Chinese Hamster Ovary Cells Expressing a Cell Surface-anchored Form of Hepatic Lipase

CHARACTERIZATION OF LOW DENSITY LIPOPROTEIN AND CHYLOMICRON REMNANT UPTAKE AND SELECTIVE UPTAKE OF HIGH DENSITY LIPOPROTEIN-CHOLESTERYL ESTER*

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The enzyme hepatic lipase may play several roles in lipoprotein metabolism. Recent investigation has suggested a role for the enzyme in lipoprotein and/or lipoprotein lipid uptake. To study this, a simple isolated system that mimics the in vivo system would be desirable. The enzyme is secreted by the hepatic parenchymal cell but exists, and presumably exerts its effects, while bound to capillary endothelial cells in the liver, adrenal gland, and the ovary. We constructed a cell that encodes the expression of a chimeric protein composed of rat hepatic lipase and the signal sequence for the addition of the glycosphatidylinositol (GPI) anchor from human decay-accelerating factor. When transfected into Chinese hamster ovary (CHO) cells this gave rise to a cell population that had immunoreactive hepatic lipase on the cell surface. Cloning of the transfected cells produced several cell lines that expressed the chimeric protein bound to the cell surface by a GPI anchor. This was documented by demonstrating incorporation of [3H]ethanolamine into anti-hepatic lipase immunoprecipitable material; in addition, hepatic lipase was released from the cells by phosphatidylinositol-specific phospholipase C but not by heparin. Phosphatidylinositol-phospholipase C treatment of cells expressing the anchored lipase released material that comigrated with hepatic lipase on SDS-polyacrylamide gel electrophoresis and was immunoreactive with antibody to the cross-reacting determinant of GPI anchors. Cell lysates containing the anchored protein contained salt-resistant lipase activity, a known feature of the secreted hepatic lipase; thus it appears that these cells have a surface-anchored hepatic lipase molecule. Although it was not possible to demonstrate lipolysis by the enzyme while it was on the cell surface for technical reasons, the protein produced by these cells was active when studied in cell membranes. The ability of the cells to take up lipoproteins was studied. The cells demonstrated an increased affinity for low density lipoprotein (LDL) receptor mediated uptake of LDL. They did not, however, demonstrate any enhanced binding or removal of chylomicron remnants. With respect to LDL and remnants, the cells expressing anchored lipase behaved similarly to CHO cell that expressed secreted hepatic lipase. The cells expressing anchored hepatic lipase had a marked increase in the uptake of high density lipoprotein and high density lipoprotein cholesteryl ester when compared to that seen with CHO cells secreting hepatic lipase. This increase occurred primarily via the selective pathway, and was not reduced by addition of anti-LDL receptor or anti-hepatic lipase antibodies or the receptor-associated protein. Together the results suggest that hepatic lipase, when bound to the cell surface by a GPI anchor, plays a role in enhancing lipoprotein uptake. For LDL this may involve the provision of a second foot for particle binding, thus enhancing affinity for the LDL receptor. For chylomicron remnants an additional molecule or molecules are necessary to mediate this effect. For HDL, the enzyme facilitates uptake of cholesteryl ester primarily by the selective pathway.

The physiological role of hepatic lipase has never been fully elucidated. Until recently, most speculation focused upon its role in the metabolism of circulating lipoproteins and specifically on its ability to convert intermediate density lipoprotein to low density lipoprotein (LDL) and high density lipoprotein (HDL$_2$) to HDL$_3$. Hepatic lipase is synthesized and secreted by liver parenchymal cells, and in most species the enzyme binds to the capillary endothelial cell lining the liver, adrenal gland, and the ovaries (1). These organs, the major sites of utilization of exogenous sterol, are presumably the physiological sites of enzymatic function. This tissue localization suggests that the enzyme may play a role in providing sterols to these tissues.

Several groups have recently begun to explore a possible role for hepatic lipase in cellular lipoprotein metabolism, and in particular on the ability of the enzyme to facilitate lipoprotein removal and lipid uptake by the tissues where it is localized. Work by Borensztajn and colleagues (2) suggests that the phospholipase activity of the enzyme may be involved in chylomicron metabolism; furthermore, hepatic lipase may be involved in chylomicron remnant uptake, since it has been shown that,

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† Deceased March 25, 1996. His contributions will be missed by all in the lipid and lipoprotein field of research.

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in humans, congenital absence of hepatic lipase activity results in the accumulation of remnant-like particles in the circulation (3, 4). Anti-hepatic lipase antibodies have been reported (5, 6) to delay chylomicron remnant removal, as well as to inhibit hepatic remnant uptake, in the rat (7) and mouse (6). It has also been reported (8, 9) that hepatic lipase can stimulate the delivery of cholesterol from HDL to hepatoma cells and to the perfused liver. This suggests roles for the enzyme in cholesterol transport that is consonant with its localization in tissues that use large quantities of cholesterol.

The mechanism for lipase binding to endothelial cells is not known with certainty. It is likely that hepatic lipase is bound to the surface of the cells by proteoglycans. Hepatic lipase is released into the circulation following the injection of heparin, as has been observed with lipoprotein lipase, a related enzyme whose binding to cell surfaces is mediated by glycosaminoglycans. In the latter instance the binding involves heparan sulfate, or at least a heparinase-sensitive linkage. Because of the complexity of the in vivo system, in which hepatic lipase is synthesized by one cell type and bound to a second cell type, it has been difficult to construct a cell culture model system with which to study the role of the enzyme in lipid transport.

In the present report such a system is described. In order to allow the study of the effects of hepatic lipase on the transport of various lipids and lipoproteins, we have prepared a relatively undifferentiated cell line that has hepatic lipase bound to its surface. Rat hepatic lipase cDNA was modified to contain the human decay-accelerating factor signal sequence for the addition of a glycosphatidylinositol (GPI) anchor; thus, expression of the construct in eukaryotic cells results in the addition of a GPI anchor to the COOH terminus of hepatic lipase. The resulting cell line to remove LDL, chylomicron remnants, and HDL was studied.

**EXPERIMENTAL PROCEDURES**

**Materials**

All reagents for cell growth, lipase purification, and lipase assay were as described previously (10). Oligonucleotides were synthesized by the Oligonucleotide Synthesis Core of the Stanford Digestive Diseases Center. Fluorescein isothiocyanate-conjugated goat anti-rabbit F(ab')2, nonspecific rabbit IgG, and heparin (sodium salt, from porcine intestinal mucosa, 169.1 units/mg) were purchased from Sigma. Phosphatidylinositol-specific phospholipase C (PI-PLC) was a generous gift of Dr. Mark Englund (Columbia University). Antibody to the COOH-terminal (CRD) was a generous gift of Dr. Paul Englund (Johns Hopkins).

**Methods**

Construction of Clones and Expression in CHO Cells—Rat hepatic lipase cDNA under the control of the metallothionein promoter (11) was as modified as outlined in Fig. 1. Cloning sites were inserted using site-directed mutagenesis (12), followed by the insertion of the signal sequence for the addition of a GPI anchor. The latter was from human decay-accelerating factor (DAF), and has been thoroughly characterized (13, 14). The signal sequence was amplified by polymerase chain reaction (PCR) using primers that incorporated the desired restriction sites at the ends, and fused to the modified hepatic lipase cDNA. The resulting construct was cloned into the metallothionein-promoter vector pMTSV40pAdy(A/B)Bam, which we have used previously to express se- creted rat hepatic lipase in CHO cells (10), to yield the expression construct D206E621-2. CHO cells were co-transformed with D206E621-2 and pSV2neo (15) by CaPO4 precipitation, cells were selected in 500 μg/ml G418, and single cell isolates were dosed as described (10).

Fluorescence-activated Cdl Sorting (FACS)—FACS analysis was done at the Stanford Digestive Diseases Core Center FACS Facility. Cells were grown and induced overnight by incubation in medium containing 30 μM ZnSO4, as described previously (10). Following removal of induction medium and washing with phosphate-buffered sa-

line (PBS), the cells were rinsed twice with PBS containing 5 mM EDTA and incubated at 25°C for 10 min. The cells were resuspended in FACS buffer (PBS containing 2.5% (v/v) fetal bovine serum plus 0.1% (w/v) sodium azide) at 107 cells/ml and incubated with primary antibody or control immunoglobulins at 2 μg/ml for 30 min at 4°C. Cells were then washed as described above. Following pelleting, cells were resuspended in PBS containing 1% (w/v) parafomaldehyde at 4°C and stored in the dark at 4°C until analysis within the next 24 h. Analysis was performed on a Becton Dickinson FACSscan flow cytometer, and measurements and analysis were done essentially as described (16).

**Cdl Labeling and Analysis—**Cells were grown in 6-well plates (Costar) until almost confluent and treated with induction medium containing 30 μM ZnSO4 for 8 h. The medium was replaced with 1 ml/well induction medium containing 100 μCi of [1-13C]ethanolamine (Amersham, 25 Ci/mmol) and incubated at 37°C for 16 h. Cells lysates were immunoprecipitated using anti-hepatic lipase antibody and StaphA in a two-cycle procedure to nonspecific binding by dialysing the immunoprecipitate into the initial immune complex (17), and the immunoprecipitates were run on 8% SDS-PAGE. Gels were treated for fluorography using sodium salicylate as described (18) and exposed to prefixed Kodak XAR-5 X-ray film at −80°C.

Heparin Release—Cells were grown in 6-well plates as above, induced for 24 h, and washed twice with Coon's F-12:Dulbecco's modified Eagle's medium (1:1). Two ml of induction medium containing 20 units/ml heparin was added to each well and the plates were incubated for 20 min at 4°C with gentle shaking. The supernatant (heparin released material) were removed, run on SDS-PAGE, transferred to nitrocellulose membranes, and developed with anti-hepatic lipase followed by alkaline phosphatase-conjugated goat anti-rabbit IgG (Protobind IgG, Hybridoma, Madison, Wisconsin) as described (10).

Treatment of Cells with PI-PLC—Cells were grown, induced, washed, and removed with EDTA as described for FACS analysis above. Cells were pelleted, washed with 1 ml of PBS, repelleted, and resuspended in 200 μl of PBS plus 10% fetal bovine serum. PI-PLC was added to a final concentration of 0.5 units/ml, the mixture was incubated at 37°C for 1 h and the cells were pelleted by centrifugation.

**Preparation of Lipoproteins—Rat mesenteric lymph chylomicrons and chylomicron remnants were prepared in vivo by the modification of the method of Redgrave and Martin (19) previously described (20). Human LDL (d 1.019–1.063 g/ml) and high density lipoprotein (HDL) (d 1.063–1.210 g/ml) were isolated from EDTA-containing plasma by sequential ultracentrifugation. The chylomicron remnants and LDL were isolated by a modification (21) of the iodide monochloride method. The distribution of radioactivity between lipid and protein was monitored on all batches as described previously (22) and fell within the range reported (22). Total protein concentration of lipoproteins was determined by the BCA procedure.

Triglyceride and cholesterol contents were determined using test kits from Sigma, Antibodies and Inhibitors. A plasmid containing the cDNA for human receptor-associated protein fused with glutathione S-transferase in Escherichia coli was kindly provided by Dr. D. Strickland (23). The protein was purified in our laboratory as described earlier (24). The anti-rat LDL receptor antibodies used have been previously described and characterized (25). Antiserum to rat hepatic lipase fusion protein (11) was prepared in New Zealand White rabbits. The standard procedures utilizing 100 μg of hepatic lipase fusion protein for initial and subsequent injections. Immunoglobulins (IgG) were isolated by use of protein A-agarose (Bio-Rad). Nonimmune IgG were isolated from normal rabbit serum using the same procedure.

Degradation and Binding Assays of Lipoproteins by CHO Cells—Binding assays were carried out as described previously (26). Control and transfected CHO cells were cultured in Dulbecco's modified Eagle's medium:Coon's F-12 (1:1) supplemented with 10% fetal calf serum containing 30 μM ZnSO4 overnight to induce hepatic lipase. Non-adherent cells were removed by rinsing 3 times with binding buffer containing 30 μM ZnSO4, 0.5% bovine serum albumin, and 10 μM HEPES (pH 7.4). 1–13C Labeled LDL (10 μg/ml) or chylomicron remnants (1 μg/ml) were added to the medium in the presence or absence of unlabeled lipoproteins or
anti-LDL receptor antibody at 37°C for 4 h. The extent of lipoprotein degradation was assessed by measuring the amount of trichloroacetic acid and silver nitrate soluble radioactive present in the incubation medium. The small amount of degradation products generated in the absence of cells was also measured and subtracted from the corresponding samples incubated with cells. The amount of lipoproteins associated to the cells was determined by dissolving the cells with 0.1 N NaOH after washing the cells three times with PBS. The amount of total cell protein was determined by the method of Lowry et al. (27).

HDL Binding and Uptake—Uptake of HDL cholesterol was determined essentially as described (28). Briefly, cells were grown and induced as above, and medium from 24-h induced cells was replaced with fresh induction medium containing HDL (1.065–1.21 g/ml), which had been radiolabeled with non-releasable apoprotein (28, 29) and cholesteryl ester tags (cholesteryl deoxy ether (COE)) that would accumulate within the cells even when degraded (29). Incubations were carried out with 125 μg of protein/ml of 125I-dilactitolethanolamine (125I) labeled ethanol HDL (I–III-DLT-(125I)COE-HDLe for 5 h at 37°C. At the end of the incubation, the cells were washed four times with PBS, 0.1% bovine serum albumin, once with PBS and subsequently solubilized in 2 ml of 0.1 N NaOH. One-ml aliquots were precipitated with an equal volume of 20% (w/v) trichloroacetic acid to determine acid insoluble and soluble radioactivities or extracted with organic solvents (28, 29) to determine 3H radioactivity.

Under the conditions used, trichloroacetic acid-insoluble 125I radioactivity was assumed to represent 125I-labeled protein remaining bound to the cell surface as part of intact lipoproteins (28, 29); trichloroacetic acid-soluble 125I radioactivity was taken to be internalized, degraded, and accumulated residualizing protein 125I label. Since the 125I and 3H labels are on the same lipoprotein particles, it follows that the relative amounts of surface-bound 125I and 3H radioactivity must be equal. Thus, the amount of cholesteryl ester selectively internalized can be computed as the difference between total cholesteryl ester uptake and trichloroacetic acid-insoluble (i.e. surface-bound) 125I. Likewise, the amount of cholesteryl ester internalized via the selective pathway was calculated as the difference between total cholesteryl ester internalized and cholesteryl ester internalized through the endocytic pathway. The results are expressed as nanograms of cholesteryl ester internalized per milligram of cellular protein.

Assay of Lipolytic Activity—Total cell lysates were prepared for assay of lipolytic activity. Total cell lysates were prepared by the method of Tavanger et al. (30), and samples were assayed as described previously in the presence of 1 M NaCl to suppress lipoprotein lipase activity (1).

Miscellaneous Methods—SDS-polyacrylamide gels and Western blots were done as described previously (10).

RESULTS

Preparation of CdsLs That Have Hepatic Lipase Anchored to the Cell Surface—A DNA construct (Fig. 1) was prepared, as described under “Experimental Procedures,” in which the stop codon in the hepatic lipase cDNA was mutated to encode a BglII site, and a downstream sequence was mutated to a MluI site. The sequence that signals the addition of a GPI anchor to the COOH terminus of rat hepatic lipase, despite the presence of substantially more protein A were added and the immunoprecipitates were subjected to SDS-PAGE and blotting, membranes were probed with anti-hepatic lipase antibodies to determine if heparin depleted the amount of hepatic lipase on the membranes. The results (not shown) demonstrate that there is no heparin-releasable material bound to the cell surfaces.

In order to determine if a radiolabeled precursor to GPI anchors could be incorporated into a hepatic lipase-bound form, cells were labeled with [1–3H]lithocholamine as described under “Experimental Procedures.” Following solubilization of washed cells, anti-hepatic lipase antibody and Staphylococcus aureus-protein A were added and the immunoprecipitates were subjected to SDS-PAGE and autoradiography. Only the cloned cells that had been transfected with the hepatic lipase-DAF chimera contained labeled immunoprecipitable material (Fig. 4). This material had an electrophoretic mobility similar to hepatic lipase. Neither untransfected CHO cells nor CHO cells that expressed secreted recombinant hepatic lipase were recognized by the antibody, while the cell pools that had been transfected with the GPI construct contained a subpopulation that bound to the antibody. Cells from this subpopulation were cloned by limiting dilution as described previously (10). Northern blot analysis of several clones, using a probe for hepatic lipase, showed that the cloned cell lines contained a single RNA band that hybridized with hepatic lipase cDNA and was of the size expected for the chimera (Fig. 3). Quantitation, by enzyme-linked immunosorbent assay using anti-hepatic lipase antibody, of hepatic lipase mass in various cell lines demonstrated that one of the anchored cell lines (that was used in Fig. 3) contained approximately 3.7 times as much cellular hepatic lipase mass/mg of total cell protein, as did the cells secreting hepatic lipase, despite the presence of substantially more lipoprotein-specific mRNA in the latter cells (data not shown). This suggests that the lipase being produced in the chimera-transfected cells is being retained by the cells, presumably by membrane anchor binding.

Characterization of the Anchor—A number of experiments were carried out to establish that the hepatic lipase was anchored to the cells by a GPI anchor. Cells were grown, induced, and washed, and bound lipase was released with heparin as described under “Experimental Procedures.” Following SDS-PAGE and blotting, membranes were probed with anti-hepatic lipase antibodies to determine if heparin depleted the amount of hepatic lipase on the membranes. The results (not shown) demonstrate that there is no heparin-releasable material bound to the cell surfaces.

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Anchored Hepatic Lipase and Uptake of LDL, HDL, and Chylomicron Remnants

**Fig. 2. FACS analysis of pools of transfected cells.** Cells were incubated with antibodies as noted below and run on the FACS. Total number of events per sample was 50,000. Pools were transformed with the following DNAs plus pSV2neo: Sample 1, HL/DAF GPI-anchored orientation pool; Sample 2, HL/DAF GPI-anchored orientation pool; Sample 3, vector alone pool. Sample 4 contained untransformed CHO cells; Sample 5 contained CHO cells secreting recombinant rat hepatic lipase. Cells were incubated with two different primary antibodies: either Fab from rabbit anti-rat HL or Fab from preimmune IgG. The second antibody in each case was fluorescein isothiocyanate-conjugated goat anti-rabbit Fab. Only the results from the incubation with immune antiserum is shown; none of the incubation with preimmune serum showed a shift in intensity. The vertical axis is the number of cells, while the horizontal axis is the relative fluorescence. Integration of the shifted peak in the sense-orientation pool showed that 16.2% of the total population in Pool 1 was shifted. The vertical line in each panel is an arbitrary event marker.

**Fig. 3. Northern blot analysis of transformed cell lines.** Cells were grown and induced, and total RNA was prepared, electrophoresed, and blotted as described under “Experimental Procedures.” The blot was probed with cDNAs for rat hepatic lipase and for rat glyceraldehyde-3-phosphate dehydrogenase (G3PDH). Lane 1 is total rat liver RNA; lane 2 is total RNA from CHO cells producing secreted rat hepatic lipase; lane 3 is total RNA from a cloned cell line producing GPI-anchored hepatic lipase; and lane 4 is total RNA from untransformed CHO cells.

**Fig. 4. Incorporation of a precursor to GPI membrane anchors into hepatic lipase by CHO cells.** Cells were grown to confluence in 6-well dishes and induced with ZnSO\(_4\) (30 \(\mu\)M) as described (10) for 8 h. The induction medium was removed, 100 \(\mu\)Ci of \(^{3}H\)lathionamide in 1 ml of fresh induction medium was added to each well. Cells were incubated at 37°C for 16 h, washed, lysed, and immunoprecipitated as described in the text. Samples were run on SDS-PAGE, soaked in salicylate for fluorography as described (12), dried, and exposed for 172 h. The sample in lane 1 is from untransformed CHO cells, the samples in lanes 2, 3, 4, and 5 are from cloned cell lines expressing anchored hepatic lipase; sample 6 is from cells expressing secreted hepatic lipase. The arrow indicates the electrophoretic mobility of secreted hepatic lipase as determined separately by Western blotting.

**Fig. 5. Release of hepatic lipase from CHO cells expressing chimeric hepatic lipase by PI-PLC.** Cells were grown and induced for 24 h as described under “Experimental Procedures.” Following induction, cells were washed twice with cold PBS, twice with PBS containing 5 mM EDTA, incubated at room temperature for 5 min, and removed from the plate by pipetting. Cells were pelleted and resuspended in 200 \(\mu\)l of PBS containing 10% fetal bovine serum. PI-PLC, 0.1 unit (a generous gift of Dr. M. Low), was added to each and samples were incubated at 37°C for 60 min. Cells were pelleted and supernatant samples were run on SDS-PAGE and blotted. Following incubation with primary antibodies, the transferred proteins were visualized using goat anti-rabbit, alkaline phosphatase-conjugated second antibody. The samples in lanes 1 and 2 are from, respectively, untransformed CHO cells and CHO cells secreting hepatic lipase, the samples in lanes 3 are from a cloned cell line expressing anchored hepatic lipase, and the samples in lanes 4 are partially purified recombinant secreted rat hepatic lipase. Molecular weight marker sizes are shown on the left (arrows). A, Blot developed with rabbit anti-rat hepatic lipase IgG; B, blot developed with rabbit anti-CRD antibody.

Direct immunological evidence for the presence of the GPI anchor was also obtained. The CRD is an epitope that is common to most GPI-anchored proteins (31). It is cryptic in the membrane-bound protein and is exposed when the protein is released by PI-PLC. The epitope, in mammalian GPI-linked proteins, is the inositol 1,2-cyclic phosphate that is formed by hydrolysis of the anchor, and remains bound to the protein following release. The presence of the CRD in a released protein is direct evidence for a GPI anchor (31). Cells were treated with PI-PLC and lysates were electrophoresed and blotted as above but developed with an antibody to the CRD. In these cells the major band in the released material that reacted with the anti-CRD antibody had the same molecular weight as hepatic lipase (Fig. 5B).

An experiment identical to that of Fig. 5 was carried out except that heparin was used rather than PI-PLC. In this experiment none of the cells transfected with the hepatic...
lipase-DAF chimera released any immunoreactive hepatic lipase (not shown).

Localization and Reactivity of the Hepatic Lipase—In order to further demonstrate the cellular localization of the chimeric lipase, cells were disrupted and plasma membranes were prepared. After SDS-PAGE and transfer to nitrocellulose, membranes from cells transfected with the chimera, cells transfected with the secreted form of the enzyme, and control cells were probed with anti-hepatic lipase antibody. Only the membranes from the cells transfected with the chimera had immunodetectable hepatic lipase (Fig. 6). Total cell lysates from the three cell types described above were assayed for hepatic lipase activity utilizing a triolein emulsion as substrate. There was detectable salt-resistant lipolytic activity in the cells transfected with the cDNA for the chimera as well as in the cell line producing secreted hepatic lipase: 13.4 ± 0.04 and 12.1 ± 0.9 pmol of triolein hydrolyzed per min/mg of protein (± S.E.) for secreted and anchored, respectively. This activity was inhibited to 0.5 ± 0.2 and 0.8 ± 0.3 pmol of triolein hydrolyzed per min/mg of protein (± S.E.) for the two cell types in the presence of anti-hepatic lipase antibody. There was no detectable salt-resistant lipolytic activity in untransfected CHO cells (not shown). There was some nonspecific lipase activity and this was subtracted from the total lipolytic activity. It was not possible to analyze for hepatic lipase lipolytic activity using whole cell lysates largely because of secretion of lipoprotein lipase-like activity by all of the lines of CHO cells. Inhibition of this with high salt lysed the cells. Thus, it is not proven that the hepatic lipase anchored to the cell has lipolytic activity, although it is clear that the chimeric form is active at least in broken cell and membrane preparations.

The results of the above experiments demonstrate that CHO cells containing a cDNA encoding a chimera of hepatic lipase and the decay-accelerating factor signal sequence for GPI anchor express a protein that is anchored to the plasma membrane by a GPI anchor and has the immunological and enzymatic properties of hepatic lipase.

Effect of the Anchored Hepatic Lipase Chimera on the Uptake of LDL—In previous experiments we (26) and Aviram et al. (32) established that the presence of hepatic lipase accelerates the uptake of LDL by cells. In our experimental system using cells that secreted rat hepatic lipase, the effect of the lipase was to enhance the affinity of the particle for the LDL receptor. To learn if the cell surface-anchored hepatic lipase chimera retained this property the cell association and degradation of $^{125}$I-LDL were studied. Compared to untransfected cells, at LDL concentrations (10 μg/ml) below the K$_{d}$, the cell association (Fig. 7A) and degradation (Fig. 7B) of the lipoprotein were doubled. The degree of enhancement was at least as large as that observed with cells that secrete hepatic lipase (Fig. 7). In general, there was at least a 50% increase in cell association and a doubling of degradation. As with the secreting cells all of the enhancement of uptake could be attributed to LDL receptor-mediated uptake, demonstrated by the fact that a monospecific anti-LDL receptor antibody was as effective as unlabeled LDL in displacing cell association and degradation (Fig. 8A). Interestingly, the anti-hepatic lipase antibody used in these studies did not affect LDL cell association or degradation (Fig. 8B), even though this antibody inhibits lipolysis catalyzed by the enzyme as documented in the previous section and in a previous publication (6).

Effect of the Anchored Hepatic Lipase Chimera on the Uptake of Chylomicron Remnants—Experiments similar to those described above were carried out using $^{125}$I-chylomicron remnants rather than LDL. Neither the cells expressing secreted nor those with bound hepatic lipase exhibited altered chylom
The former result is consistent with our previous observation with hepatic lipase-secreting CHO cells (26) and in contrast with results obtained using transfected McA-RH7777 cells (33) using \( \beta \)-VLDL as a model for chylomicron remnants. These observations suggest that hepatic lipase alone, even if present on the cell surface, is not sufficient to accelerate chylomicron remnant uptake, at least in CHO cells.

The effect of Expression of Anchored Hepatic Lipase on Selective Uptake of HDL Cholesterol Esters—Evidence accumulated over the last decade (34–38) suggests that there is a mechanism that results in the uptake of cholesterol ester from HDL independent of the removal of the whole particle. This is referred to as selective uptake (36), and is measured by using HDL labeled in both the apolipoprotein and cholesteryl ester moieties. Selective uptake is calculated as the amount of cholesteryl ester that is removed in excess of that which can be accounted for by the removal of the whole particle as determined by the uptake of labeled apolipoproteins. The molecular mechanism for selective uptake remains obscure, although it has recently been reported that a member of the scavenger receptor family may play a role in this (39). Selective uptake of HDL cholesteryl ester was examined in cell lines that produce either secreted or membrane-anchored hepatic lipase. Secreted hepatic lipase reduced the amount of cholesteryl ester uptake that could be attributed to either the selective or whole HDL particle (endocytic) uptake pathway (Fig. 9). It is important to note that the magnitude of the selective pathway is at least 10-fold greater than the endocytic pathway in CHO cells. A striking contrast was seen using the cell lines that express the anchored form of hepatic lipase. Depending upon the cell line expressing the anchored hepatic lipase, there was a 25–67% increase in the selective uptake of HDL cholesteryl ester. In further experiments using the cell line that expresses the most anchored hepatic lipase, this increase was as great as 3-fold compared to non-transfected cells. The degree of increase correlated well with the amount of hepatic lipase expressed. There was also an increase in the amount of whole particle HDL uptake (i.e. via the endocytic pathway), although this remained a relatively small portion of the total cholesteryl ester delivered.

The Mechanism of the Increase in HDL Uptake in Cells Expressing Anchored Hepatic Lipase—In order to explore the possible involvement of other effectors in the increased selective uptake, anti-LDL receptor antibody and the receptor-associ- associated protein (40, 41) were used to inhibit the members of the LDL receptor family. In contrast to our results with anchored lipase-mediated uptake of LDL, these inhibitors had no effect on selective uptake of HDL cholesteryl ester (Fig. 10, A and B). This excludes a role for these proteins in the selective uptake of HDL cholesteryl esters, at least as mediated by hepatic lipase in these cells. The same was true for the HDL taken up by the whole particle pathway. In order to determine if particle binding to cell surface proteoglycans was involved in selective uptake, heparin was used to inhibit binding. Again, no effect on uptake was observed (Fig. 10C).

To learn if lipolytic activity of the enzyme was needed for enhanced uptake, the effect of the anti-hepatic lipase antibody was studied. This antibody did not affect either selective or whole particle uptake; thus, with the caveat that the antibody may not inhibit the enzyme on the cell surface, lipolysis may...
not be required for increased selective uptake (Fig. 10A). These data suggest that when hepatic lipase is present on the surface of a cell it facilitates the selective uptake of HDL cholesteryl esters by a mechanism that does not require hydrolysis of the particle.

**DISCUSSION**

The role of hepatic lipase in lipoprotein metabolism has been the subject of considerable speculation and study. Most of the focus has been on its roles in converting triglyceride-containing apoC-II-poor intermediate density lipoprotein to LDL, and in removing triglyceride from HDL₂ to convert it to HDL₃. In recent years, however, there has been considerable interest in a possible role for this enzyme, and the related enzyme lipoprotein lipase, in the uptake of lipoprotein by cells. In the case of hepatic lipase the localization of the enzyme to liver, adrenal, and ovary, tissues with high demand for sterols, has provided impetus for such speculation. It has been somewhat difficult to study this phenomenon because the enzyme is synthesized in hepatic parenchymal cells from which it is secreted, and then bound to endothelial cells. The liver is the only site of synthesis but the enzyme binds to endothelial cells that are remote from, as well as within, the liver. The objective of this investigation was to create a cell line in which the enzyme is linked to the surface of the cell that produced it. In principle, this should create a model system for studying the role of the enzyme on the cell surface under tissue culture conditions.

In order to accomplish this, the enzyme was linked to the human decay accelerating factor signal sequence for the addition of a GPI anchor. This approach has been used to anchor other molecules to cell surfaces (13). The GPI anchor was chosen instead of a protein transmembrane sequence for two reasons. First, the presence of the carbohydrate moiety allowed the bound protein to protrude from the cell surface in a manner analogous to binding to a proteoglycan, rather than to be in closer proximity to the cell surface. Second, the use of the GPI anchor required a minimal modification of the amino acids at the C terminus of the protein; thus, instead of terminating at Asp⁴⁷², the protein contains two additional amino acids, leucine and serine. The latter is presumably the attachment site for the GPI anchor. The addition or deletion of between one and five amino acids at the C-terminal does not have an effect on either the secretion or catalytic activity of rat hepatic lipase.² The use of a protein transmembrane anchor would have involved the addition of an entire membrane-spanning domain, and the effects on protein folding and catalytic activity would be difficult to predict. In addition, we and others have determined that the heparin-binding region of hepatic lipase resides, at least in part, in the C-terminal moiety of the enzyme; thus, anchoring via the C-terminal region appears to maximize the possibility that the enzyme is presented to the extracellular environment in as physiological a manner as possible.

It is not known whether hepatic lipase is active as a monomer or as a larger complex. Previous data have demonstrated that the molecular size of rat hepatic lipase, as determined by gel filtration in the presence of detergents, is between 180 and 200 kDa (42–44), as opposed to the monomeric molecular mass of 53 kDa (45). It has been postulated that lipoprotein lipase and pancreatic lipase, two closely-related enzymes (11), are active as dimers that are bound together in a head-to-tail conformation. It is likely, but not certain, that the use of a C-terminal anchor to tether the lipase to the cell surface abolishes head-to-tail dimer formation. We were able to demonstrate hepatic lipase-specific lipolytic activity in lysates from cells containing

² M. Komoromy, unpublished results.
the anchored construct; thus, if dimerization is prevented in our construct while it is bound to membranes, this implies that hepatic lipase activity does not depend on the formation of a head-to-tail dimer, although it does not exclude the possibility that this is the physiological configuration. These cells will be useful for determining if hepatic lipase functions more efficiently when dimerized in future studies.

The results from the LDL uptake studies conducted were comparable to those previously reported by our laboratories (26), using cells expressing a secreted form of the hepatic lipase. The presence of hepatic lipase on the cell surface accelerates the uptake of LDL. The increase in the uptake of LDL treated with soluble hepatic lipase was initially described by Aviram et al. (32). They and collaborators ascribed the observation to an alteration in the physical properties of the LDL induced by lipolysis. The previous and present studies from our laboratory do not support this mechanism. The anti-hepatic lipase antibody almost completely inhibits hepatic triglyceride lipase activity (see above) but did not abolish, or even attenuate, the effect on LDL uptake. It thus appears possible that catalytically inactive hepatic lipase is able to participate in the uptake of lipoproteins, and that the processes of lipolysis and uptake are not necessarily connected. Since we could not devise an assay for lipase activity with intact cells, it is possible, but unlikely, that the antibody does not inhibit the enzyme activity when it is on the cell surface. Consistent with the above hypothesis is the report (46) that the targeted inactivation of lipoprotein lipase, by site-directed mutagenesis of the active site serine, does not affect the uptake of β-VLDL, suggesting that this closely related enzyme does not require lipolytic activity in order to enhance lipoprotein uptake. The mechanism for hepatic lipase acceleration of lipoprotein uptake is now under intense investigation in several laboratories, and the reported and related cell lines will be useful for these studies.

Also consistent with our previous results was the finding that all of the uptake of LDL was via the LDL receptor, arguing against the possibility that a hepatic lipase-LDL complex is formed and removed by another receptor such as the LDL receptor-like protein (LRP). It thus appears that hepatic lipase enhances LDL uptake by increasing its affinity for the LDL receptor in some manner. The simplest mechanism to account for all of the data is that hepatic lipase has a binding site for LDL and that this, combined with the LDL receptor-binding site, results in multifooted binding. Such binding has a much higher affinity than single footed binding and has been documented to have a multiplicative effect on the $K_d$ of a ligand. This has been previously demonstrated for antibodies, where Fab$_b$ fragments have many fold higher affinities than Fab' fragments, as well as for lipoproteins, where multiple copies of apoE on a liposome markedly enhance its affinity for the LDL receptor (47). This explanation requires that we propose that, in the case of the hepatic lipase-secreting cells, some enzyme must be bound to the cell surface, perhaps to a proteoglycan. In our experiments, the CHO cells used did not re-bind immunologically detectable amounts of secreted hepatic lipase to the cell surface. It is thus possible that cell surface binding of hepatic lipase to CHO cells occurs after the lipase binds to the LDL. It has also been shown that hepatic lipase binds to the LRP, that this binding is dependent on cell-surface proteoglycans, and that the hepatic lipase thus bound is internalized and degraded (48). It is not known if this process is related to lipoprotein uptake or if it instead functions as a breakdown pathway for hepatic lipase. Recent studies (49) argue against the latter; since the LRP does not seem to be important in lipoprotein lipase degradation by CHO cells. This is consistent with our observation that the lipase-stimulated LDL uptake is mediated via the LDL receptor, but does not exclude the possibility that an LDL-hepatic lipase complex uses the LRP for one foot of binding and the LDL receptor for a second foot and for uptake. The nature of the binding site for hepatic lipase on LDL is currently under investigation.

An explanation of the results with chylomicron remnants requires further consideration. A possible role for hepatic lipase in the hepatic uptake of chylomicron remnants has been documented by other groups and by our laboratories. In a liver-derived cell line McA-RH7777 cells, the secretion of hepatic lipase increased apoE-rich β-VLDL binding (33) and to a considerably lesser degree chylomicron remnant binding (33). This did not appear to be the result of increased affinity for the LDL receptor, suggesting that the already very high affinity of apoE-rich lipoproteins for the LDL receptor was not further enhanced by binding to hepatic lipase. It was thus possible that hepatic lipase itself was capable of becoming a binding site. Although this was our working hypothesis, it is not supported by our observations using cells expressing the anchored lipase. We instead postulate that the formation of a complex involving hepatic lipase and other molecules is necessary to reproduce the effect of hepatic lipase in McA-RH7777 cells, and that one or more of these interacting molecules is missing or is expressed in insufficient quantities in CHO cells. The LRPN would not appear to be one of these ancillary molecules, since CHO cells express a level of the LRPN that is adequate to mediate the rapid uptake of activated α2-macroglobulin (26), although it may be that the anchor prevents an interaction between hepatic lipase and the LRPN. It is more likely that the complex involves specific heparran sulfate glycosaminoglycans, as postulated by Ji et al. (50). CHO cells are not derived from a cell lineage that are known to bind hepatic lipase, while McA-RH7777 cells are relatively differentiated hepatoma cells, and are thus more likely to express the appropriate glycosaminoglycan(s). The limited and specific tissue distribution of hepatic lipase (51–53) supports the contention that the binding site is not expressed in a wide variety of cell types, as does the finding that the major heparran sulfate proteoglycan in liver is also expressed in a variety of tissues (54), suggesting that the lipase-binding, and perhaps chylomicron remnant-binding, proteoglycan is a limited subset of the family.

One other possible explanation for the difference in the results reported here and those observed by Ji et al. (50) could be in the particles used. Those authors used β-VLDL, while we used chylomicron remnants. It is possible that the remnants might have already undergone sufficient lipolysis, and that additional activity of hepatic lipase does not result in the further production of lysophospholipid, which, as suggested by Borensztajn et al. (2), might be needed for remnant uptake. The β-VLDL might, however, be subject to further phospholipolysis, and thus the effect of the lipase would be more apparent if lysophospholipid is required for the effect. The chimera-expressing cell line should prove useful for elucidating the precise molecular requirements for this process.

Perhaps the most significant finding in this study was the markedly increased rate of selective uptake of HDL cholesteryl esters in the cell lines expressing the anchored hepatic lipase. Our understanding of the mechanism of the cellular removal of HDL and is constituents is incomplete, and the mechanism whereby HDL and its components enter cells has been the subject of considerable debate. A substantial body of recent evidence using a number of mammalian tissues and cultured cells (34, 37, 55–59) and in organ perfusion systems (60–62), suggests that there is a mechanism that results in the uptake of cholesteryl ester from HDL independent of the removal of the whole particle. This is referred to as selective uptake, and
is measured by using HDL labeled in both the apoprotein and cholesteryl ester moieties and computing the amount of cholesteryl ester that is removed in excess of that which can be accounted for by the removal of the whole particle as determined by the uptake of labeled apolipoproteins. The molecular basis for selective uptake has been difficult to delineate. The recent discovery (39) that a member of the scavenger type B receptor family, SR-B1, is abundant in liver, adrenal, and ovary and can facilitate this process when it is translocated into CHO cells, begins to provide a molecular basis for this process. The present observation that the expression of cell surface hepatic lipase increases this process provides further details concerning this pathway. The SR-B1 receptor appears to be most highly expressed in organs that have high rates of sterol synthesis and that depend on exogenous cholesterol for sterol production, the adrenal gland, ovary, and liver; interestingly, these are the same organs where hepatic lipase is located, presumably on the surfaces of the capillary endothelial cells (52, 63–65). This suggests that the observations of the present experiments are likely to have physiological relevance. Whether there is an interaction between hepatic lipase and SR-B1 remains to be elucidated.

Based upon the present experiments, and as suggested by Acton et al. (39), selective uptake of HDL cholesteryl ester does not appear to require either the LDL receptor or the LRP; nor does it appear to require lipoplysis. The same caveat regarding the effectiveness of the antibody to the surface lipoplysis applies here as in the LDL experiment. Others have reported that hepatic lipase treatment of HDL stimulates the delivery of cholesterol from HDL to hepatoma cells (8) and perfused rat livers (9). Since it has been shown that hepatic lipase binds to HDL (6, 66–68), it is possible that treatment of HDL with hepatic lipase results in the binding of lipase to the HDL rather than just phospholipolysis, and that this bound lipase acts as a ligand for cell binding. Whether hepatic lipase itself catalyzes selective uptake by allowing egress of cholesteryl ester from the HDL particle or whether it acts in conjunction with another molecule such as SR-B1 remains to be established. The cell line described in these experiments will be useful for such studies as well as for other studies of hepatic lipase action.

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