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Reconstitution of the Low Density Lipoprotein Receptor

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The receptor for low density lipoprotein was purified from bovine adrenal cortex in the presence of the nonionic detergent octylglucoside. Receptors were incorporated into the bilayer of egg phosphatidylcholine vesicles by a detergent-dialysis method. Reconstituted receptors were functional in that they bound low density lipoprotein as well as a monoclonal antibody directed against the receptor in a specific, saturable fashion. Binding activity of reconstituted receptors was measured by a gel chromatography assay. The orientation of the receptor molecule within the phospholipid bilayer was investigated by binding assays following proteolytic digestion. Reconstituted receptors showed an orientation that was functionally indistinguishable from that of low density lipoprotein receptors in the plasma membrane of intact human fibroblasts.

Key words: reconstitution, LDL receptor, octylglucoside, monoclonal antibody

The low density lipoprotein (LDL) receptor is a surface membrane glycoprotein that mammalian cells synthesize when they require cholesterol. The cholesterol-rich lipoprotein, LDL, binds to this receptor and is taken into the cell by receptor-mediated endocytosis. The LDL is delivered to and digested in lysosomes, and the liberated cholesterol is used in cellular metabolism. In patients with a disease called familial hypercholesterolemia, LDL receptor function is diminished. As a result, affected individuals do not remove LDL from the circulation normally, and the lipoprotein accumulates in the plasma where it produces premature atherosclerosis [1].

The LDL receptor has been studied extensively in cultured cells and in membranes from various animal tissues. The highest concentration of LDL receptors in the body is found in the cortex of the adrenal gland, in which the cholesterol for steroid hormone synthesis is supplied by uptake of LDL via these receptors. The LDL receptor has been purified from the bovine adrenal cortex [2], and shown to be an acidic glycoprotein of approximately 160,000 daltons. LDL receptors from normal human fibroblasts [3]; a human epidermoid cancer cell line, A-431 [4]; and rabbit

Abbreviations used: LDL, low density lipoprotein; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonylfluoride.

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adrenal gland [4] share these properties with the bovine receptor. In human fibroblasts the LDL receptor is synthesized as an intracellular precursor of $M_r = 120{,}000$, which upon maturation to a molecule of $M_r = 160{,}000$ becomes inserted into the plasma membrane [5,6]. Information on the conformation of this large protein structure within the bilayer of the plasma membrane is not available. However, functional domains have been postulated based on biochemical and genetic studies (cf [7]). At least three domains must be considered: (1) domain(s) for binding of LDL, (2) domain(s) that allow receptors to cluster in discrete regions of the surface membrane called coated pits, and (3) domain(s) that enable internalized receptors to escape immediate degradation after delivery of LDL cholesterol and recycle back to the surface, ready to bind new LDL particles.

Studies aimed at the identification of these structural domains are now underway in two systems. In one, receptors in normal fibroblasts and in cells from subjects with structurally abnormal receptors, such as in familial hypercholesterolemia [1], are radiolabeled with ^{35}S-methionine and immunoprecipitated. With this approach, several structural mutations in the gene for the LDL receptor have been identified [5,6]. We hope to be able to correlate structural divergences in mutant receptors with known functional abnormalities. However, this approach is severely limited by the amounts of LDL receptors present in cultured cells and relies on the ability to identify portions of the receptor by immunological means. Thus, we would like to study chemical amounts of receptor in its membrane environment and to dissect the protein into its domains. Specific assays will then allow one to directly assign functions to the isolated portions of the receptor protein.

In the current paper, I report the reconstitution of bovine adrenal LDL receptors into the bilayer of phospholipid vesicles. These reconstituted receptors bind LDL with the same characteristics as the LDL receptor on the surface of intact human fibroblasts and thus provide a convenient means of measuring the number of pure receptors. A rapid gel chromatography assay for the determination of LDL receptor activity is described. Furthermore, a monoclonal antibody that binds to the receptor on intact cells also binds to the reconstituted bovine receptor. Thus, reconstituted LDL receptors share important functional properties with native receptors embedded in the plasma membrane.

MATERIALS AND METHODS

Materials

Sodium [^{125}I] iodide (11 to 17 mCi/μg) was obtained from Amersham; L-α-dipalmitoyl [choline-methyl-^{3}H] phosphatidylcholine (20-40 Ci/mmol) from NEN; octyl-β-D-glucoside and pronase (Cat. No. 53702) from Calbiochem Behring; egg phosphatidylcholine (Cat. No. 820051) from Avanti Biochemicals, Inc, Birmingham, AL; agarose Bio-Gel A-15m (100-200 mesh) from Bio-Rad; and sucrose (Cat. No. 5503-UB) from BRL, respectively. All other materials were obtained from sources as previously reported [2].

Monoclonal Antibodies

A monoclonal antibody directed against the LDL receptor was prepared from ascitic fluid of BALB/c mice injected with cells of the C7 clone [3], and the IgG fraction was isolated as described [3]. The antireceptor antibody is designated IgG-

C7. A control antibody, designated IgG-2001, was obtained as previously described [8]. Both antibodies contain heavy chains of the IgG2b subclass.

Lipoproteins

Human LDL (density, 1.019–1.063 g/ml) was prepared from plasma by ultracentrifugation [9]. Methyl-LDL was prepared by treatment of LDL with formaldehyde plus sodium borohydride [10]. Lipoprotein concentrations are expressed in terms of protein content as measured by the method of Lowry et al [11].

Protein Iodination

LDL was radiolabeled with ^{125}I by the iodine monochloride method [12]. Radioiodination of IgG-C7 was performed as described [8].

Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli [13] on slab gels (14 × 8.5 × 0.15 cm). Samples were applied in buffer containing 0.5% SDS, 10% (v/v) glycerol, 100 mM dithiothreitol, and 5% (v/v) 2-mercaptoethanol after heating to 90°C for 3 min. Electrophoresis was carried out at 25–50 mA/slab gel at 10°C for 3–4 hr. Gels were calibrated with the following molecular weight standards: myosin, 200,000; β-galactosidase, 116,000; phosphorylase b, 97,000; bovine serum albumin, 68,000; and ovalbumin, 43,000. Proteins were stained with Coomassie blue or with silver by a modification of the procedure of Merril et al [14] as described previously [2].

Purification of Bovine Adrenal Receptor

All operations were carried out at 4°C. The procedure used was the one reported previously [2], except that immunoaffinity chromatography was performed in the presence of detergents throughout as follows: the diethylaminoethyl (DEAE)-cellulose fraction (30-37 mg protein) was diluted with 3 vol of a buffer containing 50 mM Tris-chloride (pH 7), 2mM $CaCl_2$, 40 mM octylglucoside, and 1mM PMSF (buffer A), after which it was spun at 100,000g for 60 min at 4°. The supernatant (27–33 mg protein) was applied to a column containing 14–17 mg monoclonal IgG-C7 coupled to Sepharose 4B as described [2] equilibrated in buffer A. The flowrate of the column was adjusted so as to allow application of the sample in a total of 90 min to 2 hr. The column was washed for 16 hr with a total of 500 ml of buffer A, followed by 2 column volumes of 40 mM octylglucoside in H_2O. Subsequently, a solution of 40 mM octylglucoside in 0.5 M NH_4OH (25% of the settled bed column volume) was added to the top of the column and the eluate discarded. Finally, one column volume of the same solution was used to elute the LDL receptors. The eluate contained 200–250 μg protein of purified LDL receptors.

Reconstitution

All operations were carried out at 24°C. In a glass tube, 5 mg of egg phosphatidylcholine in ethanol, 6 mg of octylglucoside in acetone, and, where indicated, 10 μCi of ^3H-dipalmitoylphosphatidylcholine were dried under a stream of nitrogen. The mixture was taken up in ether and dried again twice so as to form a film covering the walls of the tube. One milliliter of a solution containing 100 μg of pure bovine adrenal LDL receptors in 40 mM octylglucoside was added. The solution cleared up under

slight agitation and was dialyzed against three changes of 4 liters each of buffer containing 50 mM Tris-maleate (pH 6) and 2 mM $CaCl_2$ for a total of 36 hr at 24°C. The resulting opalescent material is designated as the reconstituted LDL receptors.

Assays for Binding of ^{125}I-LDL and ^{125}I-IgG-C7

Binding of radiolabeled ligands to soluble LDL receptors was determined by the standard filtration assay described in [15]. In short, coprecipitation of receptors together with phosphatidylcholine was achieved by the addition of acetone, the precipitate was resuspended and incubated with either ^{125}I-LDL or ^{125}I-IgG-C7, and free ^{125}I-labeled ligand was separated from receptor-bound ligand by filtration on cellulose acetate membrane filters [15].

Ligand binding to the reconstituted LDL receptors was measured by a gel column chromatography procedure. Unless otherwise stated, assay mixtures (100 μl) contained 1–10 μg of receptor protein, 10 mM Tris-chloride (pH 8), 30 mM NaCl, 1 mM $CaCl_2$, 33 mg/ml of bovine serum albumin, and 15 μg/ml of radiolabeled ligand. After incubation for 60 min at the indicated temperature, 60-μl aliquots of the assay mixture were applied to the top of gel columns prepared in glass pasteur pipettes. The columns contained a total of 1.6 ml settled bed volume of agarose Bio-Rad A-15m (100–200 mesh); molecular sieve beads, 8–12 mesh (Aldrich, Cat. No. 20,858-2) were used as bed support. The gels had been equilibrated previously in buffer containing 50 mM Tris-chloride (pH 8), 20 mM NaCl, 0.5 mg/ml bovine serum albumin, and 1 mM $CaCl_2$ and were eluted with this buffer (column buffer). After application of the sample, column buffer was added in 100-μl aliquots, the eluate was collected in corresponding individual 100-μl fractions, and their radioactivity was measured.

For determination of nonspecific binding in the filtration as well as in the column chromatography assays, incubation mixtures contained the following: for ^{125}I-LDL binding, 10 mM EDTA; for ^{125}I-IgG-C7 binding, 400 μg/ml of unlabeled IgG-C7. Specific binding was calculated by subtracting the value of nonspecific binding from the value for total binding [15]. For the calculation of the amounts of bound ligand in the column chromatography assay, the total radioactivity in the peak representing bound ligand (see Fig. 3) was determined.

RESULTS

Purification of Bovine Adrenal LDL Receptors

Previously, a two-step procedure consisting of ion-exchange chromatography and immunoaffinity column chromatography was used to isolate radiolabeled LDL receptor protein [2]. In this procedure, receptors are detergent-solubilized and partially purified, the protein is labeled with ^{125}I with concomitant removal of the detergent, and pure ^{125}I-labeled LDL receptors are obtained by affinity chromatography [2]. For the studies reported here, this procedure was modified for preparation of milligram amounts of unlabeled LDL receptor protein. This modified procedure makes possible the continuous presence of the detergent octylglucoside, a prerequisite for successful reconstitution of the receptor. The DEAE-cellulose fraction (see Materials and Methods) was subjected to affinity chromatography (without prior removal of detergent) on a column containing covalently bound monoclonal antibody IgG-C7. For each mg of protein of DEAE-cellulose fraction, the use of 0.5 mg of immobilized

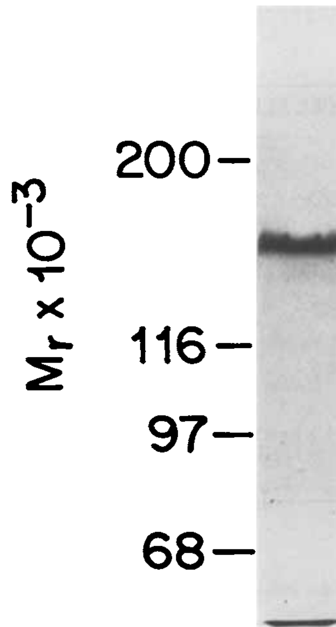

Fig. 1. SDS-polyarcylamide gel of the LDL receptor from bovine adrenal cortex. Receptor was purified and 40 μg protein were subjected to SDS-gel electrophoresis on 5% slab gels, followed by staining with Coomassie blue as described in Materials and Methods. Molecular weight markers are indicated.

IgG-C7 protein was found to result in maxium yields of pure receptors. After extensively washing the column as described under Materials and Methods, receptors were eluted with a solution containing 0.5 M NH_4OH and 40 mM octylglucoside. In a typical experiment, 250 μg of purified LDL receptors were recovered from 30 mg of protein of DEAE-cellulose fraction. Figure 1 shows a Coomassie blue-stained sodium dodecyl sulfate (SDS) polyacrylamide gel of 40 μg of a typical receptor preparation. Densitometric scans of SDS-polyacrylamide gels stained with Coomassie blue as well as with silver demonstrated that all preparations used in this study were greater than 95% pure. The receptor solution was immediately used for reconstitution.

Reconstitution

When LDL receptors were purified in the presence of octylglucoside, mixed with egg lecithin, and the detergent was subsequently removed, a slightly opalescent solution was recovered and designated as reconstituted LDL receptors. These preparations contained 80–90% of the protein and lipid present in the starting detergent-solubilized mixture. Reconstituted receptors were subjected to density gradient centrifugation and fractions were analyzed for their content of protein by SDS polyacrylamide gel electrophoresis and phospholipid by scintillation counting. When reconstituted receptors were prepared from mixtures with molar ratios of detergent to lipid of 10:1 or greater, the phospholipid was found in a single peak near the top of the gradient, corresponding to a median buoyant density of 1.03 g/cm^3. When each of the gradient fractions was analyzed for protein content, the only protein detected

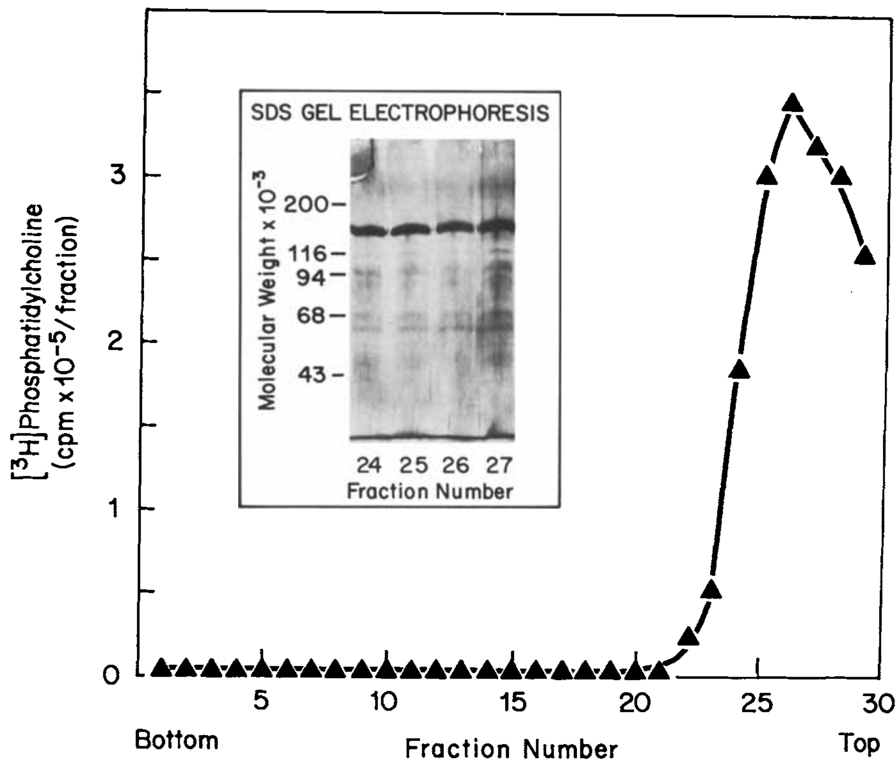

Fig. 2. Density gradient centrifugation of LDL receptor reconstituates. LDL receptor was incorporated into egg phosphatidylcholine in the presence of 5 μCi of ^{3}H-dipalmitoylphosphatidylcholine as described in Materials and Methods. Gradients of 5–15% (w/v) sucrose in buffer containing 50 mM Tris-maleate (pH 6), 2 mM $CaCl_2$, and 0.5 M NaCl were prepared, and 1 ml of the reconstituates containing 95 μg receptor protein was layered on top of the gradient (total volume 14 ml). Centrifugation was for 20 hr at 38,000g in a SW 40.1 rotor at 4°. Fractions of 0.5 ml each were collected from the bottom of the tube and analyzed for ^{3}H-radioactivity and density. Aliquots (100 μl) from each fraction were subjected to SDS-polyacrylamide gel electrophoresis on 7% gels, followed by silver staining as described [2]; fractions Nos. 24–27 are shown in the insert.

was the LDL receptor, exclusively found in the fractions containing phospholipid (fractions 24 to 27, Fig. 2). When pure LDL receptors were subjected to the reconstitution protocol, but phospholipid was omitted, and the resulting solution analyzed as above, receptor protein was found in a peak in the middle of the gradient as well as at the bottom of the gradient (data not shown). In the standard reconstitution protocol, the molar ratio of detergent to lipid was 10:1 (average M_r of egg phosphatidylcholine, 800). When the molar ratio of detergent to lipid in the reconstitution mixture was adjusted to 5:1 or less, the distribution of receptor protein on density gradients was identical to that in the absence of phospholipid.

The molar ratio of phospholipid to receptor protein in the standard reconstitution mixture was 10^4:1. Under these conditions and at higher lipid to protein ratios, all of

the phosphatidylcholine and LDL receptors comigrated on sucrose density gradients. Experiments to separate reconstituted receptors from excess phospholipid by increasing the buoyant density of the reconstituted receptors were performed. This might be possible through decreasing the ratio of lipid to protein in the reconstitution mixture. Therefore, the ratio of lipid to protein was varied over a wide range, keeping the octylglucoside concentration at a constant 60 mM. At molar ratios smaller than 10^3:1 (ie, 0.5 mg of egg lecithin and 100 μg of receptor protein), LDL receptors did not reconstitute with lipid, but instead formed aggregates that sedimented to the bottom of the sucrose gradients (data not shown). Only at molar ratios of lipid to protein in range of 5×10^3 to 2×10^4 did reconstituted receptors show the behavior demonstrated in Figure 2. Thus, all further studies were performed with reconstituted receptors obtained under the standard conditions described in the "Materials and Methods" section.

Ligand Binding to Reconstituted LDL Receptors

To test the ability of reconstituted LDL receptors to bind LDL or the monoclonal antibody, IgG-C7, a gel chromatography assay was employed. Reconstituted receptors were incubated with ^{125}I-labeled ligands, and unbound ligand was separated from the bound ligand by chromatography on small columns of 4% agarose. Figure 3 shows a series of gel filtration assays performed with reconstituted LDL receptors that were isolated by density gradient centrifugation (fraction No. 26, Fig. 2). These vesicles were labeled in their lipid portion with ^{3}H-dipalmitoylphosphatidylcholine. When they were passed over these analytical columns, all of the radioactivity emerged as a single peak in the excluded volume (Fig. 3, dashed line). When the ^{125}I-labeled ligands, LDL or IgG-C7, were passed over the columns, they were retained by the gel and eluted in a position well separated from the excluded volume. However, when ^{125}I-labeled ligands were incubated with reconstituted LDL receptors and aliquots of the incubation mixtures were passed over the agarose columns, bound ligand eluted in the excluded volume and was well separated from free ligand which eluted in the included volume. When ^{125}I-LDL (5 μg/ml) was used as the ligand, 55% of the recovered ligand eluted in the excluded volume. Virtually all of the ^{125}I-LDL was included in the column, ie, was not bound in the presence of 10 mM EDTA, a known inhibitor of LDL-receptor binding (Fig. 3A). Similarly, ^{125}I-IgG-C7 bound to reconstituted receptors, and could be competed for by an excess of unlabeled IgG-C7 (Fig. 3B). In experiments not shown, methylated LDL, which does not bind to the LDL receptor [16], was unable to displace bound ^{125}I-LDL from reconstituted LDL receptors. Moreover, a large excess of a control monoclonal antibody, IgG-2001 [8] did not compete for the binding of ^{125}I-IgG-C7. Furthermore, phosphatidylcholine vesicles formed in the absence of LDL receptors did not bind ^{125}I-LDL or ^{125}I-IgG-C7 as determined by the gel chromatography assay.

At the concentrations of labeled ligands used in the experiment of Figure 3, high-affinity binding (see Materials and Methods) was 200 μg of LDL and 207 μg of IgG-C7, respectively, per mg of reconstituted LDL receptor protein. In an effort to compare this assay procedure with the previously described filtration assay [15], these reconstituted LDL receptors were solubilized with 40 mM octylglucoside and subsequently precipitated by the use of acetone and excess phosphatidylcholine as described [15]. Binding of ^{125}I-LDL and ^{125}I-IgG-C7 to the phosphatidylcholine/acetone precipitate was then determined by the filter assay procedure [15]. When ligand binding

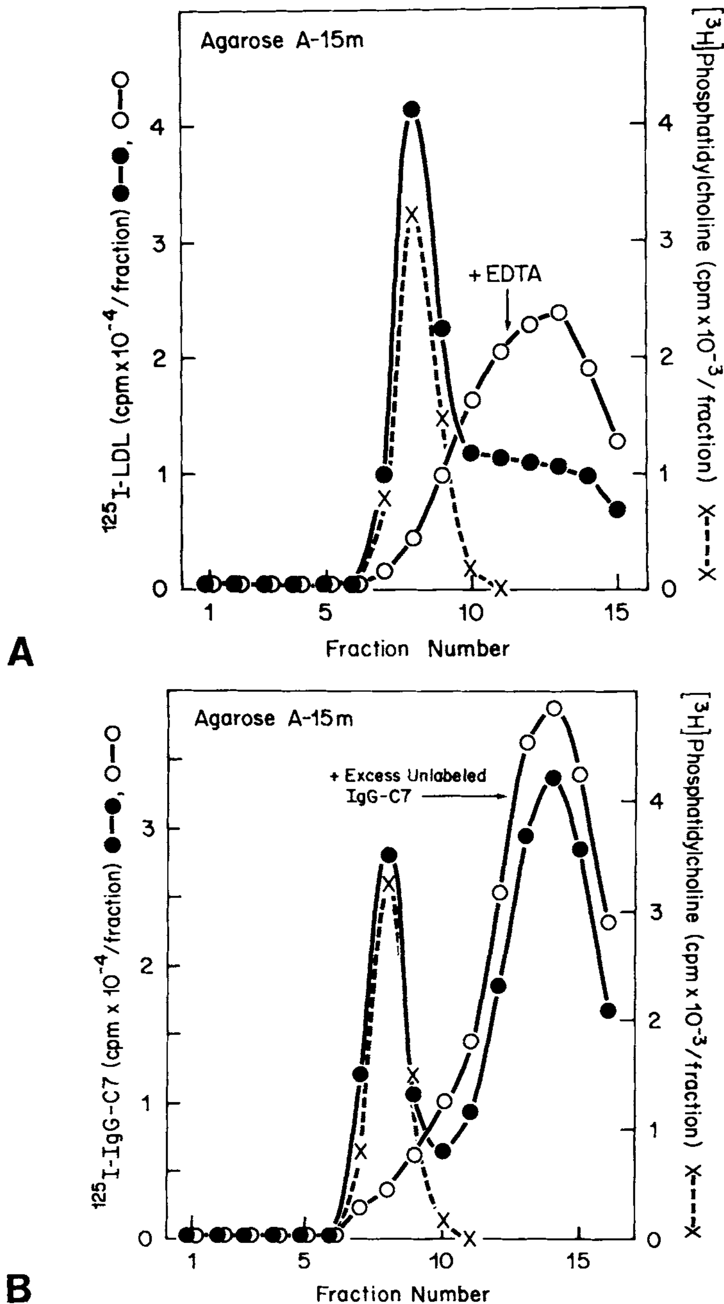

Fig. 3. Gel chromatography assay for the binding of: ^{125}I-LDL(A) and ^{125}I-IgG-C7(B) to LDL receptor reconstituates. Reconstituates (40 μl of fraction No. 26, Fig. 2) were incubated with or without ^{125}I-labeled ligand and subjected to gel chromatography on agarose A-15m as described in Materials and Methods. Radioactivity was measured in each fraction, x---x, gel chromatography of ^{3}H-phosphatidylcholine-containing reconstituates in the absence of ligand. A. Incubation with 5 μg/ml of ^{125}I-LDL (283 cpm/ng) in the absence (●-●) and presence (O-O) of 10 mM EDTA. B. Incubation with 10 μg/ml of ^{125}I-IgG-C7 (204 cpm/ng) in the absence (●-●) and presence (O-O) of 400 μg/ml unlabeled IgG-C7.

was measured in this fashion at the same ligand concentrations that were used for the column chromatography assay, the resulting specific activities were 293 μg/mg for LDL, and 302 μg/mg for IgG-C7, respectively. Thus, the amounts of binding in the two assays were comparable. Significantly, the phosphatidylcholine/acetone precipitates did not display activity when assayed for ligand binding by the column chromatography assay, and, vice versa, reconstituted LDL receptors could not be assayed by the filtration procedure (see Discussion).

The LDL receptor has been shown to lose its ability to bind LDL upon pronase treatment of solubilized receptors [17] and of intact fibroblasts [5,18]. In the current study, if ligand binding sites were exposed to the outside of the vesicles containing reconstituted LDL receptors, as suggested by the results, pronase treatment should reduce, if not abolish, the binding activity of reconstituted LDL receptors. Indeed, when reconstituted receptors were treated with pronase, they rapidly lost their ability to bind ^{125}I-LDL, as determined by the column chromatography assay. Pronase treatment also abolished binding of ^{125}I-IgG-C7 completely. When the vesicles were solubilized after pronase treatment, reprecipitated by the phosphatidylcholine/acetone procedure, and assayed for ligand binding by the filtration assay, about 2% of the binding activity for ^{125}I-LDL as well as for ^{125}I-IgG-C7 present in the starting reconstituates were recovered.

DISCUSSION

Reconstitution of membrane protein function has proven to be an important tool for the elucidation of protein structure and function in diverse systems. These include the structure of viral proteins and the mechanism of virus entry into cells [19–22], the action of ion transport systems (reviewed in [23]), the response of adenylate cyclase to hormones [24,25], and the function at the molecular level of receptors for insulin [26], and acetylcholine [27]. The present paper describes the reconstitution of purified receptors for low density lipoprotein into egg phosphatidylcholine vesicles. The reconstitution of receptor function by detergent-dialysis was achieved through the use of the nonionic detergent octylglucoside, which offers several advantages. First, octylglucoside has been shown to solubilize LDL receptors effectively from bovine adrenal cortex and human fibroblasts [17] and to facilitate purification of the bovine receptor [2,15]. Second, octylglucoside has been used extensively to reconstitute hydrophobic proteins by the detergent-dialysis method in several systems [19, 21, 28, 29]. In another study, we have used this simple method to incorporate an isolated apolipoprotein, apoprotein E, into phospholipid vesicles [30]. In addition, model studies on the formation of phospholipid vesicles by the octylglucoside-dialysis method in the absence or presence of glycophorin A have been reported [31].

In agreement with the results obtained in this model study, it was found that the critical parameter for reconstitution of LDL receptors into phospholipid vesicles was the detergent-to-lipid ratio. When this ratio was too low, receptors formed aggregrates upon removal of the detergent. These aggregates did not contain phospholipid. On the other hand, when the molar ratio of detergent to lipid was greater than 5:1 (the highest ratio examined was 20:1), protein stably associated with phospholipid.

Besides the detergent-to-lipid ratio, the lipid-to-protein ratio was considered as a parameter that might influence the properties of reconstituted LDL receptors. In particular, incorporation of receptor protein into phospholipid could be achieved only with a several thousandfold molar excess of egg phosphatidylcholine over the amount

of receptor protein. Under these circumstances, one would expect that lipid would be in large excess, and in addition to receptor-containing vesicles, protein-free vesicles should be present after removal of detergent from the reconstitution mixture. Thus, experiments to separate pure lipid vesicles from reconstituted LDL receptors were performed based on two properties in which these particles might differ, ie, buoyant density and size. Surprisingly, both density gradient centrifugation (Fig. 2) and gel filtration chromatography on Sephacryl S-1000 [32] (data not shown) did not resolve two populations of particles. Thus, it is conceivable that under the conditions that result in reconstitution, very few, if any, protein-free vesicles exist. Support for this notion comes from two observations. First, when the lipid-to-protein ratio was decreased to below 10^3:1, receptors could not associate with lipid, indicating that the amount of phospholipid is the limiting factor for successful reconstitution. Second, based on preliminary gel filtration experiments on Sephacryl S-1000 [32], the size of receptor-containing vesicles ranges from 200 to 270 nm in external diameter. Mimms et al [31] calculated that vesicles of this size prepared from egg phosphatidylcholine by the octylglucoside-dialysis method contain 3.5×10^5 to 6.4×10^6 (mean, 5×10^5) phospholipid molecules per vesicle. In the current study, if one assumes an average of 5×10^5 phosphatidylcholine molecules per reconstituted vesicle and takes into account the molar ratio of lipid to protein of 10^4:1 used in the standard protocol, one can calculate that there are 50 times more receptor molecules than vesicles. Thus, all of the vesicles may contain receptors. Alternatively, the low buoyant density of reconstituted LDL receptors (1.03 g/ml) might not differ sufficiently from that of pure lipid vesicles to allow separation on density gradients, and the same might be true for the size difference, resulting in a lack of resolution on sizing columns.

What Is the Evidence That Receptors Are Incorporated Into the Lipid Bilayer?

The evidence comes from observations made during evaluation of the gel chromatography assay for ligand binding. Previously, we measured binding of ^{125}I-LDL and ^{125}I-IgG-C7 by sequentially coprecipitating solubilized receptors with egg phosphatidylcholine liposomes by the use of acetone, incubating the precipitate with radiolabeled ligand, and separating free from receptor-bound ligand on cellulose-acetate membrane filters [15]. This procedure has been successfully adopted to measure binding of β-hexosaminidase B to bovine liver phosphomannosyl receptors [33], of ^{125}I-labeled nerve growth factor to receptors solubilized from a human melanoma cell line [34], and of ^{125}I-labeled epidermal growth factor to human placental extracts [unpublished observations]. Here, it was found that binding of ^{125}I-labeled ligands to phosphatidylcholine/acetone precipitates of LDL receptors could not be measured by the gel chromatography assay. Gel chromatography of incubation mixtures containing ^{125}I-LDL or ^{125}I-IgG-C7 and phosphatidylcholine/acetone precipitates resulted in elution of radioactivity exclusively in the position of unbound ligand. When purified LDL receptors were dialyzed to remove detergent in the absence of phospholipid, the resulting receptor preparation also did not display receptor activity when assayed by the gel chromatography procedure. However, after precipitation of these detergent-free receptors with phosphatidylcholine/acetone, receptor activity could be measured by the filtration assay. It appears that receptors in the phosphatidylcholine/acetone precipitate are associated with liposomes very loosely such that the liposomes get stripped of receptors upon gel chromatography. Thus, we can draw

two conclusions: first, phosphatidylcholine/acetone precipitation of detergent-solubilized LDL receptors does not result in stable reconstitution of receptors into liposomes, and second, the gel chromatography assay for ligand binding provides a reliable tool to investigate the nature of LDL receptor-phospholipid complexes. For example, when detergent-free purified receptors were incubated under a variety of conditions with protein-free vesicles prepared by the octylglucoside-dialysis method, the gel chromatography assay revealed that receptors did not stably associate with the vesicles. In addition, analysis of the resultant protein-lipid mixtures on density gradients confirmed that these attempts did not lead to the reconstitution of LDL receptors.

By the two criteria, density gradient centrifugation (Fig. 2) and gel chromatography assay procedure (Fig. 3), it is clear that LDL receptors become incorporated into the bilayer of phospholipid vesicles only by the octylglucoside-dialysis method.

The orientation of the major domains of the LDL receptor in the reconstituted preparations appears to be similar to, if not identical with, that of receptors in the plasma membrane of cells. Both ligands that bind to the receptor on intact cells, ie, LDL and IgG-C7, also bind to reconstituted LDL receptors. Pronase destroys the binding sites for LDL as well as for IgG-C7 on intact human fibroblasts [5], and also abolishes these binding activities on reconstituted LDL receptors. When reconstituted LDL receptors were dissolved in detergent with and without prior treatment with pronase, and receptor activity was assayed subsequently by the filtration assay, 95% of the initial activity was found destroyed by pronase (data not shown). Thus, only 5% of the total number of binding sites in the reconstituted LDL receptors were pronase resistant. These observations suggest, but — in the absence of the demonstration that pronase did not penetrate into the vesicular lumen — cannot prove that the majority of the ligand binding sites of the LDL receptor are exposed to the outer surface of the reconstituted vesicles. Studies to establish this point are now in progress. We plan to correlate studies on the structure of the LDL receptor in intact fibroblasts with investigations using chemical amounts of reconstituted LDL receptors.

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