Solubilization of the low density lipoprotein receptor

(octyl- β -D-glucoside/membranes/adrenal gland/fibroblasts/familial hypercholesterolemia)

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ABSTRACT The low density lipoprotein (LDL) receptor was solubilized from membranes of bovine adrenal cortex and cultured human cells by incubation with the nonionic detergent octyl- β -D-glucoside. Receptor activity released into the 100,000 $\times$ g supernatant was assayed by a solid-phase procedure: an aliquot of the soluble extract was removed, the detergent was diluted below its critical micellar concentration, causing the receptor to precipitate as a lipid-protein aggregate; the precipitate was collected by centrifugation and incubated with 125 I-labeled LDL (125 I-LDL); and the receptor-bound 125 I-LDL was separated from free 125 I-LDL by filtration. The 125 I-LDL binding site that was precipitated from the soluble extract of bovine adrenocortical membranes appeared to be the same as the functional LDL receptor of cultured bovine adrenocortical cells and human fibroblasts. It exhibited high affinity and specificity (affinity for LDL more than 200-fold greater than for acetylated LDL, methylated LDL, or high density lipoprotein), dependence on calcium, and susceptibility to destruction by Pronase. The amount of 125 I-LDL binding activity in solubilized membranes from cultured cells was proportional to the number of receptors on the surface of the intact cells. Thus, the number of solubilized receptors was 1/20th of normal in mutant fibroblasts from a subject with homozygous familial hypercholesterolemia and was 1/4th of normal in human epithelioid carcinoma A-431 cells when they were grown in the presence of 25-hydroxycholesterol plus cholesterol. While in the soluble form in the presence of octyl- β -D-glucoside, the LDL receptor can be carried through several steps of purification.

The low density lipoprotein (LDL) receptor on the surface of mammalian cells binds plasma LDL and thereby initiates a chain of events culminating in the internalization of the LDL by endocytosis with concomitant delivery of cholesterol to the cell (1). Interest in this receptor stems from several of its characteristics: (i) it plays a major role in controlling the level of plasma and cellular cholesterol in man (1, 2); (ii) its absence constitutes the basic defect in the disease familial hypercholesterolemia (FH), an important cause of premature atherosclerosis (2); and (iii) its localization in coated pits on the cell surface of fibroblasts has called attention to the role of these specialized structures in mediating the uptake of receptor-bound macromolecules through adsorptive endocytosis (3).

The LDL receptor has been studied most extensively in cultured cells, including human fibroblasts (1) and steroid-secreting adrenal cells (4), where it exhibits several characteristic properties: (i) it binds human LDL with a 200-fold higher affinity than human high density lipoprotein (HDL) (1, 5); (ii) it fails to bind LDL whose lysine residues have been acetylated (6, 7) or methylated (7); (iii) it requires a divalent cation (1); (iv)

it is extremely sensitive to destruction by proteolytic enzymes (1); and (v) its activity is suppressed by incubation of cells with 25-hydroxycholesterol plus cholesterol (1).

The major obstacle to the solubilization and purification of the LDL receptor has been the difficulty of devising an assay to separate free 125 I-labeled LDL (125 I-LDL) from 125 I-LDL that is bound to a solubilized receptor. Traditional biochemical assays are not applicable because of the large size of the LDL particle (molecular weight, $\approx 3 \times 10^6$) and because the LDL protein cannot be obtained in an active lipid-free form. Three recent developments have suggested a new approach to the solubilization of the LDL receptor in a form that allows its assay. First, membranes prepared from bovine adrenal cortex have been shown to possess a large number of LDL receptors that are indistinguishable from the functional receptors on cultured bovine adrenocortical cells (4, 8), thus providing a readily available source of tissue for biochemical studies. Second, octyl- β -D-glucoside (OG), a nonionic detergent with a high critical micellar concentration, has been found to dissociate readily from solubilized membrane proteins upon dilution or dialysis (9, 10), allowing the formation of protein aggregates. Third, studies by Simons, Helenius, and coworkers (11, 12) have defined the useful properties of protein aggregates that form when detergents are removed from solubilized proteins, thereby suggesting a solid-phase assay for the soluble LDL receptor. In the current studies, we used OG to solubilize the LDL receptor from bovine adrenocortical membranes as well as from cultured human cells and developed a solid-phase assay to measure its activity.

METHODS

Materials. Nuflow cellulose acetate membrane filters (pore size, 0.45 μ m; diameter, 25 mm) were obtained from Oxoid Ltd. (Basingstoke, England; catalog no. N25/45). OG and Pronase from *Streptomyces griseus*, B grade (45,000 units/g) were purchased from Calbiochem. Bovine serum albumin (no. A4378) was obtained from Sigma. Sodium iodide- 125 I (11-17 mCi/ μ g; 1 Ci = 3.7×10^{10} becquerels) was obtained from Amersham.

Lipoproteins. Human LDL ($\rho = 1.019$ -1.063 g/ml) and HDL ($\rho = 1.125$ -1.215 g/ml) were prepared from plasma by ultracentrifugation (4). Acetyl-LDL and methyl-LDL were prepared by treatment of LDL with acetic anhydride (6) and with formaldehyde plus sodium borohydride (7), respectively. LDL was labeled with 125 I as described (4). Lipoprotein concentrations are expressed in terms of protein content (13).

Abbreviations: FH, familial hypercholesterolemia; HDL, high density lipoprotein; LDL, low density lipoprotein; OG, octyl- β -D-glucoside.

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Preparation of Bovine Adrenocortical Membranes. Bovine adrenal glands were obtained 15–30 min after slaughter and placed in ice-cold 0.15 M NaCl. The cortex was separated from the medulla and placed in ice-cold buffer A (20 mM Tris-HCl/0.15 M NaCl/1 mM CaCl₂, pH 8) at 5 ml/g of tissue. All operations were carried out at 4°C. The tissue was homogenized with two 15-sec pulses of a Polytron homogenizer (settings 5 and 8) and centrifuged at 800 × *g* for 5 min. The supernatant was filtered through cheesecloth and centrifuged again at 800 × *g* for 5 min. The resulting supernatant was centrifuged at 100,000 × *g* for 60 min. The 800–100,000 × *g* pellet (membrane pellet) was frozen in liquid nitrogen. Pellets could be stored at –190°C for at least 1 month without loss of activity.

Preparation of Soluble Bovine Adrenocortical Extract. All operations were carried out at 0–4°C. Each membrane pellet was suspended in 0.25 M Tris maleate/2 mM CaCl₂, pH 6, by aspiration through a 25-gauge needle. The suspension was adjusted to 7–9 mg of protein per ml and then sonicated twice for 15 sec (Sonifier model W 185, Heat-System/Ultrasonics, Plainview, NY) by using a microprobe at setting 6. Reagents were then added to adjust the suspension to the following concentrations: 3.5–4.5 mg of protein per ml, 0.125 M Tris maleate, 1 mM CaCl₂, 0.15 M NaCl, and 40 mM OG, pH 6. The mixture was agitated by vortexing. After incubation at 4°C for 10 min, undissolved material was removed by centrifugation at 100,000 × *g* for 60 min.

Solid-Phase Assay for Soluble ¹²⁵I-LDL Binding Activity.
Step 1. Precipitation of LDL binding sites. The concentration of OG in an aliquot of the soluble extract (100,000 × *g* supernatant) was adjusted to 5 mM by addition of 7 vol of buffer B (50 mM Tris maleate/1 mM CaCl₂, pH 6), and the precipitate was collected by centrifugation at 100,000 × *g* for 60 min at 4°C. The precipitate was resuspended by aspiration with a 25-gauge needle in 20 mM Tris-HCl/50 mM NaCl/1 mM CaCl₂, pH 8, and used for measurement of LDL receptor activity. The concentration of precipitated extract is expressed in terms of its protein content (13).

Step 2. Filter assay for bound ¹²⁵I-LDL. The standard assay was conducted at pH 8 in 80 μl of buffer C (60 mM Tris-HCl/1 mM CaCl₂/25 mM NaCl/20 mg of bovine serum albumin per ml) in 250-μl polyethylene tubes. Unless otherwise stated, assays contained 20–240 μg of protein of precipitated extract and 12.5 μg of ¹²⁵I-LDL (292–413 cpm/ng) per ml in the absence or presence of 10 mM EDTA as indicated. The tubes were incubated for 50 min at 0°C or room temperature. Duplicate aliquots (30 μl) of each reaction mixture were then applied to 25-mm cellulose acetate membrane filters as follows. Prior to use, filters were soaked for at least 30 min at room temperature in buffer D [20 mM Tris-HCl/50 mM NaCl/1 mM CaCl₂/bovine serum albumin (1 mg/ml), pH 8]. Each filter was then placed on a stainless steel holder connected to a vacuum line and washed once with 3 ml of buffer D; 3 ml of buffer D was then added to each filter holder without suction, and a 30-μl aliquot of the reaction mixture was added. Suction was instantly applied, after which the filter was washed four times under suction with cold buffer D (3 ml per wash). The filter was then transferred to a glass tube for ¹²⁵I radioactivity determination. 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. Specific binding was calculated by subtracting the value for nonspecific binding from the value for total binding.

Cultured Cells. Skin fibroblasts were obtained from a normal subject and a patient with the receptor-negative form of homozygous FH (14). Human epithelioid carcinoma A-431 cells were kindly provided by S. Cohen (15).

RESULTS

When bovine adrenocortical membranes were incubated with increasing concentrations of OG at pH 6, increasing amounts of membrane protein were extracted. At an OG concentration of 40 mM, about 85% of the ¹²⁵I-LDL binding activity was removed from the membrane and no longer sedimented at 100,000 × *g* (Fig. 1A). To assay the binding activity that was released into the soluble fraction, an aliquot of the 100,000 × *g* supernatant was diluted to decrease the OG concentration to 5 mM, thereby forming a fine precipitate consisting of aggregated lipid and protein. This precipitate was collected by centrifugation, resuspended, and incubated with ¹²⁵I-LDL. The amount of ¹²⁵I-LDL bound to the precipitate was determined by trapping the precipitate on a cellulose acetate filter. All of the ¹²⁵I-LDL binding activity removed from the membrane fraction was recovered when the soluble fraction was diluted and precipitated. The precipitate that was generated when the extract was diluted and spun at 100,000 × *g* is hereafter referred to as the "precipitated extract."

Fig. 1B shows the recovery of ¹²⁵I-LDL binding activity and total protein in the precipitated extract as a function of the concentration of OG to which the membranes were exposed. Little binding activity was found in the precipitated extract until the OG approached its critical micellar concentration at about 25 mM. Thereafter, the binding activity increased markedly. At 40 mM OG, 80% of the initial binding activity and 40% of the total protein of the intact membranes were recovered in the precipitated extract.

Binding of ¹²⁵I-LDL to the precipitated extract was inhibited competitively by an excess of unlabeled LDL (Fig. 2A), suggesting a limited number of specific binding sites. EDTA at concentrations greater than 0.5 mM abolished nearly all specific ¹²⁵I-LDL binding. The addition of excess calcium restored ¹²⁵I-LDL binding in the presence of EDTA (Fig. 2B). EDTA and calcium did not affect the small amount of non-

FIG. 1. Solubilization of LDL receptor from bovine adrenocortical membranes. The membrane pellet (10.9 and 4.2 mg of protein in A and B, respectively) was treated with OG as indicated and separated by centrifugation into membrane and soluble fractions. To measure LDL binding in the soluble fractions, the 100,000 × *g* supernatants were diluted to 5 mM OG with 3–9 vol of buffer B and the resulting precipitated extracts were collected and assayed at 0°C for 50 min. In membranes not treated with OG, the 100,000 × *g* supernatant was arbitrarily diluted with 5 vol of buffer B and centrifuged at 100,000 × *g* for 60 min; no precipitate was found. (A) Specific LDL binding was determined in the membrane fraction (○) and the soluble fraction (●). X---X, Total amount of specific binding recovered. (B) Membranes were treated as in A and the percentage of specific ¹²⁵I-LDL binding (▲) and protein (△) recovered in the precipitated extract is depicted. The "100% values" refer to the membrane fraction treated with no OG: 2.5 mg per pellet for protein (△) and 1190 ng per pellet for specific ¹²⁵I-LDL binding (▲).

FIG. 2. Requirement for Ca^{2+} in binding of ^{125}I -LDL to precipitated extract of adrenocortical membranes. The precipitated extract was prepared as described in *Methods* except that CaCl_2 was omitted from all solutions. (A) Each assay tube contained 80 μl of buffer C without CaCl_2 , 69 μg of precipitated extract, 12.5 μg of ^{125}I -LDL per ml in the absence ($\bullet$) or presence ($\circ$) of unlabeled LDL at 1 mg/ml, and the indicated concentration of EDTA. (B) Each assay tube contained 80 μl of buffer C without CaCl_2 , 0.5 mM EDTA, 69 μg of precipitated extract, 12.5 μg of ^{125}I -LDL per ml in the absence ($\bullet$) or presence ($\circ$) of unlabeled LDL at 1 mg/ml, and the indicated concentration of CaCl_2 . After incubation for 50 min at 0°C , bound ^{125}I -LDL was determined by filtration.

specific ^{125}I -LDL binding observed in the presence of excess unlabeled LDL.

Binding of ^{125}I -LDL to the precipitated extract reached completion within 20 min at 4°C (Fig. 3A). Binding was largely prevented by inclusion of EDTA in the incubation medium. When binding was allowed to proceed in the absence of EDTA and then EDTA was added, the ^{125}I -LDL was rapidly released from the binding site. The binding site showed saturation with increasing concentrations of ^{125}I -LDL (Fig. 3B). In the presence of either excess unlabeled LDL or EDTA, the saturable component of ^{125}I -LDL binding was obliterated, and only a non-saturable component was observed. The concentration of ^{125}I -LDL giving half-maximal binding to the saturable site was 30 $\mu\text{g}/\text{ml}$ as calculated from Lineweaver-Burk plots. The number of binding sites and their affinity were similar at 4, 24, and 37°C . The pH optimum for binding was 8.0, and the amount of binding was proportional to the amount of protein in the precipitated extract up to at least 450 μg per assay tube (data not shown).

In the presence of 40 mM OG, the solubilized adrenocortical

FIG. 3. ^{125}I -LDL binding to precipitated extract of adrenocortical membranes. (A) Time course. Each tube contained 80 μl of buffer C, 110 μg of precipitated extract, and 12.5 μg of ^{125}I -LDL per ml in the absence ($\bullet$) or presence ($\triangle$) of 5 mM EDTA. Some of the assay tubes that contained no EDTA at zero time received 5 mM EDTA (delivered in 5 μl) after 5, 10, 20, or 60 min ($\triangle$). After incubation at 0°C for the indicated time, bound ^{125}I -LDL was determined by filtration. (B) ^{125}I -LDL concentration curve. Each assay tube contained 80 μl of buffer C, 75 μg of precipitated extract, and the indicated concentration of ^{125}I -LDL in the absence ($\bullet$) or presence of 10 mM EDTA ($\triangle$) or unlabeled LDL at 1 mg/ml (Δ). After incubation at 0°C for 50 min, bound ^{125}I -LDL was determined by filtration. (---), Specific ^{125}I -LDL binding.

FIG. 4. Destruction of ^{125}I -LDL binding activity of precipitated extract of adrenocortical membranes by Pronase treatment. The precipitated extract was suspended in Dulbecco's phosphate-buffered saline (GIBCO, no. 310-4190) with 1 mM CaCl_2 (0.6 mg of protein per ml) and incubated for 10 min at 37°C with the indicated concentration of Pronase. The action of Pronase was stopped by dilution with ice-cold buffer containing bovine serum albumin (20 mg/ml) and the binding assays were performed. Each assay tube contained 80 μl of buffer C, 20 μg of precipitated extract, and 12.5 μg of ^{125}I -LDL per ml in the absence ($\bullet$) or presence ($\circ$) of 10 mM EDTA. After incubation at 0°C for 50 min, bound ^{125}I -LDL was determined by filtration.

membranes retained all ^{125}I -LDL binding activity for at least 1 week when stored at -190°C . Binding activity of the solubilized membranes was destroyed by heating to 100°C for 4 min (data not shown). In addition, the activity was destroyed by brief incubation of the precipitated extract with Pronase in a concentration-dependent fashion (Fig. 4). Pronase had no effect on the nonspecific binding observed in the presence of EDTA.

Whereas the binding of ^{125}I -LDL to the precipitated extract was inhibited competitively by unlabeled LDL, it was not decreased significantly by unlabeled HDL (Fig. 5A). Moreover, LDL that had been modified by methylation or acetylation lost its ability to compete with ^{125}I -LDL for binding to the precipitated extract (Fig. 5B).

The amount of ^{125}I -LDL binding per mg of membrane protein was about 3-fold higher in intact membranes prepared from the adrenal cortex than in membranes prepared from the adrenal medulla (Table 1). The same relative difference was observed when the two membranes were solubilized and assayed.

The amount of ^{125}I -LDL binding activity solubilized from

FIG. 5. Competition by unlabeled lipoproteins for binding of ^{125}I -LDL to precipitated extract of adrenocortical membranes. (A) Each assay tube contained 80 μl of buffer C, 35 μg of precipitated extract, 12.5 μg of ^{125}I -LDL per ml, and the indicated concentration of either unlabeled LDL ($\bullet$) or unlabeled HDL ($\circ$). (B) Each assay tube contained 80 μl of buffer C, 98 μg of precipitated extract, 12.5 μg of ^{125}I -LDL per ml, and the indicated concentration of unlabeled LDL ($\bullet$), unlabeled methyl-LDL (Δ), or unlabeled acetyl-LDL ($\circ$). After incubation for 50 min at either 0°C (A) or room temperature (B), bound ^{125}I -LDL was determined by filtration. The "100% of control" values ($\blacksquare$) were 530 and 915 ng/mg of protein for A and B, respectively.

Table 1. ^{125}I -LDL binding activity of solubilized membranes from bovine adrenal cortex and medulla

Tissue	^{125}I -LDL bound, ng/mg protein			
	Intact membranes		Precipitated extract	
	Total	Specific	Total	Specific
Cortex	590	380	1530	1330
Medulla	220	114	550	350

From membrane pellets of adrenal cortex and medulla, one aliquot was removed for assay of ^{125}I -LDL binding and the remainder was solubilized with 40 mM OG. An aliquot of the solubilized material was removed, a precipitate was prepared by dilution, and its ^{125}I -LDL binding activity was assayed. Each assay tube contained 80 μl of buffer C, 120–240 μg of either intact membranes or precipitated extract as indicated, and 25 μg of ^{125}I -LDL per ml in the absence or presence of 10 mM EDTA. After incubation for 50 min at room temperature, bound ^{125}I -LDL was determined by filtration.

fibroblasts cultured from an FH homozygote was only 1/20th of that solubilized from normal fibroblasts as judged by assay of precipitated extracts (Table 2). When human epithelioid carcinoma A-431 cells were grown in the presence of a mixture of 25-hydroxycholesterol plus cholesterol, the ^{125}I -LDL binding activity of intact cells was decreased by approximately 75% (Table 3). A similar 75% reduction was observed in the amount of binding activity that was solubilized from membranes of the sterol-treated cells.

DISCUSSION

In the current experiments the LDL receptor has been solubilized from bovine adrenal membranes, cultured human fibroblasts, and cultured human epithelioid carcinoma A-431 cells by incubating the membranes with 40 mM OG. When maintained in the presence of the detergent, binding activity could not be sedimented at 100,000 $\times g$. However, when the concentration of OG was decreased to below its critical micellar concentration, the receptor formed a fine precipitate in conjunction with lipids and other proteins that had been extracted from the membrane. This precipitated extract retained its ability to bind ^{125}I -LDL. This observation allowed us to develop a solid-phase assay to measure the amount of LDL binding activity that was solubilized by OG. For these purposes, an aliquot of the detergent-solubilized extract was removed and

Table 2. ^{125}I -LDL binding activity of solubilized membranes from normal and FH homozygote fibroblasts

Cell	^{125}I -LDL bound by precipitated extract, ng/mg protein		
	Without		Specific
	unlabeled LDL	unlabeled LDL	
Normal	338	39	299
FH homozygote	33	16	17

Fibroblasts were grown in 100-mm petri dishes as described (16). Before reaching confluence, the cells were incubated for 48 hr in medium containing 10% human lipoprotein-deficient serum (16). Cells from 90 dishes were then harvested, pooled, and used to prepare a membrane pellet. The membranes (7.8 and 5.2 mg of protein for normal and FH homozygote cells, respectively) were solubilized with 40 mM OG, and a precipitated extract was prepared as described for bovine adrenocortical membranes. Each binding assay contained 80 μl of buffer C, 235 μg of precipitated extract, and 12.5 μg of ^{125}I -LDL per ml in the absence or presence of unlabeled LDL at 1 mg/ml as indicated. After incubation at 0°C for 50 min, bound ^{125}I -LDL was determined by filtration.

Table 3. ^{125}I -LDL binding activity of intact monolayers and solubilized membranes from human carcinoma A-431 cells grown in the absence and presence of sterols

Treatment	^{125}I -LDL in monolayers, ng/mg protein	^{125}I -LDL bound by precipitated extract, ng/mg protein		
		No		Specific
		EDTA	With EDTA	
None	220	1270	260	1010
25-Hydroxycholesterol + cholesterol	51	520	230	290

On day 0, 10^5 A-431 cells were seeded into each of 30 petri dishes (100 mm) containing 7 ml of Dulbecco's modified Eagle's medium and 10% fetal calf serum. On days 2 and 4, the medium was replaced with fresh medium containing 10% fetal calf serum. On day 6, each dish received 6 ml of fresh medium containing 10% human lipoprotein-deficient serum (14) and 15 μl of ethanol containing either no sterols or 0.6 μg of 25-hydroxycholesterol plus 14 μg of cholesterol per ml. On day 7, one dish from each group was washed once with phosphate-buffered saline, and then it received 6 ml of medium containing 10% human lipoprotein-deficient serum and 10 μg of ^{125}I -LDL per ml. After incubation for 5 hr at 37°C, the monolayers were washed and the total cellular content of ^{125}I -LDL (surface-bound + internalized) was determined (14). The remaining 14 dishes in each group were harvested, pooled, and used to prepare a membrane pellet. The membranes (5 mg of protein) were solubilized with 40 mM OG, and a precipitated extract was prepared as described for bovine adrenocortical membranes. Each binding assay contained 80 μl of buffer C, 52 μg of precipitated extract, and 12.5 μg of ^{125}I -LDL per ml in the absence or presence of 10 mM EDTA as indicated. After incubation at room temperature for 50 min, bound ^{125}I -LDL was determined by filtration.

diluted so as to form a precipitate, and the precipitate was incubated with ^{125}I -LDL. Receptor-bound ^{125}I -LDL could be separated from free ^{125}I -LDL by trapping on a cellulose acetate membrane filter.

The ^{125}I -LDL binding activity of the precipitated extract reflected the behavior of the physiologic LDL receptor of intact cells and membranes (1, 5–7). The LDL binding activity of the precipitated extract: (i) required a divalent cation, (ii) was specific for LDL as opposed to HDL, (iii) was abolished when lysine residues of the LDL were modified by reductive methylation or acetylation, and (iv) was sensitive to Pronase. Moreover, the relative binding activity of the precipitated extract was proportional to the number of LDL receptors present on intact membranes or cells. Thus, the number of soluble binding sites: (i) was nearly 20-fold higher in extracts from normal human fibroblasts than in extracts from cells from an FH homozygote; (ii) was suppressed in parallel with the receptors in intact cells when cultured epithelioid carcinoma A-431 cells were incubated with 25-hydroxycholesterol plus cholesterol; and (iii) reflected the 3-fold higher number of LDL binding sites per mg of protein in membranes from the adrenal cortex compared to membranes from the adrenal medulla. For these reasons, we believe that the ^{125}I -LDL binding activity that is solubilized by OG and assayed in the precipitated extract represents the physiologic LDL receptor.

The LDL receptor could not be extracted from adrenocortical membranes by treatments that remove extrinsic proteins, such as freeze-thawing or sonication, or by washing with high concentrations of salts, urea, guanidine hydrochloride, or EDTA. Of more than 15 detergents tested, only OG was effective in solubilizing the receptor in an assayable form. Other nonionic detergents such as Triton X-100, Nonidet P-40, various Tweens, and digitonin adhered to the membrane and bound ^{125}I -LDL in a nonspecific fashion, preventing reliable assay of the receptor. Similar artifacts were generated with positively

and negatively charged detergents such as cetylpyridinium bromide and deoxycholate.

LDL binding activity of the precipitated extract could be assayed either by centrifugation or by filtration. Comparable results were obtained, but the latter assay is more convenient. Of more than 30 different types of membrane filters tested, the Nuflow cellulose acetate filters were the only ones that gave acceptably low backgrounds (<0.2% of applied ¹²⁵I-LDL radioactivity trapped on the filter in the absence of the precipitated extract). Increasing the pore size above 0.45 μ m led to significant loss of bound radioactivity through the filter.

While in solution in the presence of 40 mM OG, the LDL receptor is amenable to purification. In studies to be reported elsewhere, we have found that the receptor can be precipitated from OG solution by 50% ammonium sulfate and resolubilized. Moreover, in the presence of OG the solubilized receptor is quantitatively adsorbed to lentil lectin-Sepharose and can be eluted with α -methyl-D-mannoside. The highest purification so far obtained with these procedures is about 40-fold when compared with the binding activity of the intact membranes. Even in its partially purified form, the receptor continues to form an assayable precipitate upon dilution. All of the data obtained so far are consistent with the earlier suggestion that the LDL receptor is an intrinsic (probably transmembrane) glycoprotein (1).

The method described here may have general utility for the purification of receptors that are intrinsic membrane proteins. Like the LDL receptor, such receptors can be solubilized with OG and purified in the presence of the detergent. During the early stages of purification, aliquots can be assayed by dilution of the detergent to form a precipitate that serves as a solid-phase binding site.

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