Endothelial lipase (EL) is a new member of the triglyceride lipase gene family, which includes lipoprotein lipase (LpL) and hepatic lipase (HL). Enzymatic activity of EL has been studied before. Here we characterized the ability of EL to bridge lipoproteins to the cell surface. Expression of EL in wild-type Chinese hamster ovary (CHO)-K1 but not in heparan sulfate proteoglycan (HSPG)-deficient CHO-677 cells resulted in 3–4.4-fold increases of [125I]-low density lipoprotein (LDL) and [125I]-high density lipoprotein 3 binding (HDL3). Inhibition of proteoglycan sulfation by sodium chloride or incubation of cells with labeled lipoproteins in the presence of heparin (100 μg/ml) abolished bridging effects of EL. An enzymatically inactive EL, EL-S149A, was equally effective in facilitating lipoprotein bridging as native EL. Processing of LDL and HDL differed notably after initial binding via EL to the cell surface. More than 90% of the surface-bound [125I]-LDL was destined for internalization and degradation, whereas about 70% of the surface-bound [125I]-HDL3 was released back into the medium. These differences were significantly attenuated after HDL clustering was promoted using antibody against apolipoprotein A-I. At equal protein concentration of added lipoproteins the ratio of HDL3 to VLDL bridging via EL was 0.092 compared with 0.174 via HL and 0.002 via LpL. In summary, EL mediates binding and uptake of plasma lipoproteins via a process that is independent of its enzymatic activity, requires cellular heparan sulfate proteoglycans, and is regulated by ligand clustering.

Lipoprotein lipase (LpL), and hepatic lipase (HL), two members of the triglyceride lipase gene family, have well-established roles in regulating lipid and lipoprotein metabolism and are implicated in atherosclerosis (1–3). LpL is synthesized primarily in adipose and skeletal muscle and is transported to the endothelial surface, where it is bound to heparan sulfate proteoglycans (HSPGs). LpL is predominantly a triacylglycerol hydrolase, and its enzymatic action is mainly related to hydrolysis of triglycerides (TG) in TG-rich apolipoprotein B-containing lipoproteins, chyomicrons and very low density lipoproteins (VLDL) (reviewed in Ref. 4). HL is synthesized primarily in hepatocytes (5, 6) and is bound mostly to hepatic and endothelial HSPGs in the hepatic sinusoids (7). Like LpL, HL has substantial TG lipase activity, but unlike LpL, HL also has significant phospholipase activity (8). This increased phospholipase activity may play an important role in the ability of HL, as opposed to LpL, to directly modulate HDL metabolism (2).

In addition to their lipolytic activities, LpL and HL have been shown to mediate “bridging” between lipoproteins and HSPGs on the cell surface, which results in increased cellular uptake and degradation of lipoproteins (9–16). In several studies LpL dramatically enhanced binding, internalization, and degradation of VLDL and LDL by cultured cells (10–14, 17, 18). In contrast, HL had relatively small, if any, effects on LDL binding and holoparticle uptake, but significantly increased selective uptake of cholesterol esters from HDL particles (19–22). Like LpL, HL has been shown to enhance binding and uptake of chyomicrons, chyomicron remnants, VLDL, and LDL by different cell types in vitro (15, 16, 23, 24) via a HSPG-dependent process (15, 16, 23). In addition, in several studies HL efficiently increased holoparticle cellular uptake of HDL as well as selective uptake of cholesterol esters (16, 25, 26). These bridging effects of LpL and HL require HSPGs and heparin-binding domains of the lipases but do not depend on their catalytical activities. There is also evidence for nonenzymatic effects of LpL and HL on lipoprotein metabolism in vivo (27–32).

Endothelial lipase (EL), a 480-amino acid protein (M, ~68,000), is a new member of the triglyceride lipase gene family (33–36). Many typical features of this gene family are conserved in EL: the catalytic triad residues, the lid that controls access of substrate to the hydrolytic pocket, and the cysteine residues that form intramolecular disulfide bonds. EL is unique in the triglyceride lipase family in that it is synthesized by endothelial cells, however, a number of other cell types also express EL (33, 34). EL has detectable triglyceride lipase ac

novirus encoding lipoprotein lipase; apo, apolipoprotein; CHO, Chinese hamster ovary; EL, endothelial lipase; HL, hepatic lipase; HDL, heparan sulfate; HSPG; heparan sulfate proteoglycan; HL, high density lipoprotein; LpL, low-density lipoprotein; LDL, very low density lipoprotein; TG, triglyceride; GFP, green fluorescent protein; Dil, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine.
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Activity, but this activity is significantly less relative to its phospholipase activity compared with HL and especially LpL (33, 37). In vitro, conditioned medium containing EL had the ability to substantially hydrolyze HDL phospholipids but had little activity toward LDL phospholipids, suggesting relative selectivity for HDL (37). In vivo, even low levels of EL overexpression in the livers of wild-type and human apoA-I transgenic mice dramatically reduced HDL cholesterol and apoA-I levels and increased HDL catabolism (33). As a result of EL overexpression, plasma levels of apoB-containing lipoproteins were also reduced, although to a lesser extent (33). Recent studies in EL knockout mice (38) and in mice injected with specific antibody against EL (39) provided additional evidence for a physiological importance of EL for lipid and lipoprotein metabolism in vivo.

The clusters of positively charged residues in LpL and HL have been implicated in binding of these molecules to heparin and HSPGs. Because the putative heparin-binding and lipoprotein-binding sites present in LpL and HL are highly conserved in EL, we hypothesized that EL may also serve as a bridging molecule between lipoproteins and cell surface and matrix HSPGs. Therefore, the primary focus of this study was to examine the ability of EL to mediate binding and holoparticle uptake of plasma lipoproteins and to compare EL-dependent metabolism of apoB-versus apoA-I-containing lipoproteins in vitro. We also compared EL with HL and LpL in their abilities to facilitate bridging of different major classes of plasma lipoproteins with cells. In this study, we demonstrated that EL can function as an efficient bridging molecule between plasma lipoproteins and cells in a process that requires intact cell surface HSPGs, but does not depend on EL enzymatic activity. Moreover, we found that compared with LpL and HL, EL has distinct preferences in bridging individual classes of lipoproteins.

EXPERIMENTAL PROCEDURES

Preparation of Reagents—Unless otherwise indicated, chemicals of analytical grade were purchased from Sigma. VLDL (d < 1.006 g/ml), LDL (1.019 < d < 1.063 g/ml), and HDL (1.21 g/ml) were purchased from fresh human plasma by ultracentrifugation as described previously (40). 125I-Labeled VLDL (125I-VLDL), 125I-labeled LDL (125I-LDL), and 125I-labeled HDL (125I-HDL) were iodinated using the iodine monochloride method (41). Dilabeled HDL was purchased from Invitrogen Corp. (Rockville, MD). Polyclonal goat antibody against apolipoprotein A-I (anti-apoA-I), which are able to cross-link human HDL, were obtained from Wako Chemical USA, Inc. (Richmond, VA). Cultured Cells—COS-7 and two types of Chinese hamster ovary cell lines, wild-type CHO-K1 and the CHO mutant line pgsn-677 (CHO-677), which is specifically deficient in both N-acetylglucosaminyltransferase and glucuronyltransferase activities and hence lacks heparan sulfate (42, 43), were obtained from the American Type Culture Collection (Manassas, VA). COS-7 cells were grown and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Both CHO cell lines were grown in Ham’s F-12 medium supplemented with 10% fetal bovine serum. These three cell lines were chosen because they are readily infected with recombinant adenovirus constructs and were successfully used in the past to characterize effects of LpL and HL on lipoprotein metabolism (16, 37, 44, 45).

Expression of Enzymes—Wild-type EL or enzymatically inactive EL, in which the active site serine was substituted with alanine (EL-S149A), LpL, and HL were expressed using recombinant adenoviruses. Recombinant adenoviruses encoding human EL (AdEL), human LpL (AdLpL), and human HL (AdHL) were a generous gift from Dr. Nicolas Duverger (Aventis Pharmaceuticals).

Adenoviral infections of cells were performed as described (37). Briefly, prior to infection cells were grown to 80–90% confluence in 12-well plates (22 mm/well). Cells were then incubated with recombinant adenoviruses encoding EL, EL-S149A, LpL, HL, or GFP (control virus) in 0.3 ml of serum-free medium at a multiplicity of infection of 3000 particles/cell. Two hours later, 0.7 ml of fresh growth medium containing serum was added to each well and incubations were continued for 2 days. At 48 h post-infection, cells were washed twice with phosphate-buffered saline and used for lipid binding experiments with 125I-labeled lipoproteins.

Expression of lipases by infected cells was confirmed by Western blotting and by assay of triglyceride lipase activity of conditioned medium according to the protocols described previously (37). Antibody against human EL was generated to a peptide in the N-terminal region of EL, as previously described (33). Antibody to rat HL that cross-reacts with human HL was also previously described (46). A polyclonal antibody to human LpL was a generous gift from Dr. Mark H. Doolittle. Cellular Metabolism of 125I-VLDL, 125I-LDL, and 125I-HDL—For binding studies, cells grown in 12-well plates were incubated with 0.5 ml of the serum-free medium supplemented with 0.2% bovine serum albumin (Sigma number A-8806) and 125I-labeled lipoproteins (5 μg of protein/ml) unless otherwise stated. To assess the effects of endogenous expression of lipases on surface binding of lipoproteins, cells were incubated with labeled lipoproteins for 1 h at 4°C. To measure cellular uptake and degradation of lipoproteins, cells were incubated for 3 h at 37°C in the presence of labeled lipoproteins, then surface-bound (heparin-releasable), intracellular (heparin-resistant), and degraded (assessed by trichloroacetic acid-precipitable radioactivity in medium) ligand was measured as described (40, 41). To examine the time course of cellular processing of 125I-LDL versus 125I-HDL, labeled lipoproteins were incubated with cells in serum-free medium for 1 h at 4°C to allow surface binding without further catabolism. Cells were washed at 4°C to remove unbound material. Fresh medium at 37°C with no ligands was then added, and incubations were performed at 37°C for the indicated times. Alternatively, non-adiabatic, intracellular, and degraded ligands were then performed as above. Additionally, trichloroacetic acid-precipitable radioactivity in the medium was quantified as an indication of lipoproteins released into the medium via retroendocytosis or desorption from the cell surface during the incubation at 37°C. To test potential effects of ligand clustering on cellular metabolism of 125I-HDL, cells were incubated with 125I-HDL, at 4°C for 1 h, to allow cell-surface binding. Cells were rinsed at 4°C to remove unbound ligand and incubated for an additional 30 min at 4°C with or without antibody against human apoA-I (10 μg/ml final concentration). Cells were then washed briefly and incubated with pre-warmed media at 37°C for the indicated times followed by measurements of surface-bound, intracellular, degraded and medium released radioactivity. All results for 125I-lipoprotein metabolism were normalized to cellular protein. Lipase-dependent catabolism was calculated by subtracting the values obtained in control cells infected with adGFP from those obtained in cells expressing lipases (total catabolism). HSPG-mediated catabolism was calculated by subtracting the values obtained in the presence of heparin (100 μg/ml) from those obtained in its absence. Assay of EL Protein—In addition to assays with labeled lipoproteins, a separate set of cells was used to measure EL or EL-S149A protein available for bridging of labeled lipoproteins. For this purpose, cells were not exposed to radiolabeled lipoproteins, but instead were incubated in the presence of heparin (100 μg/ml) to release surface-bound lipoproteins into the medium. EL protein concentration was evaluated by densitometric analysis of Western blots of the conditioned medium using a Bio-Rad imager (model GS-700) and Quantity One program (Bio-Rad). Protein concentration was defined in arbitrary units as determined by densitometric analysis. Several dilutions of conditioned medium were used to make sure the intensity of the bands was proportional to the amount of protein applied on the gel.

Role of HSPGs in Lipoprotein Metabolism—We used three approaches to test the contribution of HSPGs in EL-mediated processing of lipoproteins. First, we blocked sulfation of cellular proteoglycans by preincubating cells for 18 h at 37°C in sodium chloride (50 mM), an inhibitor of sulfate adenyltransferase (47), thus preventing sulfation of glycosaminoglycan side chains of HSPGs. Control cells were exposed to 50 mM sodium chloride. Second, we compared lipoprotein metabolism in wild-type versus HSPG-deficient CHO cells. Third, we incubated cells with labeled lipoproteins in the presence or absence of heparin at 100 μg/ml. Specifically blocks into HSPGs (40), but not lipoprotein binding to the members of the LDL receptor family or other lipoprotein receptors (48).

Statistical Analyses—All results are displayed as mean ± S.E., n = 3. Error bars that appear absent indicate S.E. values smaller than the drawn symbols.
RESULTS

Effects of EL Expression on Binding of 125I-LDL and 125I-HDL3—We examined the effects of EL on the binding of 125I-LDL and 125I-HDL3 in CHO-K1 cells. For these experiments CHO-K1 cells were infected with control (GFP) or EL-encoding adenoviral constructs. 48 h after infection, cells were incubated with radiolabeled 125I-LDL or 125I-HDL3 for 1 h at 4 °C, and cell binding of labeled lipoproteins was measured as described under “Experimental Procedures.” Expression of EL was assayed in parallel wells by Western blotting with anti-EL antibody and by measuring triglyceride lipase activity in the conditioned medium. Incubation with the control virus (adGFP) did not affect binding of either 125I-LDL or 125I-HDL3 compared with uninfected cells (91.3 ± 4.8 and 95.6 ± 7.4% of values for uninfected cells, respectively). In contrast, cells infected with adEL demonstrated a 4.4-fold increase in binding of 125I-LDL (Fig. 1A) and a 3.9-fold increase in binding of 125I-HDL3 (Fig. 1B) compared with control, adGFP-infected cells. Of interest, although 125I-LDL or 125I-HDL3 were added to the cells at equal protein concentrations, about 2.3 times more 125I-LDL protein was bound to the cells compared with 125I-HDL3 via EL, the values for the EL-mediated binding of 125I-LDL versus 125I-HDL3 (Fig. 1A) and the values for 125I-HDL3 versus 125I-LDL (Fig. 1B) are similar. EL-dependent component of 125I-LDL and 125I-HDL3 was internalized via the EL-mediated process (114.3 ± 7.6 versus 49.4 ± 2.3 ng/mg of cell protein, respectively) indicating that a higher percentage of LDL particles was bound to the cells compared with HDL.

Role of Cell Surface HSPGs in EL-mediated Increase of 125I-LDL and 125I-HDL3 Metabolism—We next tested if cell surface HSPGs are responsible for the observed effects of EL on binding of lipoproteins by incubating cells with labeled lipoproteins in the presence of 100 μg of heparin/ml. At this concentration heparin selectively blocks ligand binding to heparan sulfate side chains of HSPGs (40) but not lipoprotein binding to the members of the LDL receptor family or other lipoprotein receptors (48). The EL-dependent component of 125I-LDL or 125I-HDL3 binding in control CHO-K1 cells infected with adEL was completely abolished by heparin (Fig. 1, panels A and B). Cell-surface binding of both 125I-LDL and 125I-HDL3 via EL was also inhibited by more than 90% when cells were pretreated for 18 h in the medium containing 50 mM sodium chloride, which blocks sulfation of glycosaminoglycan side chains of HSPGs (data not shown). Additional support for the role of HSPGs in EL-mediated binding of lipoproteins was obtained when we tested effects of adEL infection on 125I-LDL and 125I-HDL3 binding in HSPG-deficient CHO-67777 cells. The level of EL secretion by HSPG-deficient cells was similar or slightly higher than the level of EL secretion in control cells, but there was no EL-dependent increase in 125I-LDL and 125I-HDL3 binding in HSPG-deficient cells (Fig. 1, panels A and B). As expected, heparin (100 μg/ml) also had no effect on lipoprotein binding by HSPG-deficient cells (Fig. 1, panels A and B). Taken together, these results indicate that HSPGs are crucial for EL-dependent increases in binding of 125I-LDL and 125I-HDL3 to the cell surface.

To verify if enzymatic activity is required for the bridging effects of EL, we compared binding of 125I-LDL and 125I-HDL3 by cells infected with adEL versus those infected with recombinant adenovirus encoding enzymatically inactive EL (adEL-S149A). In four independent experiments, adEL-S149A-infected cells demonstrated significant increases in binding of 125I-LDL and 125I-HDL3, compared with cells infected with control virus (Fig. 2). When normalized to protein expression, increases in binding of 125I-LDL and 125I-HDL3 caused by expression of EL-S149A were 99.2 ± 2.0 and 102.8 ± 3.90%, respectively, of those resulting from the expression of wild-type EL. This indicates that enzymatic activity of EL is not required for bridging of either 125I-LDL or 125I-HDL3.

Intracellular Processing of 125I-LDL and 125I-HDL3 by Cells Expressing EL—To compare effects of endogenous EL on cellular metabolism of 125I-LDL and 125I-HDL3, cells were incubated with labeled lipoproteins for 3 h at 37 °C, a temperature that allows internalization and degradation of lipoproteins after their binding to cell receptors. Under these conditions, expression of EL by COS cells resulted in the increased binding, intracellular accumulation, and degradation of both 125I-LDL and 125I-HDL3 compared with control cells infected with adGFP (Fig. 3, A and B, respectively). EL-dependent components of binding, cellular accumulation, and degradation of lipoproteins were inhibited by more than 95% when cells were incubated with labeled lipoproteins in the presence of heparin (100 μg/ml). Interestingly, the relative portion of 125I-LDL that was internalized via the EL-mediated process in 3 h was much higher compared with that of 125I-HDL3, suggesting that the nature of the ligand may affect metabolism of the ligand after initial binding to the cell surface. Additionally, in the experiments performed at 37 °C the values for the EL-mediated binding of 125I-LDL, expressed in nanograms of metabolized lipoprotein per mg of cell protein, were much higher compared with those for 125I-HDL3 suggesting significantly more efficient intracellular processing of LDL versus HDL via an EL-dependent pathway.

We next compared kinetics of cellular processing of 125I-LDL versus 125I-HDL3. About 75% of 125I-LDL initially bound to the cell surface through the EL-mediated pathway was internalized, and ~50% of internalized material was degraded by the end of the 2-h incubation (Fig. 4A). Less than 10% of 125I-LDL-related radioactivity was recovered from the medium as trihaloacetic acid-precipitable material. In contrast, the majority of 125I-HDL3 was released back into the medium in trihaloacetic acid-precipitable particles and only 20–25% of 125I-HDL3 was eventually internalized (Fig. 4B). By the end of the 2-h incubation ~80% of internalized 125I-HDL3 was degraded. Interestingly, compared with 125I-LDL, degradation of 125I-HDL3 started earlier and proceeded at a higher rate when expressed as a percentage of the internalized material, but it could be related to a considerably lower rate of internalization of 125I-HDL3.

We sought to explain the observed differences in the cellular processing of LDL versus HDL by comparing affinities of binding of these lipoproteins to EL. For this purpose, COS cells expressing EL were incubated for 1 h with increasing concentrations of 125I-LDL or 125I-HDL3. Incubations were performed at 4 °C to minimize the enzymatic activity of EL. At all concentrations tested (0.37–90 μg of lipoprotein protein/ml), binding of 125I-LDL or 125I-HDL3 to EL-expressing cells was signifi-
ligation, and degradation of lipoproteins were measured as described under “Experimental Procedures.” The results show the absolute increases in binding, intracellular accumulation, and degradation of lipoproteins attributable to EL. EL-dependent catabolism was calculated by subtracting the values obtained in control cells (infected with adGFP) from those obtained in cells expressing EL (infected with adEL). The absolute values for cell-surface binding, intracellular accumulation, and degradation of 125I-LDL in control cells infected with adGFP were 60.4 ± 9.6, 235.6 ± 7.7, and 171.0 ± 17.4 ng/mg of cell protein, respectively. The corresponding values for 125I-HDL₃ metabolism in control cells were 19.8 ± 5.5, 19.3 ± 1.0, and 12.4 ± 2.6 ng/mg of cell protein, respectively.

Fig. 2. Effects of endogenous expression of enzymatically inactive EL mutant, EL-S149A, on binding of 125I-LDL (A) and 125I-HDL₃ (B) by CHO-K1 cells. CHO-K1 cells were infected with adGFP or adEL-S149A. 48 h after infection, cells were incubated at 4 °C for 1 h with 5 μg of 125I-labeled lipoproteins per ml of bovine serum albumin-containing medium. Displayed is the total binding of lipoproteins.

Fig. 3. Metabolism of 125I-LDL (A) and 125I-HDL₃ (B) by COS-7 cells expressing EL. COS-7 cells infected with adEL were incubated with 5 μg of 125I-labeled lipoproteins per ml of medium at 37 °C for 3 h. At the end of the incubation, cell-surface binding, intracellular accumulation, and degradation of lipoproteins were measured as described under “Experimental Procedures.” The results show the absolute increases in binding, intracellular accumulation, and degradation of lipoproteins attributable to EL. EL-dependent catabolism was calculated by subtracting the values obtained in control cells (infected with adGFP) from those obtained in cells expressing EL (infected with adEL). The absolute values for cell-surface binding, intracellular accumulation, and degradation of 125I-LDL in control cells infected with adGFP were 60.4 ± 9.6, 235.6 ± 7.7, and 171.0 ± 17.4 ng/mg of cell protein, respectively. The corresponding values for 125I-HDL₃ metabolism in control cells were 19.8 ± 5.5, 19.3 ± 1.0, and 12.4 ± 2.6 ng/mg of cell protein, respectively.

Fig. 4. Time course of the catabolism of surface-bound 125I-LDL (A) and 125I-HDL₃ (B) via the EL-mediated pathway in COS-7 cells. COS-7 cells infected with adGFP or adEL viruses were incubated with 125I-labeled lipoproteins (10 μg/ml final concentration) in serum-free medium for 1 h at 4 °C to allow surface binding without further catabolism. Cells were washed at 4 °C to remove unbound material. Fresh medium at 37 °C with no ligands was then added, and incubations were continued at 37 °C for the indicated time. Assays for EL-dependent surface-bound (open diamonds), intracellular (closed squares), and degraded (closed triangles) were then performed. Measurements of 125I-lipoproteins remaining on the cell surface (open diamonds), intracellular accumulation (closed squares), and degraded ligand (closed triangles) are displayed. Additionally, trichloroacetic acid-precipitable radioactivity in the medium was quantified as an indication of lipoproteins released into the medium via retroendocytosis or desorption from the cell surface during the incubation at 37 °C (open circles). The results are displayed as percentage of the total amount of lipoproteins initially bound via the EL-mediated process to the cells during incubation at 4 °C. The absolute values for initial binding of 125I-LDL and 125I-HDL₃ to the control cells infected with adGFP were 25.5 ± 0.7 versus 22.6 ± 8.1 ng/mg of cell protein, respectively. EL-dependent binding of 125I-LDL and 125I-HDL₃, calculated as described before, were 153.4 ± 22.6 versus 46.3 ± 2.5 ng/mg of cell protein, respectively.

By subtraction analysis, the binding curve demonstrated the presence of only one class of binding site, with an apparent Kᵦ of 161.9 nM; Bmax of 1.80 pmol/mg of cell protein) and another with much higher affinity but lower capacity (apparent Kᵦ of 1.3 nM; Bmax of 0.27 pmol/mg of cell protein). In contrast, under similar experimental conditions the Scatchard curve for EL-dependent binding of 125I-HDL₃ was linear (Fig. 5B) and nonlinear regres-
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**Comparison of Lipoprotein Bridging Preferences of EL Versus LpL and HL**—EL, HL, and LpL discretely hydrolyze different lipoprotein classes (37). We next explored whether lipoprotein bridging preferences of the three members of the lipase family might also be different. Parallel sets of COS-7 cells were infected with equal amounts of control vector or adenoval constructs encoding EL, HL, and LpL. Expression of the active enzymes was verified by Western blotting and by measuring triglyceride lipase activity. TG lipase activities in the medium from adEL-, adHL-, and adLpL-infected cells were 26.5 versus 161.1 versus 337.5 versus 215.9 nmol/ml/h, respectively. Infected cells were incubated with 125I-VLDL, 125I-LDL, or 125I-HDL, at equal protein concentrations (10 μg/ml). Lipase-dependent cellular metabolism of lipoproteins was measured by subtracting values obtained in control, adGFP-infected cells from those observed in EL-, HL-, and LpL-expressing cells. As shown in Fig. 8A, expression of EL resulted in very similar increases in metabolism of 125I-VLDL and 125I-LDL, whereas its effect on 125I-HDL was relatively lower. Similar to EL, HL was able to enhance the metabolism of all three lipoprotein classes (Fig. 8B). Under our experimental conditions, LpL caused pronounced increases of 125I-VLDL and

**Fig. 6. Effect of anti-apoA-I on distribution of HDL3 on the cell surface.** COS-7 cells grown in the glass-bottom 35-mm Petri dishes (MatTek corporation, Ashland, MA) and infected with adEL were incubated with DiI-labeled HDL3 (10 μg/ml final concentration) in serum-free medium for 1 h at 4 °C. Then, cells were washed to remove unbound material and incubated for an additional 30 min at 4 °C without (A) or with anti-apoA-I (B). At the end of the incubation, cells were washed briefly, fixed with 3% paraformaldehyde in phosphate-buffered saline overnight at room temperature, and blocked in phosphate-buffered saline/bovine serum albumin for 1 h at room temperature. Samples were analyzed using a Zeiss Axiosvert 100TV microscope (Germany) with 40X Plan-Apochromat lenses, a precisely controlled XYZ stage (Applied Precision), and a scientific grade cooled CCD camera (MicroMax, Princeton Instruments, Trenton, Nj). To maximize resolution along the optical axis, illumination from a mercury lamp was directed through a fiber optic scrambler to provide high intensity, homogenous illumination to the back aperture plane of the objective lens.

**Fig. 5. Scatchard analysis of EL-dependent binding of 125l-LDL (A) and 125l-HDL3 (B) to COS-7 cells. COS-7 cells infected with adGFP or adEL viruses were incubated with different concentrations of 125I-labeled lipoproteins (0.37–90 μg of protein/ml) in serum-free medium for 1 h at 4 °C to allow surface binding without further catabolism. At the end, cells were washed to remove unbound ligands and total radioactivity associated with the cells was measured. Lipoprotein binding to control cells infected with adGFP was used to estimate nonspecific binding. EL-dependent binding of lipoproteins was calculated by subtracting values obtained in adGFP-infected cells from those obtained in EL-expressing cells. The data are plotted as bound/free versus bound, where bound is picomole of lipoproteins bound per mg of cell protein and free is nanomole of added lipoproteins per ml of medium. Dotted and dashed lines represent curves for low and high affinity binding sites, which were calculated using nonlinear regression analysis.
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Fig. 7. Effect of anti-apoA-I on EL-mediated catabolism of $^{125}$I-HDL$_3$ by COS cells. A, fractional distribution of $^{125}$I-HDL$_3$ after pre-incubation in the absence or presence of anti-apoA-I. COS-7 cells infected with adGFP or adEL viruses were incubated with $^{125}$I-labeled lipoproteins (10 µg/ml final concentration) in serum-free medium for 1 h at 4°C to allow surface binding without further catabolism. Cells were washed at 4°C to remove unbound material and incubated for an additional 30 min at 4°C in fresh medium in the absence of IgGs. In the presence goat polyclonal antibodies against human apoA-I (10 µg/ml) or matching concentrations of irrelevant goat IgGs. Then, cells were briefly washed and incubations were continued for 40 min at 37°C in fresh medium with no ligands. Measurements of EL-dependent surface binding, intracellular accumulation, degradation and medium release were taken very efficiently into the cells after initial cell-surface binding via the EL-mediated process. In contrast, more than 70% of HDL3 bound to the cell surface after incubation at 4°C (Fig. 3). These values and the results of kinetic studies presented in Fig. 4 are consistent with the rate of LDL internalization and degradation via syndecan-mediated pathway described previously (40, 52, 63), suggesting that HSPGs play a major role not only in surface binding, but also in the uptake and degradation of lipoproteins via an EL-mediated process.

The fate of lipoproteins bound to the cell surface HSPGs via EL may also depend on characteristics of the lipoproteins themselves. For example, apoB-containing VLDL and LDL were taken very efficiently into the cells after initial cell-surface binding via the EL-mediated process. In contrast, more than 70% of HDL3 bound to the cell surface after incubation at 4°C was subsequently released into the medium during incubation at 37°C (Fig. 4). A number of factors may contribute to the observed differences. We found that compared with HDL3, LDL is able to bind much more avidly to EL. Interestingly, analysis of $^{125}$I-LDL interaction with cell surface EL revealed the presence of two types of binding sites, which differ significantly by their apparent $K_d$ and $B_{max}$. We speculate that the low affinity, high capacity type reflects interactions between EL and lipid components of LDL, whereas interaction between EL and apolipoprotein B is likely to be associated with the second class, characterized by high affinity and low capacity. Furthermore, our finding, that interaction of HDL with EL could be described by only one class of binding sites with low affinity and high capacity is in line with the inability of the structurally and functionally related HL and LpL to bind directly to protein components of LDL (49, 64). Several studies demonstrated that HL and LpL had significantly higher affinities toward LDL than HDL (49, 64–66), although there was disagreement on the relative importance of the protein-protein interaction between these lipases and apoB.

Different affinities of LDL versus HDL toward EL could be one of the factors responsible for the differences in uptake and degradation of these lipoproteins. The lower efficiency of HDL uptake may also be related to a relatively smaller HDL surface area and, therefore, fewer sites available for binding of EL on a
FIG. 8. Comparison of preferential bridging properties of EL versus LpL versus HL in COS-7 cells. COS-7 cells infected with adEL (A), adHL (B), or adLpL (C) constructs were incubated with 
\(^{125}\text{I}-\text{VLDL}, ^{125}\text{I}-\text{LDL}, \text{or } ^{125}\text{I}-\text{HDL}_3 \text{, for 1 h at 37 } ^\circ \text{C. At the end of the incubation, the total amount of } ^{125}\text{I}-\text{HDL}_3 \text{ to } ^{125}\text{I}-\text{VLDL} \text{ metabolism calculated for each of the lyses.}

Overall, our data indicate that EL is able to bridge plasma lipoproteins with the cell surface in a process that requires intact HSPGs but does not rely on enzymatic action of the lipase. Moreover, we demonstrated that compared with HL and LpL, EL has distinctively different bridging preferences toward plasma lipoproteins offering further evidence of a unique role of EL in lipoprotein metabolism.

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