, determined from equilibrium binding studies, is within the range of values reported for detergent-solubilized insulin receptors (14) and IGF-I receptors from human placenta (15), and for IGF binding to adipocytes (3) and cultured cells (3, 40). The k0 values of 4–8 × 10⁻³ min⁻¹ for the IGF-II receptor are smaller than has been reported for the insulin receptor (41, 42) and infers that the former binds its ligand with a greater affinity than the latter. Since we had

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no difficulty in measuring the dissociation of the IGF-II-receptor complex, the discrepancy between the values for $K_d$ determined by the equilibrium versus the kinetic method can be explained, in part, by our calculating a large $k_3$ for this receptor system. The $K_d$ for the binding of IGF-II to the placental receptors was about one log higher than has been reported for insulin's binding to its receptor (41) or for IGF binding to cultured chick embryo fibroblasts (40). We have ruled out methodological problems as accounting for these results and conclude that the high $k_3$ reflects our failure to measure association under pseudo first order conditions, i.e. the concentration of the ligand is assumed to remain essentially constant over the period of time binding to the receptor is determined with $k_3$ depending on receptor concentration alone (43). In these studies, we incubated 3.82 fmol of $^{125}$I-IGF-II (≈4300 cpm) with 6 or 1 fmol of receptor calculated to be present in 0.6 μg of extract protein or membrane protein, respectively. A plot of IGF-II binding with time (Fig. 4) indicates that 6 and 16% of the free $^{125}$I-IGF-II-bound to the membranes and soluble receptor, respectively, by 30 min of incubation. If one-half of the $^{125}$I-IGF-II was biologically active (this is a reasonable assumption since the maximum $B_0/T$ that could be obtained with soluble receptor was 0.6, and 20% of this binding was nonspecific), then with increasing periods of incubation, less of it would be available for binding and the reaction of the receptor with the ligand would shift from pseudo first order to second order. This explanation assumes that the interaction of IGF-II with its receptor is a reversible reaction. However, if the receptor is modified concomitant with occupancy or has cooperative binding sites, then a simple, biomolecular reaction mechanism will be inadequate to explain our results. This possibility is supported by a study of progesterone binding to cytosol receptors from chick oviduct by Hansen et al. (44). These investigators observed a 180-fold difference between the $K_d$ determined from a kinetic analysis and that from equilibrium binding experiments, e.g. 0.027 and 5 nM, respectively, when the incubations were carried out at 0 °C. Raising the temperature to 15 °C increased the difference by more than three logs. The $K_d$ from the equilibrium binding measurements did not change with temperature and was similar to the concentration of steroid that evoked 50% of a maximum physiological response. Since the $k_3$ changed only slightly while the $k_3$ increased 100-fold with a 35 °C increase in temperature, they concluded that measurements of the latter parameter are most susceptible to error. This is particularly so, if the association of ligands with receptors are complex reactions involving more than one state of the receptor.

Based on cross-linking with $^{125}$I-IGF-II (18), or $^{125}$I-MSA (19), or $^{125}$I-II.A the IGF-II receptor is thought to be a 220-kDa monomeric protein, i.e. it is not associated with other subunits. This interpretation must be viewed with caution, since we have observed that: 1) chromatography of detergent-solubilized extracts on Sephacryl S-300 resolved the binding components into two forms with Stokes radii of 7.2 and 4.3 nm; 2) cross-linking the IGF-II receptor with $^{125}$I-IGF-II revealed a 250-kDa component (reduced) in the 7.2 and 3.8 nm forms and a lightly labeled 70-kDa component (reduced) in the former; 3) centrifugation of peak 1 proteins prelabeled with $^{125}$I-IGF-II suggested multiple forms of the receptor; and 4) the molecular weight of this receptor was calculated from the Stokes radius, partial specific volume, and sedimentation constant to be 290,000.

The existence of more than one form of the insulin receptor was observed by Ginsberg et al. (45). These investigators noted that the addition of small quantities of insulin to detergent-solubilized turkey erythrocyte receptors proportionally increased the amount of a receptor with a Stokes radius of 3.8 nm and concomitantly reduced the amount of the 7.2 nm form. Using similar methodology, Maturo and Hollenberg resolved the receptors from the membranes of rat liver (46) and adipocytes and cultured fibroblasts (47) into two $^{125}$I-insulin binding regions with Stokes radii of 7.2 and 3.8 nm. The 7.2 nm form of the receptor bound insulin with 10-fold greater affinity than did the 3.8 nm form. Krupp and Livingston identified two insulin binding components in Triton X-100 extracts of rat adipocytes (48) and liver plasma membrane (49) when the extracts were electrophoresed in polyacrylamide gels under nondenaturing conditions. They too found that small quantities of insulin converted some of the larger receptors present in peak 1 into a smaller form, designated peak 2. This bound $^{125}$I-insulin with different affinities. For example, Scatchard plots of insulin binding to peak 1 were curvilinear while those to peak 2 were linear.

The insulin binding component with a Stokes radius of 7.2 nm, and which may be the receptor form present in Krupp and Livingston's peak 1, is most probably the disulfide-linked 250-kDa insulin receptor complex proposed by Maturo and co-workers from the results of affinity labeling studies (50). In their model, the receptor is composed of two 140-kDa α subunits and two 90-kDa β subunits linked by disulfide bonds in a stoichiometry of $\beta-(S-S-S)\alpha-(S-S-S)-(S-S-S)-(S-S-S)\beta$. Peak 2 of Krupp and Livingston's or the insulin binding components with a Stokes radius of 3.8 nm could result from the reduction of this complex and the generation of a smaller receptor form with the stoichiometry of $\beta-(S-S-S-\alpha$. This form of the receptor has recently been shown to be functional, e.g. it binds $^{125}$I-insulin as well as the unmodified form of the receptor, and its occupancy stimulates hexose uptake by rat adipocytes (51).

The IGF-I receptor extracted from human placenta has a Stokes radius of 7.2 nm (15) and a sedimentation coefficient of 11 s (21). A molecular weight of 402,000 was calculated for the detergent-receptor complex. This receptor and that present in cultured cells contain 130-140-, and 95-kDa subunits (15, 18-20), and others of still smaller size (21) that are disulfide bonded to form 300-350-kDa complexes similar to the hormone receptor. However, unlike the latter, an IGF-I binding species with a Stokes radius of 3.8 nm has not been observed (15).

Two IGF-II binding species were detected in detergent extracts of rat placenta (Fig. 8). Peak 1 bound the greatest quantity of $^{125}$I-IGF-II, e.g. the $R_0 = 21.8$ pmol/mg of protein, and with the highest affinity. It had a Stokes radius of 7.2 nm, which is identical with that of the insulin (45-49) and the IGF-I receptor (15). A minor peak of IGF-II binding with a Stokes radius of 4.3 nm was consistently observed in the detergent extracts. The possibility was considered that this peak reflected the presence of a contaminating somatomedin binding protein but repeated washing of the membrane prior to solubilization or the use of bovine serum albumin-free elution buffer (this protein is frequently contaminated with binding protein) did not alter the magnitude of the 4.3 nm peak. The affinity of $^{125}$I-IGF-II for the binding components on peak 2 was much less than it was for those in peak 1; e.g. the $K_d$ values were 2.6 and 0.6 nm, respectively, and numerous attempts to identify receptor complex following centrifugation in gradients of sucrose or electrophoresis in polyacrylamide gels under nondenaturing conditions were unsuccessful. $^{125}$I-IGF-II could be cross-linked to a 250-kDa protein present in peak 2, but the quantity labeled was so

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small in comparison to the proteins labeled in peak 1 (Fig. 14) that the results could be accounted for by the carry over of a small amount of the latter into peak 1. Alternatively, the cross-linking of $^{125}$I-IGF-II to a putative 4.3 nm receptor by disuccinimidyl suberate could be very inefficient. A precedent for this phenomenon was our inability to cross-link $^{125}$I-IGF-II to binding components remaining in the residue following detergent extraction of the $R_2$ even though the magnitude of this binding was significant and it was specific (Table 1).

Consistent with the observations of others (18, 19, 52), $^{125}$I-IGF-II could be cross-linked specifically to a receptor of 220-kDa (or 250-kDa when reduced) that was present in rat placental membrane (Fig. 12), a detergent extract of these membranes (Fig. 13), and peak 1 of the chromatographed extract (Fig. 14). However, we have also identified a radioactively labeled 50- or 75-kDa (when reduced with DTT) protein in the $R_2$, and Sephacryl gels and lead to anomalous estimates of its molecular weight (52). The possibility exists, with the subsequent chemical and physical characterization should be reproducible pattern could represent IGF-I receptor components in peak 1 also indicates that there may be more than one form of this receptor. Sucrose density gradient centrifugation of these components, subsequent to their incubation with $^{125}$I-IGF-II ± MSA, disclosed specific ligand binding between fractions 24–42 (Fig. 10). The greatest quantity of label was found in fraction 38. Receptors with similar sedimentation properties also were identified by incubating the fractions from the gradients with $^{125}$I-IGF-II and precipitating the complex with polyethylene glycol. The latter method did not disclose, however, the small but specific region of binding between fractions 24–29 and the shoulder around fraction 33 that had been observed for the prelabeled receptors. This reproducible pattern could represent IGF-II-receptor complexes that are either dissociating or are being degraded as a consequence of receptor occupancy. The latter effect has been reported to occur when the insulin receptor is occupied (50). Finally, the calculation of a molecular weight of 290,000 for the IGF-II receptor (Table II) is consistent with the suggestion that the ~220-kDa component may be associated with other proteins. However, this method to determine molecular size is fraught with potential errors, e.g. estimating $\bar{v}$ of a receptor-detergent complex from extrapolated $\rho$ and $s$ values, or the $s_{20w}$ from an experimentally determined $s$ value. In addition, the presumed covalent linkage of heterosaccharides with the protein of the receptor would also influence the electrophoretic and diffusional mobilities in polyacrylamide and Sephacryl gels and lead to anomalous estimates of its molecular size. Therefore, it is premature to speculate about the possible structure of the IGF-II receptor without more information. Its eventual purification to homogeneity from the rat placenta or Swarn rat chondrosarcoma (53) and subsequent chemical and physical characterization should satisfy this requirement.

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