Partial Purification and Characterization of the Low Density Lipoprotein Receptor from Bovine Adrenal Cortex*

(Received for publication, June 11, 1980, and in revised form, August 6, 1980)

Wolfgang J. Schneider, Joseph L. Goldstein, and Michael S. Brown

From the Department of Molecular Genetics and Internal Medicine, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

The low density lipoprotein (LDL) receptor has been solubilized from bovine adrenocortical membranes with octyl-β-D-glucoside and purified 350-fold in the presence of the detergent. The activity of the solubilized receptor was assayed by precipitating the receptor with receptor with receptor, in the presence of egg phosphatidylcholine liposomes. The receptor-phosphatidylcholine liposomes bound 125I-LDL with the same affinity and specificity as did the native LDL receptor of intact membranes. The complex of receptor and octylglucoside had a Stokes radius of 83.5 Å as determined by agarose gel filtration. The sedimentation coefficient, \( s_{20,w} \), of the receptor-octylglucoside complex was 7.3 as determined by metrizamide density gradient centrifugation. An identical value for the sedimentation coefficient was obtained when deuterium oxide was substituted for water in the metrizamide gradient. These data were used to derive an estimate of 163,000 for the molecular weight of the LDL receptor-octylglucoside complex (range of molecular weight, 152,000 to 170,000). The receptor is an acidic protein as determined by its behavior on ion exchange chromatography. In the most highly purified LDL receptor preparation, which had been subjected to the sequential steps of solubilization, DEAE-cellulose chromatography, agarose gel filtration, and phosphatidylcholine/acetone precipitation, the receptor was estimated to constitute about 5% of the total protein. Thus, complete purification of the LDL receptor from bovine adrenocortical membranes will require an additional 20-fold purification, or a total purification of about 7,000-fold.

The low density lipoprotein receptor functions in the delivery of cholesterol to body cells (1). The receptor binds LDL, the predominant cholesterol-carrying lipoprotein in plasma, and initiates a process by which the lipoprotein is internalized by receptor-mediated endocytosis and degraded in lysosomes, liberating cholesterol for cellular use. The LDL receptor is one of a class of cell surface receptors that carry ligands into cells by virtue of their localization in coated pits. Internalization of these receptors and their bound ligands is accomplished by the invagination of these pits into the cells and their pinching off to form coated endocytic vesicles (2). Although the LDL receptor has been studied extensively in intact cells and isolated membranes by biochemical, genetic, and ultra-structural techniques (1–3), it has not yet been purified.

Recently, a method was described for the solubilization of the LDL receptor with the use of the nonionic detergent, octyl-β-D-glucoside (4). A large number of other detergents could not be used, either because they failed to solubilize the receptor or because their presence in the binding assay gave artifactual results due to their interaction with the 125I-LDL ligand (4).

In the previous study, the solubilized LDL receptor was assayed by a dilution-precipitation method in which dilution of the octylglucoside caused the receptor to form an insoluble aggregate that retained its ability to bind 125I-LDL (4). Receptor-bound 125I-LDL was separated from unbound 125I-LDL by filtration through cellulose acetate filters. The receptor that was solubilized and assayed by this technique was shown to be the same as the physiologic LDL receptor on the basis of biochemical and genetic evidence. Like the physiologic receptor of intact cells, the solubile receptor was absolutely dependent on calcium for binding LDL, it was sensitive to proteolytic enzymes, and it showed specificity for native LDL as opposed to lysine-modified LDL and other lipoproteins (4). Moreover, the activity of the soluble receptor was not detected in extracts of cultured fibroblasts from patients with homozygous familial hypercholesterolemia whose cells have a defect in the gene for the LDL receptor (4).

In the current studies, we have begun to purify and characterize the octylglucoside-solubilized LDL receptor from bovine adrenal cortex. This purification employs a new binding assay that permits measurement of small amounts of purified LDL receptor, a measurement that was not possible with the dilution-precipitation assay used earlier. The new binding assay involves the use of acetone to produce a co-precipitation of the receptor together with phosphatidylcholine. With this assay, LDL receptor activity can be measured in extracts containing extremely small amounts of dilute protein, thus allowing a characterization of the behavior of the octylglucoside-solubilized receptor on ion exchange chromatography, agarose chromatography, and density gradient centrifugation.

**EXPERIMENTAL PROCEDURES**

**Materials**

Sodium \([^{125}I]\)iodide (11 to 17 mCi/μg) was purchased from Amer sham. \([^{1-14}C]\)Acetic anhydride (109 mCi/mmol) was obtained from New England Nuclear Corp. Nuflow cellulose acetate membrane filters (pore size, 0.45 μm; diameter, 25 mm) were purchased from Oxoid Ltd. (Basingstoke, England; catalog No. N25/45). Miracloth was purchased from Calbiochem. Octyl-β-D-glucoside was obtained from Calbiochem or Sigma Chemical Co. Bovine serum albumin (catalog No. A4378 and A7888 (for use in binding assays)), human hemoglobin, catalase, and deuterium oxide were obtained from Sigma Chemical Co. Ferritin, aldolase, bovine serum albumin, and ovalba-
was prepared by reaction of aldolase with [I-^14C]acetic anhydride (5).

Bio-Gel A-1.5m, 100 to 200 mesh (8% agarose) was from Bio-Rad.

Metrizamide (analytical grade) was obtained from Accurate Chemical and Scientific Corp., Hicksville, NY. Egg phosphatidylcholine was from Avanti Polar-Lipids, Birmingham, AL (catalog No. 820051). Sodium deoxycholate was purchased from Schwarz/Mann.

Lipoproteins

Human LDL (density, 1.019 to 1.063 g/ml) and high density lipoprotein (density, 1.215 to 1.251 g/ml) were prepared from plasma by ultracentrifugation (6). Methyl-LDL was prepared by treatment of LDL with formaldehyde plus sodium borohydride (7). LDL was radiolabeled with [14C]acetic acid (4). Lipoprotein concentrations are expressed in terms of protein content.

Preparation of Bovine Adrenocortical Membranes and Solubilization of LDL Receptor

Bovine adrenal glands were obtained 15 to 30 min after slaughter and placed in ice-cold 0.15 M NaCl. All subsequent operations were carried out at 0-4°C. The cortex was separated from the medulla by sharp dissection and placed in Buffer A (20 mM Tris/chloride (pH 8), 0.15 M NaCl, and 1 mM CaCl2) at 5 ml/g of tissue. The tissue was homogenized with two 20-s pulses of a Polytron homogenizer (settings 5 and 9) and centrifuged at 800 x g for 10 min. The supernatant was filtered through Miracloth and centrifuged again at 8,000 x g for 5 min. The resulting supernatant was centrifuged at 100,000 x g for 60 min. The surface of each 800 to 100,000 x g pellet (membrane pellet) and the walls of the centrifuge tubes were rinsed with 15 ml of Buffer A to remove a lipid film. The membrane pellets were frozen in liquid nitrogen and stored at -170°C for at least 1 month without loss of LDL receptor activity.

The membrane pellets were solubilized as previously described (4). Typically, membrane pellets prepared from 70 g of bovine adrenal cortex (16 to 20 adrenal glands) were suspended in 45 ml of buffer containing 4.25 M Tris/maleate (pH 6.0) and 90 mM CaCl2 (pH 6.0) by sonication through a 22-gauge needle. The suspension was sonicated twice for 15 s (Sonifier model W 185, Heat Systems-Ultrasonics, Inc., Plainview, NY) with a microprobe at setting 6. Reagents were then added to adjust the suspension to a total volume of 90 ml containing the following concentrations: 15 to 18.5 mg of protein/ml, 0.125 M Tris/maleate, 2 mM CaCl2, 0.16 M NaCl, and 40 mM octylglucoside at pH 6.0. The suspension was stirred at 4°C for 10 min, and undissolved material was removed by centrifugation at 100,000 x g for 60 min. The clear supernatant (85 ml) containing 400 to 420 mg of protein) was slowly added to 255 ml of stirred buffer containing 10 mM Tris/maleate (pH 6.0) and 250 mM CaCl2 (pH 6.0) with a microprobe at setting 6. After stirring for 20 min on ice, the mixture was clarified by centrifugation at 5,000 x g for 10 min. The supernatant, which contained 98% of the LDL receptor activity of the solubilized membranes, was immediately applied to a column of DEAE-cellulose. Solubilized membranes were added as described in the legend to Fig. 1, and the fractions containing the LDL receptor activity were pooled. This pooled material is designated as DEAE-fraction. The LDL receptor activity of the DEAE-fraction was stable to quick-freezing in liquid nitrogen with subsequent storage at -170°C for at least 6 weeks.

Phosphatidylcholine/Acetone Precipitation of Solubilized LDL Receptor

An egg phosphatidylcholine liposome suspension was prepared by placing 200 mg of egg phosphatidylcholine in ethanol in a 125-ml glass flask. The ethanol was removed under a stream of nitrogen. The dry phosphatidylcholine was dissolved in diethyl ether, and a 2.5 cm phosphatidylcholine covering the inner walls of the flask was formed by evaporating the ether with a stream of nitrogen. Buffer (100 ml) containing 60 mM Tris/maleate (pH 5) and 2 mM CaCl2 was then added, and a suspension of liposomes was formed by hand shaking for 5 min at room temperature. This liposome suspension was stored at 4°C for 1 month without losing its biologic activity relevant to this study.

Prior to precipitation with acetone, solutions of the LDL receptor (0.04 to 4 mg of protein/ml) in 40 mM octylglucoside, 50 mM Tris/maleate (pH 6), 2 mM CaCl2, and 0.5 to 0.5 M NaCl. To achieve this mixture, 0.6 volumes of ice-cold acetone was added rapidly with vigorous vortex agitation (final acetone concentration, 37.5% (v/v)). After 2 min on ice, the precipitate was collected by centrifugation at 20,000 x g for 20 min at 3°C. The pellet was resuspended in 150 to 800 μl of Buffer B (20 mM Tris/chloride (pH 8), 50 mM NaCl, and 1 mM CaCl2) and used for measurement of 125I-LDL binding activity.

Filter Assay for 125I-LDL Binding

The procedure was a minor modification of that previously outlined (4). The standard binding assay was conducted at pH 8 in 85 μl of buffer containing 60 mM Tris/chloride, 1 mM CaCl2, 25 mM NaCl, and 20 mg/ml of bovine serum albumin in 200 μl of tissue. Unless otherwise stated, assay mixtures contained 10 to 400 μg of protein (either membranes or solubilized extracts precipitated with phosphatidylcholine/acetone) and 18 μg/ml of 125I-LDL (300 to 400 cpm/ng of protein) in the absence or presence of 12 mM EDTA as indicated. The tubes were incubated for 50 min at room temperature (approximately 24°C). Aliquots (90 μl) of each reaction mixture were then applied to 25-mm cellulose acetate membrane filters. Free 125I-LDL was separated from receptor-bound 125I-LDL by filtration and washing exactly as described (4). Nonspecific binding represents the amount of 125I-LDL retained by the filter when incubations were performed in the presence of either EDTA or excess unlabeled LDL. High affinity binding was calculated by subtracting the value for nonspecific binding from the value for total binding (4).

Assay of Protein Content of Membranes and Extracts

Method A—The protein content of samples containing no octylglucoside and no exogenous phosphatidylcholine was determined by the method of Lowry et al. (9) with bovine serum albumin as standard.

![Fig. 1. DEAE-cellulose chromatography of solubilized bovine adrenocortical membranes. Ascending chromatography was carried out at 4°C. DEAE-cellulose in a column (2.6 × 7.1 cm) was equilibrated with Buffer C (50 mM Tris/maleate (pH 6), 2 mM CaCl2, and 40 mM octylglucoside). The LDL receptor was solubilized from 12 adrenal glands with the use of octylglucoside as described under "Experimental Procedures." The receptor solution (330 ml containing 390 mg of protein and 210 μg of high affinity 125I-LDL binding activity) was applied to the column at a flow rate of 130 ml/h, after which the column was washed with 60 ml of Buffer C. The resulting flow-through was collected as a single fraction (open bar). The column was then eluted with an 80-ml linear gradient of 0 to 250 mM NaCl in Buffer C, followed by 40 ml of Buffer C containing 250 mM NaCl (- - -). The flow rate was 35 ml/h, and fractions of 3.2 ml each were collected. Two 10-μl aliquots of each fraction were removed for duplicate assay of protein content by Method B (C), and a 150-μl aliquot was removed for phosphatidylcholine/acetone precipitation and assay of 125I-LDL binding activity in the absence and presence of 12 mM EDTA. High affinity binding of 125I-LDL (○) was determined as described under "Experimental Procedures." The amount of non-specific binding of 125I-LDL was 0.6 to 0.8 μg/fraction. The tubes containing LDL receptor activity (Fractions 16 to 21) were combined together and designated as DEAE-fraction.
Purification and Properties of the LDL Receptor

Method B—Octylglucoside interfered with the standard Lowry procedure (9), necessitating the following modifications. Samples (5 to 200 μl) containing 40 nun octylglucoside and a maximum of 20 μg of protein were diluted with water to 1 ml, 30 μl of 1% sodium deoxycholate in water was then added, and the protein was precipitated with 0.6% trichloroacetic acid. All operations were performed at room temperature. The precipitate was collected by centrifugation at 2500 rpm for 25 min in a Sorvall GLC-2B centrifuge and was dissolved in the Lowry reagent (9). Varying amounts of bovine serum albumin in 40 mm octylglucoside were precipitated in the same manner, and the results were used to generate a standard curve.

Method C—The protein content of soluble extracts that had been precipitated with acetone in the presence of phosphatidylcholine was determined as follows. Precipitates were resuspended in 150 to 800 μl of Buffer B, and aliquots (10 to 200 μl) of the suspension containing 0 to 20 μg of protein and 0.02 to 0.2 mg of phosphatidylcholine were clarified by the addition of 300 μl of 0.2 M octylglucoside in water. The volume was adjusted to 1 ml with water, 30 μl of 1% sodium deoxycholate in water was then added, the protein was precipitated with 8% trichloroacetic acid, and the amount of protein was determined as described in Method B for samples containing octylglucoside. When 0 to 20 μg of bovine serum albumin was added to 0.02 to 0.5 mg of phosphatidylcholine liposomes in Buffer B and the samples were processed as described above, the standard curves were identical with those obtained with bovine serum albumin by the standard Lowry method (9).

Other Assays

The position of elution of marker proteins after 8% agarose A-1.5m chromatography and metrizamide density gradient centrifugation was determined by the following measurements: absorbance at 420 nm for ferritin, catalase, and human hemoglobin; protein content by Method A; ferritin by liquid scintillation counting for acetylated [acetyl-14C]aldolase.

Chromatography and Density Gradient Centrifugation

The experimental details for DEAE-cellulose column chromatography, gel filtration on 8% agarose A-1.5m, and metrizamide density gradient centrifugation are given in the legends to Figs. 1, 5, and 6, respectively.

RESULTS AND DISCUSSION

DEAE-cellulose Chromatography of the Solubilized LDL Receptor

The technique that proved most useful as a first step in the purification of the LDL receptor was ion exchange chromatography on DEAE-cellulose in the presence of micellar concentrations of octylglucoside. In a typical preparation (Fig. 1), 1420 μg of bovine adrenocortical membranes was solubilized in Buffer B containing 40 mm octylglucoside (critical micellar concentration, about 22 mm) (10) and 0.16 M NaCl at pH 6. After removal of the nonsolubilized material by ultracentrifugation, the soluble extract was diluted to lower the salt concentration to 40 mM, while maintaining the octylglucoside at 40 mM. The LDL receptor activity in the extract was then adsorbed quantitatively to a column of DEAE-cellulose equilibrated at pH 6 with 40 mm octylglucoside and no salt. Most of the protein in the extract did not adsorb to the column. The receptor activity was then eluted with a NaCl gradient of 0 to 250 mm, the receptor appearing in a single peak at about 125 mm NaCl (Fig. 1). In seven similar experiments, the purification on DEAE-cellulose columns was 7- to 10-fold, and the yield of receptor activity averaged 40% of the activity applied to the column as measured by the phosphatidylcholine/acetone precipitation assay. The adherence of the LDL receptor to the DEAE-cellulose column at pH 6 in the presence of salt concentrations up to 125 mM suggests that the receptor is a strongly acidic protein.

The receptor that emerged from the DEAE-cellulose column could no longer be assayed reproducibly by the simple method of diluting the octylglucoside and measuring the binding of 125I-LDL to the precipitated material. An empirical assay method was then devised in which the receptor was precipitated from octylglucoside-containing solutions by exposure to acetone in the presence of liposomes made from egg yolk phosphatidylcholine. The precipitated liposomes containing the LDL receptor activity were then resuspended and incubated with 125I-LDL, and the bound 125I-LDL was trapped by filtration on cellulose acetate filters. Fig. 2 demonstrates that maximal recovery of the active receptor in precipitates from the DEAE-purified extract required both phosphatidylcholine and acetone. When the DEAE-extract was treated with acetone in the absence of phosphatidylcholine, less than 10% of the receptor activity was recovered in the pellet (Fig. 2A). Maximal recovery was obtained when the concentration of added phosphatidylcholine was greater than 0.1 mg/ml. Similarly, when phosphatidylcholine vesicles were added in the absence of acetone, only 10% of the LDL receptor activity was recovered in the 20,000 × g pellet (Fig. 2B). In the presence of optimal amounts of phosphatidylcholine, complete precipitation required the presence of 30% acetone (Fig. 2B).

By electron microscopy, the acetone-precipitated phosphatidylcholine was observed to form large multilamellar liposomes with diameters varying from 3 to 5 μm. It is not yet known whether the receptor is inserted into the phospholipid bilayer of these liposomal membranes or whether it is simply adherent to the outer surface.

Binding Properties of DEAE-purified Receptor

The 125I-LDL binding activity that was precipitated with phosphatidylcholine/acetone from the DEAE-cellulose column eluate retained the properties of the LDL receptor as manifest in intact membranes. The saturable component of the binding curve was abolished by EDTA (Fig. 3A). A Scatchard plot of the EDTA-sensitive binding curve showed evidence for a single high-affinity binding site with an apparent dissociation constant of 20 μg of LDL-protein/ml (Fig. 3B).
unbound lipoprotein in the reaction mixture (micrograms of protein/ml) precipitation assay as described under "Experimental Procedures." LDL (342,000 cpm/pg of protein) in the absence phosphatidylcholine/acetone plot of the high affinity binding data. acetone precipitation assay and high affinity binding protein, and the indicated concentration of either unlabeled LDL labeled as described under "Experimental Procedures." Bound/Free, the amount of bound 125I-LDL (micrograms of protein/ml) divided by the amount of unbound lipoprotein in the reaction mixture (micrograms of protein/ml).

This is similar to the $K_d$ value of 20 to 30 µg/ml for intact bovine adrenal membranes (4, 11).

The binding of 125I-LDL to the phosphatidylcholine/acetone-precipitated DEAE-extract was inhibited competitively by unlabeled LDL but not by LDL that had been modified by reductive methylation (Fig. 4). Moreover, human high density lipoprotein did not compete for binding. This specificity is identical with that of the LDL receptor of intact membranes (11, 12) and intact cells (1, 7, 13).

Purification and Properties of the LDL Receptor

Physical Characterization of the DEAE-purified LDL Receptor-Octylglucoside Complex

Gel Filtration—The LDL receptor that was eluted from the DEAE-cellulose column gave a single symmetrical peak when passed through a column of agarose A-1.5m in the presence of 40 mM octylglucoside and 0.5 mM NaCl (Fig. 5). The peak fraction appeared midway between the void volume ($V_0$) and the salt peak ($V_s$) and between the peak for ferritin (Stokes radius, 59 Å) and catalase (Stokes radius, 52 Å). A plot of Stokes radius versus the distribution coefficient ($K_d$) for a series of marker proteins gave a straight line. In four experiments in which the DEAE-purified LDL receptor was passed over the agarose column, the mean $K_d$ was 0.39, which corresponded to a Stokes radius of 53.5 ± 0.6 Å. In the same four experiments, the recovery of LDL receptor activity from the agarose column, as measured in the pooled fractions, averaged 52% of the activity applied. It should be pointed out that the gel filtration experiments were carried out in buffers containing 0.5 mM NaCl in order to decrease the loss of receptor activity due to adsorption to the gel.

Density Gradient Centrifugation—Gradients were formed

![Fig. 3. 125I-LDL binding to partially purified LDL receptor as a function of the concentration of 125I-LDL. A, each assay tube contained 13 µg of protein (DEAE-fraction precipitated with phosphatidylcholine/acetone) and the indicated concentration of 125I-LDL (342,000 cpm/µg of protein) in the absence (○) or presence (△) of 12 mM EDTA. After incubation for 50 min at room temperature, bound 125I-LDL was measured by the standard phosphatidylcholine/acetone precipitation assay and high affinity binding (○) was calculated as described under "Experimental Procedures." B, Scatchard plot of the high affinity binding data. Bound/Free, the amount of bound 125I-LDL (micrograms of protein/ml) divided by the amount of unbound lipoprotein in the reaction mixture (micrograms of protein/ml).

![Fig. 4. Competition by unlabeled lipoproteins for binding of 125I-LDL to partially purified LDL receptor. Each assay tube contained 22 µg of protein (DEAE-fraction precipitated with phosphatidylcholine/acetone), 12.5 µg/ml of 125I-LDL (344,000 cpm/µg of protein), and the indicated concentration of either unlabeled LDL (○), unlabeled methyl-LDL (△), or unlabeled high density lipoprotein (HDL) (△). After incubation for 50 min at room temperature, bound 125I-LDL was measured by the standard phosphatidylcholine/acetone precipitation assay as described under "Experimental Procedures."

This is similar to the $K_d$ value of 20 to 30 µg/ml for intact bovine adrenal membranes (4, 11).

The binding of 125I-LDL to the phosphatidylcholine/acetone-precipitated DEAE-extract was inhibited competitively by unlabeled LDL but not by LDL that had been modified by reductive methylation (Fig. 4). Moreover, human high density lipoprotein did not compete for binding. This specificity is identical with that of the LDL receptor of intact membranes (11, 12) and intact cells (1, 7, 13).

![Fig. 5. Agarose A-1.5m chromatography of partially purified LDL receptor. Ascending chromatography was carried out at 4°C. Eight per cent agarose (A-1.5m) in a column (1.8 × 55.7 cm) was equilibrated with Buffer D (50 mM Tris/maleate (pH 6), 2 mM CaCl$_2$, 0.5 mM NaCl, and 40 mM octylglucoside). Three milliliters of the DEAE-fraction, containing 3.7 mg of protein and 15.7 µg of high affinity 125I-LDL binding activity, were applied to the column. The column was eluted with Buffer D at a flow rate of 9 ml/h, and 3.1-ml fractions were collected. Aliquots of each fraction were removed for assay of protein content by Method B (○) and 125I-LDL binding activity (●, total; △, nonspecific) by the standard phosphatidylcholine/acetone precipitation assay as described under "Experimental Procedures." The tubes containing LDL receptor activity (Fractions 25 to 28) were combined together. The void volume ($V_0$) and total volume ($V_t$) were determined with blue dextran and potassium dichromate (yellow color). Arrows indicate the elution position peak of marker proteins that were used to calibrate the column: 1, ferritin (Stokes radius, 59 Å); 2, catalase (52 Å); 3, aldolase (46 Å); 4, bovine serum albumin (30 Å); and 5, ovalbumin (27 Å). Each calibration run contained two marker proteins (2 mg of ferritin and 10 mg of catalase, 10 mg of catalase and 10 mg of bovine serum albumin, or 10 mg of aldolase and 10 mg of ovoalbumin) added to the DEAE-fraction. The position of elution of the marker proteins was determined as described under "Experimental Procedures." Insert, estimation of the Stokes radius of the LDL receptor as determined by gel filtration. The known Stokes radii of the marker proteins are plotted against their distribution coefficient, $K_d = (V_t - V_0)/(V_t - V_s)$, where $V_t$ is the elution volume of the peak in question (14).
with metrizamide instead of sucrose because the recovery of receptor activity from sucrose gradients was less than 30% as compared with a 50 to 65% recovery from metrizamide gradients. When the metrizamide gradient was prepared in water containing 40 mM octylglucoside (Fig. 6A), the LDL receptor activity sedimented as a single symmetrical peak whose sedimentation velocity was slightly smaller than that of aldolase ($s_{20,w} = 7.7$) and greater than hemoglobin ($s_{20,w} = 4.2$). From a plot of $s_{20,w}$ versus sedimentation velocity for standard proteins (Fig. 6A), the $s_{20,w}$ value for the LDL receptor was estimated to be 7.3.

The above metrizamide gradient was performed in the absence of NaCl. When the gradients were prepared in the presence and absence of 0.5 M NaCl, we obtained $s_{20,w}$ values of 7.25 and 7.3, respectively, indicating that the 0.5 M NaCl had no significant effect on the sedimentation behavior of the receptor activity. Similarly, the Stokes radius of the receptor was performed in the presence of Octylglucoside Complex. Inasmuch as the gel filtration and control experiments are important inasmuch as the data obtained from the gel filtration, which was carried out in 0.5 M NaCl, and the metrizamide gradient centrifugation are combined to derive a molecular weight for the protein-detergent complex (see below).

**Estimation of the Molecular Weight of the LDL Receptor-Octylglucoside Complex**—Inasmuch as the gel filtration and density gradient experiments were performed in the presence of 40 mM octylglucoside, the measured Stokes radius and sedimentation coefficient apply to the LDL receptor-octylglucoside complex and not to the receptor per se. To calculate the molecular weight for the receptor-octylglucoside complex, the partial specific volume ($\bar{v}$) of the complex must be known (14). The $\bar{v}$ of the complex could be calculated if the $\bar{v}$ of the receptor, the $\bar{v}$ of octylglucoside, and the relative amounts of octylglucoside and protein in the receptor-octylglucoside complex were known. However, measurement of the relative amounts of octylglucoside and protein in the complex would require complete purification of the LDL receptor-octylglucoside complex, which is not yet possible.

To estimate the $\bar{v}$ for impure protein-detergent complexes, Clarke (15) developed a widely used method in which the protein-detergent complex and known water-soluble marker proteins are sedimented in density gradients of sucrose in water and in deuterium oxide (15). If the $\bar{v}$ of the protein-detergent complex differs from the $\bar{v}$ of the water-soluble standard proteins that do not bind detergent, then the behavior of the protein-detergent complex is altered in deuterium oxide relative to the behavior of the standards. The extent of this alteration is proportional to the difference in $\bar{v}$ between the protein-detergent complex and the $\bar{v}$ of the standard proteins, and this allows a calculation of the $\bar{v}$ of the complex. As shown in Fig. 6, the LDL receptor-octylglucoside complex showed the same relative sedimentation velocity in water and deuterium oxide when compared with the marker proteins. In both cases, the peak of the protein-detergent activity sedimented slightly slower than aldolase. For the seven gradients performed in water, the mean $s_{20,w} \pm$ S.D. for the receptor activity was 7.25 ± 0.08; for the five gradients performed in deuterium oxide, the mean $s_{20,w}$ was 7.32 ± 0.09. This difference was not statistically significant.

The similarity in relative sedimentation coefficients of the LDL receptor in water and deuterium oxide suggests that the LDL receptor-octylglucoside complex has a $\bar{v}$ that is similar to that of the hydrophilic marker proteins. This conclusion is consistent with the known $\bar{v}$ of octylglucoside (0.81 ml/g), which is similar to the $\bar{v}$ of water-soluble proteins (0.71 to 0.74 ml/g) (16).

If the $\bar{v}$ of the LDL receptor-octylglucoside complex is in the range of 0.71 to 0.74 ml/g, then the molecular weight of the receptor-octylglucoside complex can be computed by inserting the range of values for $\bar{v}$ into the relevant equation and by using the measured Stokes radius of 53.5 Å and the sedimentation coefficient of 7.3. The resultant range for the molecular weight of the LDL receptor-octylglucoside complex is 152,000 to 170,000, with the most likely value being 163,000 (6, 0.73 ml/g).

It is also possible to calculate the frictional ratio ($f/f_0$) of the LDL receptor-octylglucoside complex based upon the $\bar{v}$, the molecular weight, and the measured Stokes radius. This value ranges from 1.53 ($\bar{v}$, 0.71 ml/g) to 1.45 ($\bar{v}$, 0.74 ml/g). At the most likely $\bar{v}$ of 0.73 ml/g, the frictional ratio would be about 1.5, indicating an elongated protein. This value is similar to the frictional ratios of several membrane protein-detergent complexes.

Molecular weights were calculated according to the following equation (14):

$$M_r = \frac{6\pi \eta N}{1 - \phi} \bar{s}_{20,w}$$

where $N$ is Avogadro's number, $\eta$ is the viscosity of water at 20°C, $\rho$ is the density of water at 20°C, $\phi$ is the Stokes radius, $\bar{v}$ is the partial specific volume, and $\bar{s}_{20,w}$ is the sedimentation coefficient.

R. Roxby, personal communication.

Frictional ratios were calculated according to the following equation (14):

$$f/f_0 = a(4\pi N/3M_0\bar{v})^{1/3}$$

where $a$ is the Stokes radius, $N$ is Avogadro's number, $\bar{v}$ is the partial specific volume, and $M_r$ is the molecular weight. Solution was not taken into consideration.
Partial Purification of the LDL Receptor

With the techniques described above, it has been possible so far to obtain a preparation of the LDL receptor that is about 350-fold purified relative to the activity of the starting adrenal membrane fraction (Table I). The overall yield of receptor activity was about 18%. The solubilization itself achieved a 3-fold purification. DEAE-cellulose chromatography achieved a further 7-fold purification (total of 21-fold). Agarose A-1.5m chromatography increased the overall purification to 81-fold. After the agarose step, precipitation of the receptor with acetone and phosphatidylycholine increased the purification to 4-fold purification since only 25% of the mass of the receptor protein was recovered in the pellet.

Assuming that the maximum molecular weight of the LDL receptor is about 163,000, that the molecular weight of the protein component of LDL is 500,000 (19), and that one particle of LDL binds to one receptor (20), one can calculate that a pure preparation of active receptor should have a high affinity binding activity at saturation of 3.1 mg of 125I-LDL bound/mg of receptor protein. The binding assays of Table I were performed at a concentration of 125I-LDL that is half-saturating. At saturation, the purified receptor (Fraction V in Table I) bound 0.14 mg of 125I-LDL/mg of protein, which is about 5% of the activity expected in a pure receptor. If this reasoning is correct, then the receptor comprises about 5% of the protein in the purified preparation, and a 20-fold further purification will be necessary to obtain a pure protein that will then be about 7,000-fold purified relative to the starting membrane preparation. Attempts to achieve this further purification are now in progress.

Acknowledgments—Paul Harvill, Richard Gibson, and Kevin Overture provided excellent technical assistance.

REFERENCES

TABLE I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein Recovered</th>
<th>Specific activity</th>
<th>Total activity</th>
<th>Recovery of high affinity binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/fraction</td>
<td>mg/1000 mg protein</td>
<td>fold% initial activity</td>
<td></td>
</tr>
<tr>
<td>I. Intact membranes</td>
<td>1420</td>
<td>0.31</td>
<td>0.20 1</td>
<td>284 100</td>
</tr>
<tr>
<td>II. Solubilized membranes</td>
<td>408</td>
<td>0.62</td>
<td>0.54 3</td>
<td>220 77</td>
</tr>
<tr>
<td>III. DEAE-cellulose chromatography</td>
<td>24</td>
<td>4.5</td>
<td>4.3 21</td>
<td>103 36</td>
</tr>
<tr>
<td>IV. Agarose chromatography</td>
<td>3</td>
<td>17</td>
<td>16 80</td>
<td>50 18</td>
</tr>
<tr>
<td>V. Acetone precipitation</td>
<td>0.72</td>
<td>72</td>
<td>70 350</td>
<td>50 18</td>
</tr>
</tbody>
</table>