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Reconstituted Low Density Lipoprotein: A Vehicle for the Delivery of Hydrophobic Fluorescent Probes to Cells

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Previous studies have shown that the cholesteryl ester core of plasma low density lipoprotein (LDL) can be extracted with heptane and replaced with a variety of hydrophobic molecules. In the present report we use this reconstitution technique to incorporate two fluorescent probes, 3-pyrenemethyl-23,24-*dinor*-5-cholen-22-oate-3β-yl oleate (PMCA oleate) and dioleyl fluorescein, into heptane-extracted LDL. Both fluorescent lipoprotein preparations were shown to be useful probes for visualizing the receptor-mediated endocytosis of LDL in cultured human fibroblasts. When normal fibroblasts were incubated at 37°C with either of the fluorescent LDL preparations, fluorescent granules accumulated in the perinuclear region of the cell. In contrast, fibroblasts from patients with the homozygous form of familial hypercholesterolemia (FH) that lack functional LDL receptors did not accumulate visible fluorescent granules when incubated with the fluorescent reconstituted LDL. A fluorescence-activated cell sorter was used to quantify the fluorescence intensity of individual cells that had been incubated with LDL reconstituted with dioleyl fluorescein. With this technique a population of normal fibroblasts could be distinguished from a population of FH fibroblasts. The current studies demonstrate the feasibility of using fluorescent reconstituted LDL in conjunction with the cell sorter to isolate mutant cells lacking functional LDL receptors.

Key words: low density lipoprotein, cell surface receptors, receptor-mediated endocytosis, reconstitution of lipoproteins, fluorescent probes, fluorescence-activated cell sorter, familial hypercholesterolemia

Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoprotein; PMCA oleate, 3-pyrenemethyl-23,24-*dinor*-5-cholen-22-oate-3β-yl oleate; r-[PMCA oleate]LDL and r-[dioleyl fluorescein]LDL, LDL that had been reconstituted with PMCA oleate and dioleyl fluorescein, respectively.

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Receptor-mediated endocytosis is the process by which certain physiologically important molecules such as plasma transport proteins and protein hormones bind to cell surface receptor sites before being internalized by cells. For some receptor-bound molecules, efficient cellular uptake is achieved by the clustering of receptors in specialized regions of plasma membrane called "coated pits," which continually invaginate and pinch off from the cell surface to form coated endocytic vesicles [1–3]. In principle, the process of receptor-mediated endocytosis can be utilized as a system for rapid delivery of drugs to cells, provided that pharmacologically active agents can be attached to transport proteins and hormones that normally enter cells through coated vesicles.

One receptor-mediated uptake system that lends itself to drug delivery is the one that involves low density lipoprotein (LDL), the major cholesterol-transport protein in human plasma [1, 4]. LDL is a large spherical particle that has an average diameter of 220 Å and a molecular weight of approximately 3×10^6 [5]. Each LDL particle consists of an apolar core containing approximately 1,300 molecules of cholesteryl ester that is surrounded by a polar coat composed primarily of phospholipid, small amounts of unesterified cholesterol, and apoprotein B [5]. Cultured cells and body cells derive cholesterol from plasma LDL through receptors that recognize the apoprotein B component of the lipoprotein [4]. Once bound to its receptor in coated pits, LDL is rapidly internalized and delivered to lysosomes where its components undergo hydrolysis. The apoprotein B of LDL is degraded to amino acids, and the cholesteryl esters of LDL are hydrolyzed to yield unesterified cholesterol, which is used by cells for structural and regulatory purposes [4].

The feasibility of using LDL as a vehicle for delivering hydrophobic molecules to cells bearing LDL receptors has recently been established [6–8]. Krieger et al developed a method for removing the cholesteryl ester core of LDL with heptane and reconstituting the particle with exogenous cholesteryl esters [6, 7]. In addition to cholesteryl esters, a wide variety of other hydrophobic molecules can be used to reconstitute heptane-extracted LDL, including compounds that contain esters of long chain *cis*-unsaturated fatty acids (such as triolein and methyl oleate) and compounds that contain polyisoprenoid groups (such as retinyl palmitate and ubiquinone-10) [8]. These reconstituted LDL preparations retain the ability to bind to the LDL receptor and to be taken up by cultured cells [6–8]. Substances introduced into the core of LDL will not enter cells unless the lipoprotein is taken up by the receptor mechanism [6–8].

Previous studies of reconstituted LDL have used radiochemical and biochemical methods to measure cellular uptake. In the present studies we have used the reconstitution technique to incorporate two types of fluorescent molecules into LDL, allowing LDL uptake to be visualized with a fluorescence microscope. The uptake of both preparations of fluorescent reconstituted LDL was found to be dependent on the LDL receptor. With the use of a fluorescence-activated cell sorter, we show that cells that contain LDL receptors can be separated from cells that are genetically deficient in receptors.

MATERIALS AND METHODS

Materials

Sodium [^{125}I]iodide (17 mCi/μg) was purchased from Amersham/Searle. 0.05% Trypsin/0.02% EDTA solution (Cat. No. 610-5300) was obtained from Grand Island Biological Co. 3-Pyrenemethyl-23,24-*dinor*-5-cholen-22-oate-3β-yl oleate (PMCA oleate)

(Fig. 1) was synthesized from 3-pyrenemethyl-23,24-*dinor*-5-cholen-22-oate [9] and oleyl chloride in pyridine, and the compound was purified by silicic acid chromatography. Dioleyl fluorescein (Fig. 2) was synthesized by Dr. T. Y. Shen and associates at Merck Sharp & Dohme Research Laboratories. Other supplies and reagents were obtained from sources as previously reported [6, 10].

Lipoproteins

Human LDL (density 1.019–1.063 g/ml) and lipoprotein-deficient serum (density > 1.215 g/ml) were obtained from the plasma of healthy individuals and prepared by ultracentrifugation [10]. Fetal calf lipoprotein-deficient serum (density > 1.215 g/ml) was prepared by ultracentrifugation [10]. r-[PMCA oleate]LDL and r-[dioleyl fluorescein]-LDL were prepared by a previously described reconstitution method in which the endogenous neutral lipids of LDL were removed by heptane extraction and replaced with PMCA oleate and dioleyl fluorescein, respectively [6, 8]. r-[PMCA oleate]LDL was prepared by incubating 1.9 mg of heptane-extracted LDL-protein with 200 μl of benzene containing 6 mg of PMCA oleate [8]. The mass ratio of PMCA oleate to protein (mg/mg) in the final solubilized r-[PMCA oleate]LDL was 1.24. r-[Dioleyl fluorescein]LDL was prepared by incubating 1.9 mg of heptane-extracted LDL with 200 μl of heptane containing a mixture of 0.6 mg of dioleyl fluorescein and 5.4 mg of triolein [8]. The mass ratio of dioleyl fluorescein to protein (mg/mg) in the final solubilized r-[dioleyl fluorescein]LDL was 0.5. The mass ratio of triolein to protein (mg/mg) in the same preparation of r-[dioleyl fluorescein]LDL was 1.2. The triolein was mixed with dioleyl fluorescein in the reconstitution to dilute the dioleyl fluorescein so that the cellular fluorescence intensity would remain within the dynamic range of the detection system of the cell sorter (see below). The concentrations of all lipoproteins are expressed in terms of their protein content. ^{125}I-LDL was prepared as previously described [11]. Lipoprotein electrophoresis was carried out in agarose gel at pH 8.6 in barbital buffer [12].

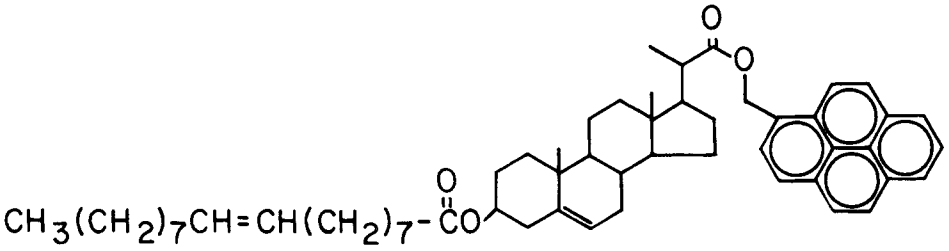

Fig. 1. Structural formula of PMCA oleate.

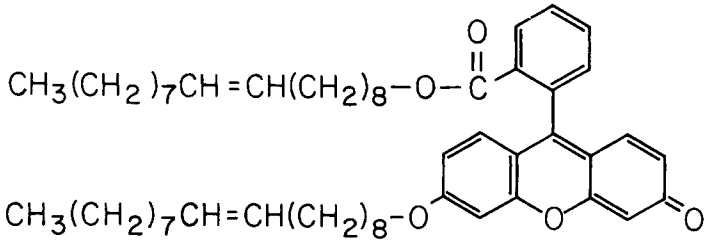

Fig. 2. Structural formula of dioleyl fluorescein.

Cells

Human fibroblasts from normal subjects and from patients with the heterozygous and homozygous forms of familial hypercholesterolemia (FH) were grown in monolayer culture as previously described [13].

Assays

The amounts of surface-bound ^{125}I-LDL (dextran sulfate-releasable ^{125}I-LDL), intracellular ^{125}I-LDL (dextran sulfate-resistant ^{125}I-LDL), and degraded ^{125}I-LDL were measured in intact fibroblast monolayers by previously described methods [9, 13]. The protein content of extracts and lipoproteins was determined by the method of Lowry et al [14], with bovine serum albumin as a standard. The content of PMCA oleate and dioleyl fluorescein in reconstituted LDL was determined by fluorescence spectrophotometry after extracting the lipids from the lipoprotein with chloroform/methanol.

Fluorescence-Activated Cell Sorter

After incubation of cells with r-[dioleyl fluorescein]LDL, the medium was removed and each monolayer was washed 6 times at 4°C with an albumin-containing buffer [13]. The cell monolayers were dissociated by incubation with 1 ml of 0.05% trypsin/0.02% EDTA solution for 1.5 min at 37°C. Nonspecific background fluorescence was reduced by illuminating each cell suspension for 2 min at 4°C with a 75 watt Xenon lamp at a distance of 5 cm. The cells were then kept on ice (3–10 min) until they were analyzed on a Becton-Dickinson FACS III fluorescence-activated cell sorter (laser settings: 488 nm line, 300 milliwatts of power in the light stabilized mode; photomultiplier setting: 500 V; flow rate: 10,000 cells per sample analyzed at approximately 200–300 cells/sec through a 50 μm aperture) [15]. The light scatter gates were set to exclude small nonfluorescent cell debris and large cell aggregates. The observed fluorescence intensity is expressed on a relative scale, which ranged from 1 to 128 units/cell.

RESULTS

Both of the fluorescent compounds used for these studies, PMCA oleate and dioleyl fluorescein, were incorporated into LDL with high yield using the reconstitution procedure [6, 8]. The structures of PMCA oleate (M_r = 825) and dioleyl fluorescein (M_r = 565) are shown in Figures 1 and 2, respectively. Mass analyses of the r-[PMCA oleate]LDL indicate that approximately 750 molecules of PMCA oleate were incorporated into each LDL particle. Similarly, 440 molecules of dioleyl fluorescein and 680 molecules of triolein were incorporated into each particle of r-[dioleyl fluorescein]LDL.

When the r-[PMCA oleate]LDL preparation was subjected to electrophoresis in agarose gel, the fluorescent lipoprotein exhibited the same electrophoretic mobility as native LDL (Fig. 3). When the gel was examined under fluorescent light, the fluorescent material (Fig. 3A) was observed in a position identical to that of the lipid components of the lipoprotein as visualized by fat red 7B staining (Fig. 3B). Similar results were obtained when r-[dioleyl fluorescein]LDL was analyzed by electrophoresis.

Figure 4 shows a series of micrographs of fibroblasts that had been incubated for 24 h with LDL reconstituted either with PMCA-oleate or with dioleyl fluorescein. When incubated with either of these reconstituted lipoproteins, normal fibroblasts accumulated large amounts of intracellular fluorescent material (Fig. 4 A, B, and E). The material appeared as granules that tended to cluster around the nucleus, a region that contains numerous lysosomes [16].

Two observations indicate that the uptake of fluorescent LDL was occurring through the LDL receptor mechanism: 1) Inclusion of a 50-fold excess of native LDL in the culture medium blocked the uptake of both r-[PMCA oleate]LDL (data not shown) and r-[dioleyl fluorescein]LDL (Fig. 4F) by competing for the limited number of LDL receptor sites [4]. 2) Fibroblasts from a patient with the receptor-negative form of homozygous FH, which have a near-total absence of LDL receptor activity [4], failed to accumulate visible quantities of fluorescent LDL when incubated with either r-[PMCA oleate]LDL (Fig. 4 C and D) or r-[dioleyl fluorescein] LDL (data not shown).

The amount of dioleyl fluorescein accumulated by individual fibroblasts incubated with r-[dioleyl fluorescein]LDL was assessed by detaching the cells from the Petri dish with trypsin and passing them through a fluorescence-activated cell sorter of the type de-

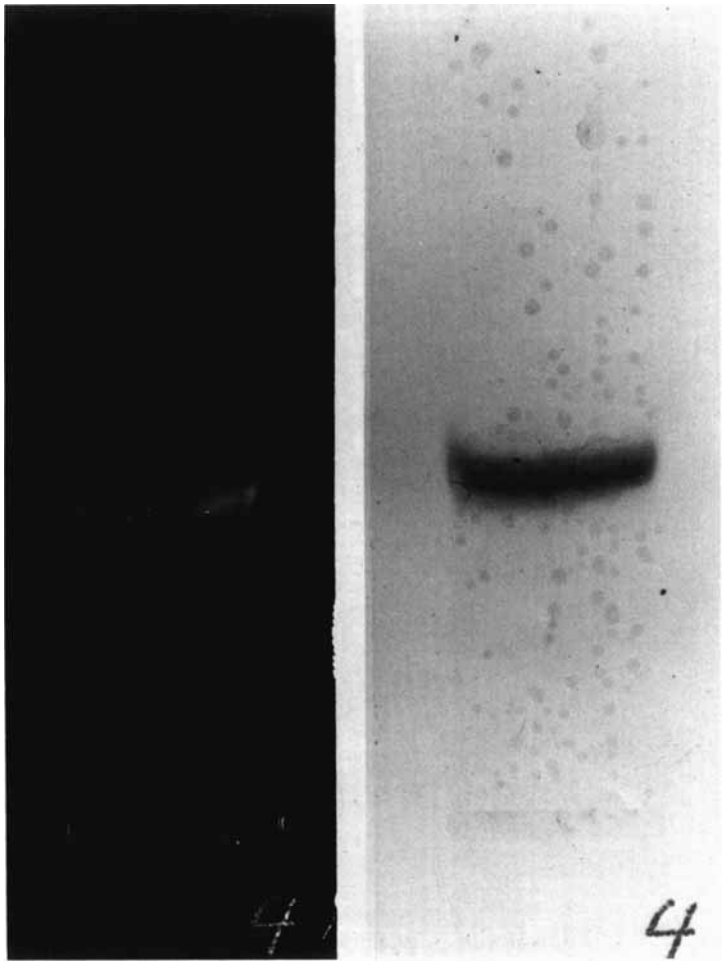

Fig. 3. Electrophoretic mobility of intact r-[PMCA oleate]LDL. A sample of r-[PMCA oleate]LDL (20 μg of protein) was subjected to electrophoresis in agarose gel (7.2 × 2.5 cm) at pH 8.6 (barbital buffer). After electrophoresis, the gel was photographed under ultraviolet light illumination (375 nm) (left), and then fixed and stained with fat red 7B (right). The point of application of the sample is indicated by the well at the bottom of the gel. The electrophoretic mobility of the r-[PMCA oleate]LDL was identical to that of native LDL, which was subjected to electrophoresis in the same study (photograph of native LDL not shown).

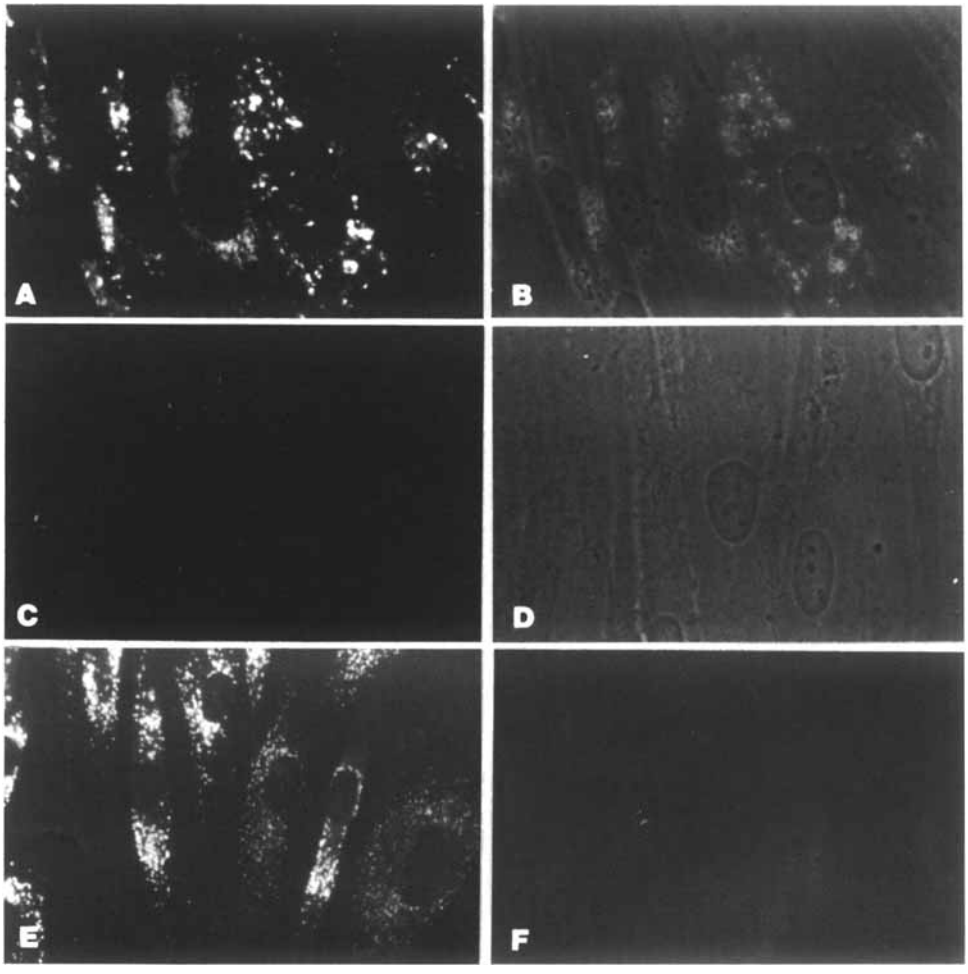

Fig. 4. Fluorescent light micrographs (A, C, E, F) and combined phase contrast-fluorescent light micrographs (B, D) of normal (A, B, E, F) and homozygous FH (C, D) fibroblasts incubated with LDL reconstituted with fluorescent compounds. Cells were seeded (day 0) onto glass coverslips contained within 60-mm Petri dishes at a concentration of 1×10^5 cells per dish in growth medium containing 10% (v/v) fetal calf serum [13]. On day 4 the medium was replaced with medium containing 10% fetal calf lipoprotein-deficient serum. On day 5 the medium was replaced with 2 ml of fresh medium containing 10% fetal calf lipoprotein-deficient serum and either 20 μg protein/ml of r-[PMCA oleate]-LDL (A–D) or r-[dioleyl fluorescein]LDL (E, F) in the absence (A–E) or presence (F) of 1 mg protein/ml of native LDL. After incubation with reconstituted LDL at 37°C for 24 h, the cell monolayers were washed 6 times [12] and fixed with 3% paraformaldehyde in 0.2 M sodium phosphate (pH 7.3) for 10 min at room temperature. The coverslips were removed from the dish, washed with water, mounted with glycerol on glass slides, and viewed in the epifluorescence and phase contrast modes of a Zeiss Photomicroscope III equipped with the appropriate filter packages (Panels A–D: exciter filter, 365/12 nm; chromatic beam splitter, 395 nm; barrier filter, 420 nm. Panels E and F: exciter filters, 455–500 nm; chromatic beam splitter, 510 nm; barrier filter, 528 nm). Panels A and B are photographs of the same cells, as are Panels C and D. Magnifications: A–D, × 600; E and F, × 400.

scribed by Loken and Herzenberg [15]. The results of one such experiment are presented in the dotplots in Figure 5. In this diagram, each dot represents the relative fluorescence (vertical axis) and the relative intensity of light scatter (horizontal axis) of a single cell. In the normal fibroblasts, virtually all cells were highly fluorescent (Fig. 5, left panel). In the FH homozygote cells, much less fluorescence was observed (Fig. 5, center panel). When the two cell populations were mixed together in approximately equal proportions prior to passage through the sorter, the normal cells were clearly distinguished from the FH homozygote cells (Fig. 5, right panel).

Cells from FH heterozygotes have been shown previously by studies using ^{125}I-labeled LDL to express approximately 50% of the normal number of LDL receptors [17, 18]. To determine whether the fluorescence-activated cell sorter could distinguish normal fibroblasts, FH heterozygote fibroblasts, and FH homozygote fibroblasts, cell strains from each of the three genotypes were incubated in the same experiment with both ^{125}I-LDL and r-[dioleoyl fluorescein]LDL at 37°C. In the case of ^{125}I-LDL, the amounts of surface binding, internalization, and degradation of the lipoprotein were measured using standard techniques [4]. Each of these parameters has been shown previously to be a direct reflection of the number of LDL receptors [4]. Fibroblasts from parallel sets of dishes that had been incubated with r-[dioleoyl fluorescein]LDL were harvested with trypsin, the cells from each dish were passed through the cell sorter, and the mean fluorescence intensity per cell was determined.

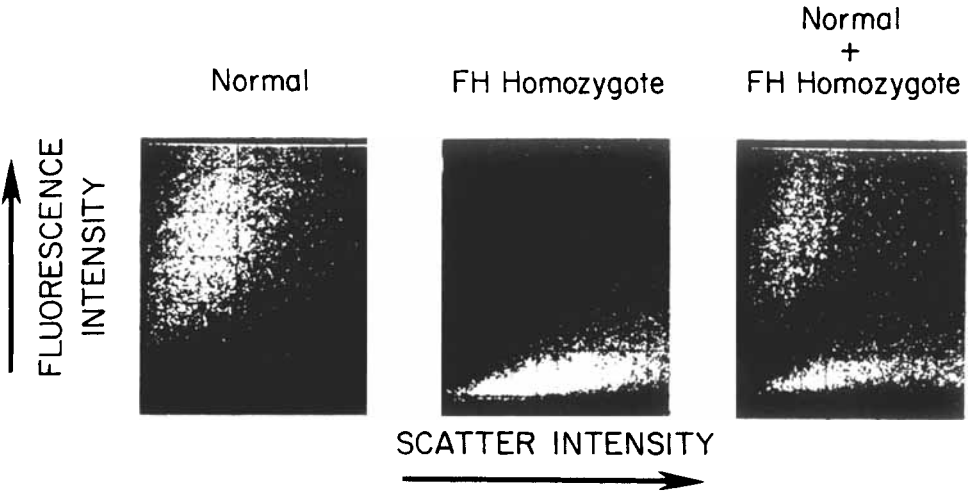

Fig. 5. Dotplots of normal, FH homoxygote, and a mixture of normal and FH homoxygote fibroblasts incubated with r-[dioleyl fluorescein] LDL. Cells were seeded (day 0) in 60-mm Petri dishes at a concentration of 7.5×10^4 cells per dish in growth medium containing 10% fetal calf serum [13]. On day 4 fresh medium containing 10% fetal calf serum was added. On day 6 the medium was replaced with 2 ml of fresh medium containing 10% human lipoprotein-deficient serum. On day 7 each dish received 2 ml of fresh medium containing 10% human lipoprotein-deficient serum and 10 μg protein/ml of r-[dioleyl fluorescein]LDL. On day 8, after incubation for 18 h at 37°C, 10,000 normal fibroblast cells (left panel), 10,000 FH homozygote cells (center panel), and a mixture of normal cells and FH homozygote cells (total of 10,000 cells) (right panel) were analyzed in the fluorescence-activated cell sorter as described in Materials and Methods.

Table I shows that LDL receptor activity, as measured by the ^{125}I-LDL techniques, was highest in normal cells, about 50% of normal in the FH heterozygote cells, and virtually undetectable in the FH homozygote cells. Similarly, the average fluorescence intensity was highest in the normal cells (89 and 65 units/cell), about 50% of normal in the FH heterozygote cells (34 and 43 units/cell), and lowest in the FH homozygote cells (17 units/cell). After incubation with r-[dioleyl fluorescein], 54% of the FH homozygote cells (subject M.C.) had fluorescence intensities that were less than 15 units/cell, whereas only 1.7% of the normal cells (subjects D.S. and A.H.) had fluorescence intensities below this value. In normal cells (subject D.S.) that had been subjected to prior incubation with a mixture of 25-hydroxycholesterol and cholesterol to reduce the number of LDL receptors [4], the average fluorescence was reduced from 89 to 22 units/cell (Table I), confirming that the major portion of the fluorescence was attributable to uptake through the LDL receptor. In contrast, prior incubation of the FH homozygote cells (subject M.C.) with 25-hydroxycholesterol plus cholesterol did not significantly reduce the small amount of fluorescence (17 units/cell) that was detected in these cells, confirming that this fluorescence was receptor-independent (Table I).

The reason for the nonspecific fluorescence present in the normal cells incubated with 25-hydroxycholesterol plus cholesterol (22 units/cell) and in FH homozygote cells incubated either in the absence (17 units/cell) or in the presence (14 units/cell) of these sterols is not known. This fluorescence was dependent upon incubation of the cells with r-[dioleyl fluorescein]LDL, but it was independent of the LDL receptor. It may represent transfer of small amounts of dioleyl fluorescein from the surface of LDL to the cell membrane. This low level of nonspecific fluorescence corresponded to a slight diffuse fluorescent glow that was present when cells of all three genotypes were initially observed in the fluorescence microscope after incubation with r-[dioleyl fluorescein]LDL. In cells of all genotypes this glow faded within seconds after exposure to light, whereas the specific fluorescence that was concentrated in the perinuclear region in the normal cells was long-lived (Fig. 4E). Accordingly, in all experiments with r-[dioleyl fluorescein]LDL, the cells were exposed to a bright light prior to being passed through the cell sorter to reduce this nonspecific fluorescence. Such receptor-independent fluorescence was not observed microscopically when cells were incubated with r-[PMCA oleate]LDL.

Heptane-extracted LDL can be reconstituted with a wide variety of nonfluorescent as well as fluorescent hydrophobic molecules [6–8]. Table II lists compounds that have been successfully incorporated into LDL. These molecules fall into several broad classes: 1) lipids that contain esters of long-chain *cis*-unsaturated fatty acids, 2) lipids that contain polyisoprenoid groups, and 3) other lipids, such as cholesteryl ethyl ether, cholesteryl nitrogen mustard, and the aromatic diazo dye fat red 7B. In general, the amount of each hydrophobic molecule that can be incorporated into heptane-extracted LDL is 1–2 mg of neutral lipid per mg of LDL-protein, a value that is approximately equal to the mass of endogenous cholesteryl ester present in native LDL. Inasmuch as most of the lipids listed in Table II do not readily enter mammalian cells in culture, their use as molecular probes has hitherto been limited. The ability to incorporate these compounds into LDL now permits their delivery to cellular lysosomes through the LDL receptor pathway.

DISCUSSION

The results presented here demonstrate that fluorescent reconstituted LDL provides an easily detected visual probe that can be used to determine whether or not a cell in culture expresses LDL receptors. The use of fluorescent LDL should enhance the ease with

TABLE I. LDL Receptor Activity in Fibroblasts From Normal Subjects and Patients With Heterozygous and Homozygous FH: Quantitation with ^{125}I-LDL and r-[dioleyl fluorescein]LDL*

Clinical phenotype	Age	Sex	Prior treatment of cells	Metabolism of ^{125}I-LDL: Surface-bound ^{125}I-LDL	Metabolism of ^{125}I-LDL: Internalized ^{125}I-LDL	Metabolism of ^{125}I-LDL: Degraded ^{125}I-LDL	Average fluorescence intensity after incubation with r-[dioleyl fluorescein]LDL
				ng/mg protein			units/cell
Normal							
D.S.	Newborn	M	None	150 (140)[a]	920 (840)	3,600 (3,300)	89
D.S.	Newborn	M	25-Hydroxycholesterol + cholesterol	17	75	240	22
A.H.	70	M	None	200 (180)	640 (580)	2,300 (2,200)	65
FH Heterozygote[b]							
G.M.	39	M	None	61 (51)	270 (240)	1,100 (1,000)	34
M.M.	31	F	None	86 (76)	310 (280)	1,200 (1,100)	43
FH Homozygote							
M.C.	8	F	None	5 (1)	21 (16)	37 (33)	17
M.C.	8	F	25-Hydroxycholesterol + cholesterol	4	17	19	14

*Cells were seeded (day 0) in 60-mm Petri dishes at a concentration of 7.5×10^4 cells per dish in growth medium containing 10% fetal calf serum [13]. On day 4 fresh medium containing 10% fetal calf serum was added. On day 6 the medium was replaced with 2 ml of fresh medium containing 10% human lipoprotein-deficient serum and either 2 μl of ethanol or a mixture of 2 μg of 25-hydroxycholesterol plus 24 μg of cholesterol added in 2 μl of ethanol as indicated. On day 7 the dishes were divided into two groups. Cells in Group A were used for studies of ^{125}I-LDL metabolism and cells in Group B were used for fluorescence analysis. Group A: On day 8 each dish received 2 ml of growth medium containing 10% human lipoprotein-deficient serum and 10 μg protein/ml of ^{125}I-LDL (98 cpm/ng protein) in the absence and presence of 400 μg protein/ml of unlabeled LDL. After incubation for 5 h at 37°C the total amounts of surface-bound ^{125}I-LDL, internalized ^{125}I-LDL, and degraded ^{125}I-LDL were measured as described in Materials and Methods. The high affinity values for these processes, which are shown in parentheses, represent the difference between the values observed in the absence and presence of unlabeled LDL. Group B: On day 7 each dish received 2 ml of fresh medium containing 10% human lipoprotein-deficient serum, either ethanol or the mixture of 25-hydroxycholesterol and cholesterol in ethanol as indicated, and 10 μg protein/ml of r-[dioleyl fluorescein]LDL. On day 8, after incubation with r-[dioleyl fluorescein]LDL for 18 h at 37°C, 10,000 cells from each dish were analyzed in the fluorescence-activated cell sorter as described in Materials and Methods.

[a]The values in parenthesis represent high affinity values.

[b]These FH heterozygotes are obligate gene carriers in that each is the parent of an FH homozygote.

which cells are screened for mutations in the LDL uptake pathway, including mutations in patients with FH as well as mutations that are created through in vitro mutagenesis in cultured cells. The ability to separate populations of normal, heterozygous, and homozygous LDL receptor-deficient cells with the fluorescence-activated cell sorter should also facilitate further studies of the somatic cell genetics of the LDL receptor pathway. For example, it should now be possible to mutagenize normal cells in culture and to isolate those that have developed a mutation in a single gene for the LDL receptor. Although one cycle of cell sorting would not be sufficient to separate completely these heterozygous cells from normal cells, it should be possible to subject the mutagenized cells to repeated cycles of growth, incubation with fluorescent LDL, and cell sorting. With each sequential sorting, the percentage of cells that are truly heterozygous should increase progressively. After a clone of cells that is heterozygous for a mutation at the LDL receptor locus has been obtained, the cells could be mutagenized again, and cells homozygous for receptor defects could be selected.

TABLE II. Hydrophobic Molecules That Have Been Incorporated Into Heptane-Extracted LDL to Yield Reconstituted LDL

Lipids containing long-chain *cis*-unsaturated fatty acyl groups and their derivatives
Cholesteryl esters (monounsaturated fatty acids with chain lengths of 14, 16, 18, 20, 22, and 24 carbon atoms; diunsaturated and triunsaturated fatty acids with chain lengths of 18 carbon atoms; tetraunsaturated fatty acid with chain length of 20 carbon atoms)
Triacylglycerols (triolein; trilinolein; dioleyl monostearyl glycerate)
Methyl esters (monounsaturated fatty acids with chain lengths of 14, 16, 18, 20, 22, and 24 carbon atoms; diunsaturated and triunsaturated fatty acids with chain length of 18 carbon atoms)
Linoleyl alcohol
19-Iodocholesteryl oleate
4-Methyl umbelliferyl oleate
25-Hydroxycholesteryl oleate
Dioleyl methotrexate
Dioleyl fluorescein
PMCA oleate
Lipids containing polyisoprenoid groups
Vitamin A (retinol)
Vitamin A palmitate (retinyl palmitate)
Vitamin E acetate (α-tocopheryl acetate)
Vitamin K_1
Coenzyme Q_{10} (ubiquinone-10)
β-Carotene
Chlorophyll (a + b)
Other lipids
Cholestene
Cholestane
Cholestan-3-one
Cholesteryl nitrogen mustard (phenesterine)
Cholesteryl ethyl ether
Fat red 7B

A second area of importance involves the use of LDL as a vehicle to deliver hydrophobic molecules of biologic interest to cells that specifically possess LDL receptors. On the basis of this study and other studies previously reported [6–8], it is clear that hydrophobic molecules other than cholesteryl esters can be introduced into LDL. In principle, any molecule (eg, fluorescein) can be incorporated into LDL provided that it can be modified so that it is sufficiently apolar and can be esterified to either an unsaturated long-chain fatty acid (eg, dioleyl fluorescein) or to a polyisoprenoid compound such as phytol (eg, chlorophyll). When taken up by cells in the form of reconstituted LDL, such hydrophobic molecules will be delivered primarily to lysosomes [1, 4, 19, 20]. Some of these probes might prove useful for studying receptor-mediated endocytosis as it relates to lysosome function.

The use of reconstituted LDL as a vehicle for the delivery of a variety of hydrophobic drugs or probes to cells adds a new dimension to the concept of receptor-mediated pharmacotherapy discussed by Neville and Chang [21]. These investigators have suggested that one can devise new approaches to drug delivery by constructing hybrid molecules in which the binding chain of one protein (such as the receptor recognition subunit of a polypeptide hormone) is covalently coupled to the active chain of a different protein that will damage cells as it enters the cytoplasm (such as the toxic subunit of a plant or bacterial toxin) [21]. Indeed, such a disulfide-linked hybrid protein (β-s-s-A) containing the cell-specific β-subunit of human chorionic gonadotropin and the toxic A subunit of ricin has recently been synthesized [22]. Studies in rat Leydig cells have shown that in order for the A subunit of ricin to inhibit protein synthesis it must first bind to cells through the chorionic gonadotropin receptors that are specific for the β subunit of the hybrid molecule. Cells that lack receptors for chorionic gonadotropin, such as mouse L cells, are resistant to the toxic effect of the hybrid molecule [22].

The unique aspect of the LDL reconstitution technique for drug delivery lies in the fact that as many as 1,000 molecules of a hydrophobic compound can be incorporated into a single LDL particle and targeted to cellular lysosomes. One limitation of this approach is that, in order to achieve the desired biologic effects, the drug incorporated into LDL must be able to survive the acidic environment of the lysosome and the action of its multiple hydrolytic enzymes. The types of biologically active molecules that can survive this lysosomal exposure must now be determined.

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