Purification and Properties of the Human Placental Insulin Receptor*

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Human placental insulin receptor was purified 11,000-fold to near homogeneity using DEAE-cellulose chromatography and affinity chromatography on insulin-Sepharose. Approximately 200 to 300 µg of purified receptor were obtained from a single placenta. In solution, the native receptor is a complex ($M_r = 440,000$) of an acidic, multi-subunit protein with a $M_r$ of 350,000 and bound detergent accounting for the remainder of the mass. The receptor protein is asymmetric ($I_f/H = 1.4$) and consists of a single Coomassie blue staining polypeptide of $M_r = 135,000$. In addition to the 135,000-dalton polypeptide, two smaller polypeptides of $M_r = 45,000$ and 90,000 were observed upon autoradiography of $^{125}$I-labeled receptor subjected to sodium dodecyl sulfate-polyacrylamide gels under reducing conditions. The smaller polypeptides did not stain with Coomassie blue but migrated with native receptor activity on isoelectric focusing gels and were communoprecipitated with the 135,000-dalton polypeptide by anti-insulin receptor antibody. The 135,000-dalton subunit was specifically labeled by $^{125}$I-insulin using the bifunctional cross-linking reagent disuccinimidyl suberate (P. F. Pilch, and M. P. Czech, (1979) J. Biol. Chem. 254, 3375-3381), suggesting that this component contains the insulin binding domain.

The initial step in insulin action is the specific binding of the hormone by a high affinity receptor at the target cell surface. Plasma membrane receptors for insulin were recognized more than a decade ago, but progress toward a detailed description of the structural components of the receptor and the elucidation of their roles in sequestering insulin and transducing hormone binding into physiological effects has been sporadic and limited because of the low concentrations of receptors in target tissues and difficulties encountered in purifying highly active forms of this integral membrane protein.

Cuatrecasas (1, 2) first demonstrated that the receptor could be quantitatively solubilized from rat liver and fat cell membranes by the nonionic detergent Triton X-100 without alteration in its affinity, specificity, and kinetics of insulin binding and susceptibility to proteases and denaturants. Subsequently, Cuatrecasas (3) and Jacobs et al. (4) isolated a highly purified preparation of rat liver insulin receptor using a combination of conventional procedures and affinity chromatography on insulin-agarose. Analysis of the insulin receptor on sodium dodecyl sulfate-polyacrylamide gels under reducing conditions revealed a single Coomassie blue-stained poly-

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1 The abbreviations used are: SDS, sodium dodecyl sulfate; insulin-SDS-agarose, insulin-succinyl-diamino-dipropyl-amino-agarose.

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putative subunits might be involved in signal transduction and receptor regulation, while others may arise from the putative subunits. Further potential complexity in the structure of insulin receptors has been suggested by the findings that (a) the minimal M, for the cross-linked insulin receptor-P41-insulin complex determined on nonreducing, SDS-polyacrylamide gels is 300,000 to 350,000 (5, 8); (b) M, for the native receptor on nondenaturing, Triton X-100-containing polyacrylamide gels is 1,000,000 (16); (c) target size analysis indicates that the functional receptor is associated with a regulatory protein of 135,000-dalton protein by partial proteolysis. Thus, the available data fail to provide a clear picture of the physicochemical properties and subunit structure of the human insulin receptor. In order to begin to establish a definitive description of the subunit structure of the receptor and to develop correlations among the biochemical and functional properties of the receptor subunits, we have (a) purified the human placental receptor to apparent homogeneity, (b) characterized the native receptor in hydrodynamic studies, and (c) qualitatively identified potential receptor subunits by combinations of SDS-polyacrylamide gel electrophoresis, isoelectric focusing, specific immunoprecipitation, and cross-linking with 125I-insulin.

**EXPERIMENTAL PROCEDURES**

**Materials**—Transferrin, thyroglobulin, apoferritin, myosin, and D,0 were purchased from Sigma; bovine γ-globulin and bovine serum albumin were obtained from Miles Laboratory; β-galactosidase and phosphorylase b were from Boehringer-Mannheim; human serum albumin was from Calbiochem; scintillation grade Triton X-100 was acquired from New England Nuclear and fluorescamine was from Miles Laboratories. Crystalline porcine insulin, porcine proinsulin, porcine desoctapeptide insulin, guinea pig insulin, and tuna fish insulin were gifts from Dr. Ronald Chance, Eli Lilly and Co. Purified multiplication stimulatory activity was kindly supplied by Dr. Matthew M. Rechler, National Institutes of Health. Rabbit antiserum against purified rat liver insulin receptor (4) was prepared as previously described (10).

**Insulin Binding Activity**—Crude and purified samples of Triton X-100-solubilized insulin receptor were incubated with 0.4 nm 125I-insulin (100,000 cpm) in a final volume of 0.2 ml of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid binding buffer (0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.12 mM NaCl, 1.2 mM MgCl2, 2.5 mM KCl, 0.5 mM EDTA, 0.5 mM sodium acetate, pH 7.9) for 90 min at 24 °C. The 125I-insulin-insulin receptor complex was separated from free 125I-insulin by the polyethylene glycol precipitation method of Naughton et al. (11). Tubes containing 20 μg/ml of nonradioactive insulin were incubated in parallel to determine the extent of nonspecific binding. All assays were performed in triplicate and the data have been corrected for nonspecific binding.

**Iodination of Insulin**—125I-insulin (175 μCi/μg) was prepared as previously described (22).

**Iodination of the Purified Insulin Receptor**—Human placental insulin receptor (1 μg) was added to an equal volume of 0.33 M sodium phosphate buffer, pH 7.4, containing 0.1% Triton X-100. Na235I (0.5 μCi) and chloramine T (0.5 mM) were then added. After 1 min at 25 °C, the amount of 125I incorporated into the receptor was determined by precipitation of an aliquot, diluted in 0.1 M sodium phosphate buffer, pH 7.4, containing 1.5 mg/ml of bovine serum albumin, with 5% trichloroacetic acid. When 30% of the radioactive iodine had been incorporated, the reaction was terminated and unreacted iodine was removed by gel filtration on a column (0.56 × 10 cm) of Sephadex G-25 equilibrated with 50 mM Tris·HCl buffer, pH 6.8, containing 0.2% Triton X-100. The iodinated rat liver insulin receptor used for comparison with the human placental receptor was purified and iodinated according to Pilch and Czech (8).

**Protein Determinations**—Prior to affinity chromatography, protein determinations were performed by the method of Lowry et al.
The tissue was minced finely with scissors, suspended in 600 ml of 0.25 M sucrose, containing 5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 25 mM benzamidine-HCl, and was homogenized with a Brinkmann polytron homogenizer (PT35K) for 3 min at 42 V. The homogenate was centrifuged at 600 × g for 5 min and the pellet was reextracted with an equal volume of homogenizing buffer. The supernatant fractions were combined and microsomal membranes were prepared by the differential centrifugation method described by Cuatrecasas (1). Insulin receptors in the 40,000 g pellet were solubilized by extraction with 30 ml of 2% (v/v) Triton X-100 in 50 mM Tris-HCl buffer (pH 7.4) at 4 °C for 60 min. After centrifugation at 130,000 × g for 60 min, the supernatant fluid contained 90 to 100% of the insulin binding activity originally observed in placental microsomal membranes. The solubilized receptor preparation was then dialyzed for 16 h against 1 liter of 50 mM sodium acetate, pH 6.3, containing 0.2% Triton X-100, and subsequently was centrifuged for 40 min at 40,000 × g to remove material which precipitated during dialysis. The supernatant fluid was applied to a column (1.5 × 30 cm) of DEAE-cellulose previously equilibrated in the same buffer. The column was washed with 1 bed volume of buffer and then was eluted with a 400-ml linear gradient (0.1 to 1 M) of ammonium acetate, pH 6.3, containing 0.2% Triton X-100.

Peak fractions of insulin binding activity which eluted between 0.15 M and 0.55 M ammonium acetate were pooled and dialyzed for 16 h against 1 liter of 0.1 M sodium phosphate, pH 7.4, containing 0.1% Triton X-100. Subsequently, the solubilized receptor (80 ml) was applied to a 1-ml column of insulin-succinyl-diaminodipropylamino-agarose (3, 29) at 23 °C at a flow rate of 20 ml/h. The column was washed overnight at 4 °C with 500 ml of 50 mM sodium phosphate buffer, pH 7.4, containing 0.5 M NaCl and 0.1% Triton X-100 and the insulin receptor was then eluted at 23 °C with 50 mM sodium acetate buffer, pH 6.3, containing 0.1% Triton X-100 and 4.5 mM urea. Immediately after elution, peak fractions were pooled, cooled to 4 °C, and concentrated by dialysis against phosphate-buffered saline, pH 7.4, containing 0.1% Triton X-100 and 40% (w/v) sucrose. The concentrated receptor (100 μg/ml) can be stored for 3 months at 4 °C without loss of insulin binding activity and without alteration in either the molecular weights or the relative proportions of the subunits (see below). In general, 200 to 300 μg of purified receptor protein were obtained from a single placenta.

A typical insulin receptor purification is summarized in Table I. Exceptional enrichment occurs in two of the steps, preparation of a microsomal membrane fraction from the homogenate (Table I, Step 2) and affinity chromatography on insulin-SDS-agarose (Table I, Step 5). The overall purification was 11,000-fold.

Subunit Composition of the Insulin Receptor—When 2 μg of the purified receptor were subjected to polyacrylamide gel electrophoresis in sodium dodecyl sulfate, only one band, with an estimated molecular weight of 135,000, was visualized by staining with Coomassie blue (Fig. 1). This contrast with the pattern observed when the same or other preparations of receptor were chemically labeled with 125I, subjected to electrophoresis in SDS-polyacrylamide gels, and examined by autoradiography. As depicted in Fig. 2, lanes A and B, the preparation of 125I-placental insulin receptor contained 3 radioactive bands with molecular weights of approximately 135,000, 90,000, and 45,000. The relative amounts of 125I radioactivity in the 135,000-, 90,000-, and 45,000-dalton peptides were determined by SDS-polyacrylamide gel electrophoresis of highly purified human placental insulin receptor. A, insulin receptor (2 μg) was subjected to electrophoresis on a 10% polyacrylamide gel containing 0.1% SDS and was subsequently stained with Coomassie brilliant blue as indicated under "Experimental Procedures." B, human placental insulin receptor was labeled with 125I via chloramine T as described under "Experimental Procedures." An aliquot containing ~40,000 cpm was subjected to electrophoresis in 0.1% SDS using a 7.5% polyacrylamide gel. An autoradiograph of the dried gel is presented.

<table>
<thead>
<tr>
<th>Step</th>
<th>Insulin binding activity</th>
<th>Protein</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Homogenate</td>
<td>0.03 pmol/mg</td>
<td>48,300 mg</td>
<td>1</td>
</tr>
<tr>
<td>2. Placental membranes</td>
<td>1.37 pmol/mg</td>
<td>468 mg</td>
<td>46</td>
</tr>
<tr>
<td>3. Triton X-100 extract</td>
<td>2.74 pmol/mg</td>
<td>234 mg</td>
<td>91</td>
</tr>
<tr>
<td>4. DEAE-cellulose chromatography</td>
<td>6.41 pmol/mg</td>
<td>100 mg</td>
<td>214</td>
</tr>
<tr>
<td>5. Affinity chromatography</td>
<td>337 pmol/mg</td>
<td>0.20 mg</td>
<td>11,200</td>
</tr>
</tbody>
</table>

* Specific insulin binding activities are based on determinations of the amount of protein required to complex 10% of the 125I-insulin present in the standard radioligand binding assay (see "Experimental Procedures"). Serial dilutions of receptor preparations were assayed in triplicate to accurately determine these values. Since Scatchard plots of insulin binding data obtained using equal receptor concentrations of highly purified receptor and crude, Triton X-100-solubilized placental membranes were essentially superimposable (though curvilinear at high insulin levels), this approach permits a valid and direct estimate of the relative purification at each step and eliminates uncertainties introduced by extrapolating the Scatchard plots to the abscissa.
Purification of the Placental Insulin Receptor

**TABLE II**

**Distribution of radioactivity in the components of the human insulin receptor**

Highly purified human insulin receptor was chemically iodinated with chloramine-T (see "Experimental Procedures") and resolved into its apparent subunits as described in Fig. 1. Gel slices containing radiolabeled polypeptides were excised and the radioactive polypeptides were visualized by autoradiography (26). The autoradiogram presented depicts the following samples: lane A, 125I-placental insulin receptor immunoprecipitated with anti-insulin receptor serum (38,000 cpm applied); lane B, 125I-human placental insulin receptor immunoprecipitated with anti-insulin receptor serum (76,000 cpm); lane C, 125I-rat liver insulin receptor immunoprecipitated with anti-insulin receptor serum (62,000 cpm); lane D, 125I-rat liver insulin receptor precipitated with nonimmune serum (1,900 cpm); lane E, 125I-placental insulin receptor precipitated with nonimmune serum (1,300 cpm); lanes F (280,000 cpm) and G (560,000 cpm), total 125I-human placental insulin receptor; lanes H (150,000 cpm) and I (150,000 cpm), total 125I-rat liver insulin receptor.

![Fig. 2. Determination of the subunit composition of 125I-labeled human placental insulin receptor.](image)

![Fig. 3. Isoelectric focusing of highly purified, undissociated human insulin receptor and subsequent analysis of its subunit composition in a second dimension gel.](image)

(125I-labeled rat liver insulin receptor, presented for comparison in Figs. 2 and 3, contained only two subunits which exhibited relative mobilities slightly higher than the largest and smallest subunits of the human placental receptor. After iodination of sufficient placental receptor to be visualized by staining with Coomassie blue, only one polypeptide band of Mr = 135,000 was observed in SDS-polyacrylamide gels, excluding the possibility that the iodination procedure generated the two additional bands (data not shown). Purification of the placental receptor in buffers containing 50 mM benzamidine, 5 mM EDTA, 1 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 0.2 mM phenylmethylsulfonyl fluoride, and 50 μM leupeptin did not alter the patterns observed on stained or autoradiographed gels.

The purified receptor bound 125I-insulin avidly between pH 7.0 and 8.0 with an optimal activity evident at pH 7.6. Using 0.1 nM 125I-insulin, hormone binding reached equilibrium after 60 min at 24 °C or 30 min at 37 °C. The approach to equilibrium was slower by a factor of three to four at 4 °C, but the amount of 125I-insulin bound after 18 h was 25% higher than that observed after 1 h at 24 °C. At equal receptor concentrations, increasing amounts of nonradioactive insulin were equipotent in competitively displacing 125I-insulin from highly purified receptors and the unpurified insulin receptor solubilized from the placental membranes (Fig. 4). The apparent K_d for the receptor-insulin complex estimated from competitive binding curves is 1 to 2 nM.

Scatchard plots (30) of the competitive binding data are nearly linear at physiological insulin concentrations (0.01 to 5
nm), but display marked curvature at supraphysiological hormone concentrations. While negatively cooperative interactions or lower affinity sites clearly make significant contributions to the overall binding activity observed at high insulin concentrations, the physiologically relevant sites are more likely to be occupied at 0.01 to 5 nm hormone. More detailed equilibrium binding analyses in this range (typical results are presented in the inset in Fig. 4) suggest that the purified placental receptor contains a high affinity binding site with a $K_r$ of 0.35 nm for insulin and a maximum capacity for sequestering 0.3 mol of insulin/molecule of receptor.²

The potencies of various insulins and insulin analogs in displacing $^{125}$I-insulin from the highly purified receptor were compared as shown in Fig. 5. Of the ligands tested, only tuna fish insulin was as effective as porcine insulin; guinea pig insulin, porcine proinsulin, and porcine desoctapeptide insulin were bound only 1 to 10% as avidly as porcine insulin. The rank order of the binding affinities parallels the established biological potencies of these peptides and is characteristic of insulin receptors in a variety of mammalian tissues (31, 32).

As anticipated, glucagon, adrenocorticotropic hormone, and epidermal growth factor failed to displace $^{125}$I-insulin, while a high concentration (0.25 µM) of the "insulin-like" growth factor multiplication stimulatory activity displaced only 30% of the radioactive ligand.

Identification of the Insulin-binding Subunit—$^{125}$I-insulin was covalently coupled to the putative hormone binding component of the purified receptor and the receptor resident in placental membranes or extracted from those membranes by Triton X-100 (Fig. 6) by employing disuccinimidyl suberate, a bifunctional cross-linking reagent (8, 9). In all three receptor preparations, $^{125}$I-insulin was cross-linked to the 135,000-dalton subunit of the receptor. The cross-linking of $^{125}$I-insulin was prevented by the addition of 1 µM nonradioactive insulin.

As predicted from its relatively poor affinity for the receptor (see Fig. 5), higher concentrations of proinsulin than insulin were required to block cross-linking of $^{125}$I-insulin.

Hydrodynamic Properties of the Insulin Receptor—The purified receptor was preincubated with $^{125}$I-insulin until equilibrium binding was achieved and then was subjected to gel filtration on a calibrated column of Bio-Gel A-1.5M agarose (Fig. 7). A sharp symmetrical peak of high molecular weight receptor-$^{125}$I-insulin complex was resolved from free $^{125}$I-insulin. Since the insulin receptor exhibits the properties of an integral, plasma membrane protein, it is likely to have hydrophobic domains embedded in the lipid bilayer. During membrane dissolution by Triton X-100, tightly bound phospholipids are often displaced from these sites by competing detergent molecules. If significant amounts of Triton X-100 are avidly bound by the receptor, its size and density will be altered and hydrodynamic measurements will be misleading unless the

² The calculation of the number of sites is based on a $M_r$ of 350,000 (see below) and estimates of both the $K_r$ and number of binding sites are not corrected for small contributions made by lower affinity receptors or cooperative interactions. Nevertheless, the data fit the model of a single set of noninteracting sites quite well and thus provide a preliminarily description of the high affinity site.
reaction mixtures were then cooled on ice and carefully layered onto Triton X-100 for the 4.8-ml gradients. Samples were centrifuged for 11 (Hz0 gradients) purified insulin receptor. Linear sucrose gradients (5 to 20%) were sonicated in bicarbonate buffer, pH 7.9, containing 10 mg/ml of albumin and 0.2% nonradioactive insulin, for 60 min at 24 °C (see “Experimental procedures”). The hormone-receptor complex was then cooled to 4 °C and subjected to gel filtration on a column (1.6 × 100 cm) of BioGel A1.5M equilibrated with 50 mM sodium phosphate buffer, pH 7.4, containing 0.5% Triton X-100. The flow rate was 6 ml/h and 1.8-ml fractions were collected and assayed for 125I radioactivity in a gamma counter. A Vo of 64 ml was determined using blue dextran and the peak of the receptor-125I-insulin complex was observed at 92 ml. Fractions were collected and assayed for 125I radioactivity in a gamma counter. A typical soluble protein peak was observed at 92 ml.

Preincubation of the receptor with 125I-insulin in the presence of 35 pmol of tightly bound detergent. Data from the sedimentation experiments were used to estimate the partial specific volume of the insulin receptor. The physical parameters of the receptor were summarized in Table III.

**FIG. 7** Determination of the Stokes radius of the highly purified placental insulin receptor. The receptor (0.6 µg) was preincubated with 2 nm 125I-insulin (500,000 cpm) alone, or with 35 µM nonradioactive insulin, for 60 min at 24 °C (see “Experimental Procedures”). The hormone-receptor complex was then cooled to 4 °C and subjected to gel filtration on a column (1.6 × 100 cm) of BioGel A1.5M equilibrated with 50 mM sodium phosphate buffer, pH 7.4, containing 0.5% Triton X-100. The flow rate was 6 ml/h and 1.8-ml fractions were collected and assayed for 125I radioactivity in a gamma counter. A Vo of 64 ml was determined using blue dextran and the peak of the receptor-125I-insulin complex was observed at 92 ml. Fractions were collected and assayed for 125I radioactivity in a gamma counter. A typical soluble protein peak was observed at 92 ml.

**FIG. 8** Sucrose density gradient centrifugation of the highly purified insulin receptor. Linear sucrose gradients (5 to 20%) were prepared in D2O or H2O containing 0.1 mM sodium phosphate buffer (pH 7.4), albumin (1 mg/ml), and 0.2% Triton X-100. The purified placental insulin receptor (0.12 µg) was incubated with 125I-insulin (1 nmol (250,000 cpm) in 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, pH 7.9, containing 10 mg/ml of albumin and 0.2% Triton X-100 for 60 min at 24 °C in a final volume of 0.2 ml. The reaction mixtures were then cooled on ice and carefully layered onto the 4.8-ml gradients. Samples were centrifuged for 11 (H2O gradients) or 25 h (D2O gradients) in a Beckman SW 50.1 rotor at 42,000 rpm at 4 °C. After centrifugation, fractions (0.1 ml) were collected and assayed for 125I radioactivity. The internal standards used were catalase (s20,w = 11.3), fumarase (s20,w = 8.9), lactate dehydrogenase (s20,w = 7.3), and hemoglobin (s20,w = 4.5). Another set of incubations was processed identically except that 20 µg of nonradioactive insulin were added 5 min before the addition of 125I-insulin. The upper panels show the distribution of 125I observed in the H2O and D2O gradients, when the receptor was preincubated in the absence (O—O) and presence (●—●) of excess nonradioactive insulin. The bottom panel illustrates the determination of apparent s20,w values for the insulin receptor (indicated with arrows) in sucrose density gradients prepared in H2O and D2O.

**DISCUSSION**

The human placental insulin receptor has been solubilized with Triton X-100 and purified 11,000-fold to near homogeneity. In solution, the native receptor appears to be a large mass of bound detergent is directly estimated and corrections are made.

The possibility that tightly bound Triton X-100 was associated with the purified insulin receptor was evaluated by sedimenting the receptor through linear sucrose gradients prepared with H2O and D2O, as described by Meunier et al. (34), Neer (35), and Clarke (36). Significant amounts of bound detergent (v = 0.94 ml/g) will increase the partial specific volume (v) of the receptor to a value higher than that of typical soluble proteins (v = 0.73 ml/g). Consequently, the apparent sedimentation coefficient of a receptor-Triton X-100 complex would shift to a lower value relative to soluble marker proteins (which do not bind Triton X-100) as solvent density is increased.

Purified insulin receptor exhibited an apparent sedimentation coefficient of 11 S in sucrose gradients prepared in H2O, but only 9.5 S in the D2O gradients (Fig. 8), clearly indicating the presence of tightly bound detergent. Data from the sedimentation experiments were used to estimate v and s20,w for the receptor-Triton X-100 complex using Equations 13 and 14 of Clarke (36). The partial specific volume was found to be 0.777 ml/g and the s20,w value was 12 S. The experimentally determined Stokes radius and s20,w and v values were employed to calculate the molecular weight and frictional ratio for the insulin receptor. The physical parameters of the receptor are summarized in Table III.

**TABLE III**

<table>
<thead>
<tr>
<th>Physical properties of the human placental insulin receptor</th>
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<tbody>
<tr>
<td>Stokes radius</td>
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<tr>
<td>Partial specific volume (v)</td>
</tr>
<tr>
<td>s20,w</td>
</tr>
<tr>
<td>Mw (receptor/Triton X-100 complex)</td>
</tr>
<tr>
<td>f/f0</td>
</tr>
<tr>
<td>Triton X-100 bound (v)</td>
</tr>
<tr>
<td>Mw (receptor protein)</td>
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<td>pl</td>
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</table>

*a The molecular weight was calculated from the equation (33):

\[ M_w = \frac{\alpha N}{1 - \frac{\bar{v}}{\bar{v}}} s_{20,w} \]

where \( \alpha = \) Stokes radius, \( N = \) Avogadro's number, \( \eta = \) viscosity of water at 20 °C, and \( \rho = \) density of water at 20 °C.

The frictional ratio was calculated from (33):

\[ f/f_0 = \alpha (4\pi N/3 \rho) \bar{v} \]

These values are based on the assumption that the \( \bar{v} \) observed represents an average of contributions made by protein (0.735 ml/g) and tightly bound Triton X-100 (0.94 ml/g).
complex \((M_r = 440,000)\) composed of an acidic, multi-subunit protein with a \(M_r\) of 350,000 and avidly bound neutral detergent accounting for an additional mass of \(-90,000\) daltons (Table III). The association of 140 mol of Triton X-100/mol receptor (Table III) is consistent with the occurrence of large hydrophobic domains in the molecule which may play an important role in integrating the receptor into the phospholipid bilayer of the plasma membrane. The \(f/v\) value of 1.4 (axial ratio \(\approx 8\)) indicates that the receptor is asymmetric, but not extraordinarily so. Comparable or higher frictional ratios have been reported for other detergent-solubilized hormone receptors (34, 37).

The \(M_r\) value of 350,000 is in good agreement with estimates obtained for the overall size (\(M_r = 290,000\) to 350,000) of the disulfide-stabilized insulin receptor in adipocyte and liver membranes as visualized by photoaffinity labeling with

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{\text{I}}^{125}\text{-insulin analogs or cross-linking with } {\text{I}}^{125}\text{-insulin and electrophoretic analyses on nonreducing SDS-polyacrylamide gels (5, 9, 12). Other studies (16) indicating a } M_r \text{ of } 0.9 \times 10^6 \text{ on Triton X-100-containing, nondenaturing polyacrylamide gels raise the possibility that the receptor-detergent complex may undergo dimerization under some circumstances.}
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The corrections made for the effects of bound detergent on the \(\bar{v}\) of the receptor explain the discrepancy between the \(M_r\) data obtained in these studies and previous reports of \(M_r\) values for insulin receptors in crude, Triton X-100 extracts of membranes, which were \(-20\%\) lower (2, 14). During the course of receptor purification, no evidence was obtained for the occurrence of significant amounts of smaller \((<7 \text{ nm})\) insulin binding species; nor did the highly purified receptor dissociate into putative subunits upon preincubation with insulin and subsequent gel filtration.

Direct staining of the receptor after reduction and SDS-polyacrylamide gel electrophoresis revealed a single polypeptide chain with a \(M_r = 135,000\) (Fig. 1). Since this subunit is specifically labeled by

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{\text{I}}^{125}\text{-insulin and disuccinimidyl suberate (Fig. 6), it appears to contain the high affinity insulin-binding domain of the receptor. Photoaffinity labeling and cross-linking experiments on intact fat cells (9), purified fat cell and liver membranes (5-9), and highly purified rat liver insulin receptor (5) have all resulted in the identification of an insulin binding moiety with essentially the same } M_r \text{ (i.e. } 125,000 \text{ to } 135,000).}
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Upon chemical labeling with

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{\text{I}}^{125}\text{-via chloramine T, two additional, putative receptor subunits, exhibiting } M_r = 45,000 \text{ and } 90,000, \text{ were identified (Fig. 2). These polypeptides tentatively appear to be authentic receptor components because they copurify with the receptor, co-migrate with native receptor activity and the } 135,000\text{-dalton protein on isoelectric focusing gels (Fig. 3) and are co-immunoprecipitated with the } 135,000\text{-dalton polypeptide by anti-insulin receptor serum (Fig. 3, inset). Subunits of similar size have also been identified in cross-linking studies on adipocyte membranes (12, 13), and an } {\text{I}}^{125}\text{-labeled } 45,000\text{-dalton subunit is coimmunoprecipitated with the } 135,000\text{-dalton protein by an antiseraum specific for the latter polypeptide (10). At present, definitive conclusions regarding subunit stoichiometries cannot be reached because the } 45,000\text{-dalton and } 90,000\text{-dalton polypeptides are refractory to Coomassie blue staining (Figs. 1 and 2), and their identification by chemical labeling of tyrosine residues with } {\text{I}}^{125}\text{ is qualitative rather than quantitative. Thus, although the observations presented in this communication are consistent with the proposal of Massague et al. (13) that the native insulin receptor is an IgG-like molecule containing two "heavy chains" (} M_r = 135,000 \text{) and two "light chains" (} M_r = 90,000 \text{ and/or } 45,000 \text{) in a structure stabilized by multiple disulfide bonds, documentation of this kind of structure awaits more definitive information about the subunit stoichiometry of the receptor.}
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The subunit composition of the human insulin receptor reported here differs considerably from that suggested by earlier reports (17, 18) of two \((M_r = 80,000 \text{ and } 75,000)\) or four polypeptides \((90,000, 67,000, 56,000, \text{ and } 35,000)\) of markedly different size. Harrison and Itin (38) have recently suggested that proteolytic cleavage of the insulin receptor in human IM-9 lymphocyte extracts accounts for the increased multiplicity and decreased size of the apparent subunits.

During the preparation of this manuscript, Harrison and Itin (38) reported the purification of the insulin receptor from human placenta by affinity chromatography on wheat germ aglutinin-agarose and anti-insulin receptor IgG-agarose. Our results support their findings that a \(-126,000\text{-dalton polypeptide is a principal component of the receptor. These workers also found apparent subunits with } M_r \text{ values of } 90,000 \text{ and } 42,000 \text{ after labeling with } {\text{I}}^{125}\text{ via lactoperoxidase. In contrast to the data reported here, the } 42,000\text{-dalton protein is heavily stained by Coomassie blue with an intensity greater than that of the } 126,000\text{-dalton protein. If staining is proportional to protein mass, this result implies a high molar ratio of } 42,000\text{-dalton protein:} 135,000\text{-dalton protein (on the order of } 5 \text{ to } 6 \text{ if staining intensity were twice as high). Since the } 125,000 \text{ to } 135,000 \text{ dalton subunit appears to possess the hormone binding site it is possible that the excess } 42,000\text{-dalton protein is involved in signal transduction or regulation of receptor function and is lost in the purification procedure employed in our studies. Alternatively, some of the excess } 42,000\text{-dalton protein and smaller peptides that migrate near the dye front in the gels presented by Harrison and Itin (38) may be generated by partial proteolytic cleavage of the } 126,000\text{-dalton chain. A } 43,000\text{-dalton fragment of the } 126,000\text{-dalton polypeptide has been recently identified in cross-linking studies by Massague et al. (13).}
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It is difficult to directly compare insulin binding specific activities of the purified placental receptor preparations because of uncertainties introduced by extrapolating curvilinear Scatchard plots, different degrees of partial receptor denaturation caused by acidic MgCl\(_2\) in one case and acidic urea in the other, and the different methods used in evaluating binding activity (saturation assays versus partial saturation-high affinity site assays). Nevertheless, the study of Harrison and Itin (38) and our results are in close agreement on both the relative purification factor achieved in proceeding from crude membranes to nearly homogeneous receptor and the observation that \(-20\%\) to \(-33\%\) of the isolated receptor retains its native functional activity. The latter result is probably a function of the necessity of employing partially denaturing conditions to elute the receptor from affinity columns. Harrison and Itin (38) did not perform hydrodynamic studies to characterize the physicochemical properties of the receptor or attempt to assign the insulin binding domain to one (or more) of the subunits.

In a general sense, we have established that the insulin receptors in man and the rat are highly conserved with respect to overall size and shape, subunit composition, and the identity of the polypeptide chain containing the insulin binding site. The availability of a procedure for isolating 0.2 to 0.3 mg of highly purified insulin receptor from a single placenta should make it possible to determine subunit stoichiometries, evaluate possible structural relationships among the subunits, and ultimately discover the physiological roles of the 90,000- and 45,000-dalton peptides.

REFERENCES

Purification of the Placental Insulin Receptor