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Lipoprotein Lipase Association with Lipoproteins Involves Protein-Protein Interaction with Apolipoprotein B*

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Sungshin Y. Choi‡, Pillarisetti Sivaram‡, Dee E. Walker‡, Linda K. Curtiss§, Daniel G. Gretch¶, Stephen L. Sturley¶‖, Alan D. Attie¶, Richard J. Deckelbaum‖, and Ira J. Goldberg‡

From the Departments of ‡Medicine and ‖Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York 10032, §Scripps Research Institute, La Jolla, California 92037, and the ¶Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706

Lipoprotein lipase (LPL) hydrolyzes chylomicron and very low density lipoprotein (VLDL) triglycerides and potentiates the cellular uptake of lipoproteins. These LPL-lipoprotein associations could involve only protein-lipid interaction, or they could be modulated by apolipoproteins (apo). ApoB is the major protein component of chylomicrons, VLDL, and low density lipoprotein (LDL). ApoB100, a large glycoprotein with a molecular mass of 550 kDa, is composed of several functional domains. A carboxyl-terminal region of the protein is the ligand for the LDL receptor. There are several hydrophobic domains that are believed to be important in lipid binding. The relatively hydrophilic amino-terminal region of apoB, however, has no known function. Using solid phase assays we quantified LPL-lipoprotein complex formation. On a molar basis, severalfold greater amounts of LPL bound to LDL and VLDL than to high density lipoprotein at all the concentrations of LPL tested (0.9–55 nM).

To assess the roles of LDL protein *versus* lipid, we performed competition and ligand blotting experiments. LDL and an amino-terminal fragment of apoB competed better for ^{125}I-LPL binding to LDL than did lipid emulsion particles. Delipidation of LDL-coated plates did not alter LPL binding. On ligand blots, LPL bound to amino-terminal fragments of apoB generated by thrombin digestion but not to apoA1, apoE, or carboxyl-terminal fragments of apoB. Further evidence for LPL interaction with the amino-terminal region of apoB was obtained using anti-apoB monoclonal antibodies. Antibodies directed against the amino-terminal regions of apoB blocked LPL interaction with LDL, whereas those against the carboxyl-terminal region of apoB did not inhibit LPL interaction with LDL. Thus, we conclude that a specific interaction between LPL and the amino-terminal region of apoB may facilitate LPL association with circulating lipoproteins.

Lipoprotein lipase (LPL)[1] is primarily responsible for hydrolyzing chylomicron and VLDL triglycerides (TG). This enzyme is synthesized in a variety of tissues and cells including adipose, muscle (1), brain (2), and macrophages (3). After synthesis, the enzyme is transported across the endothelium to its site of activity on the luminal surface of endothelial cells (4). The enzyme binds to this site via electrostatic interactions with cell surface proteoglycans (5, 6) and, perhaps, via protein-protein interaction involving a non-proteoglycan binding protein (7).

A number of experimental observations suggest that LPL has a greater affinity for LDL than for HDL. LPL is primarily a triacylglycerol hydrolase, and its enzymatic actions are usually assayed using a TG-rich substrate (8, 9). Yet LPL-mediated hydrolysis of LDL, a cholesteryl ester-rich lipoprotein, and VLDL, a TG-rich lipoprotein, had a lower K_m than HDL (10). Further evidence that LPL does not bind indiscriminately to all classes of lipoproteins was obtained from studies of human postheparin plasma. Most active LPL in postheparin plasma elutes during gel filtration in a major peak that precedes the peak of LDL cholesterol (11). Although Vilella *et al.* (12) reported that some LPL was also associated with HDL, HDL-associated LPL was less active than the LPL on apoB-containing lipoproteins. Thus, active LPL is associated primarily with LDL or buoyant LDL size lipoproteins in the circulation.

In addition to its enzymatic actions, LPL can anchor lipoproteins to cell surface and matrix proteoglycans. This molecular bridge has been postulated to increase lipoprotein retention by subendothelial cell matrix and increase cellular lipoprotein uptake. Both Saxena *et al.* (13) and Eisenberg *et al.* (14) showed that LPL anchors apoB-containing lipoproteins to subendothelial cell matrix. Saxena *et al.* (13) were unable to show any increase in LPL-mediated HDL binding to matrix. In contrast, Eisenberg *et al.* (14) reported that LPL caused a small increase in HDL association with matrix heparan sulfate proteoglycans. This increase in HDL binding was almost an order of magnitude less than that found for LDL or VLDL. Saxena *et al.* (13) performed their experiments in media containing serum lipoproteins that could have competed for LPL binding to HDL. This might explain the disparity between results. Nonetheless, LPL appears to preferentially anchor apoB-containing particles.

How does LPL interact with lipoproteins? One hypothesis is that LPL has several hydrophobic regions that bind to lipid molecules on the surface of large particles containing a TG core (15). LPL, however, does not associate well with protein-free lipid particles. Fielding and Fielding (16) reported a method of partial LPL purification by incubating postheparin plasma with 5 mg/ml TG derived from 20% Intralipid in low ionic

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** To whom correspondence should be addressed: Dept. of Medicine, Columbia University, 630 West 168th St., New York, NY 10032. Tel.: 212-305-3678; Fax: 212-305-5384.

[1] The abbreviations used are: LPL, lipoprotein lipase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; PBS, phosphate-buffered saline; BSA, bovine serum albumin; TBS, Tris-buffered saline; mAb, monoclonal antibody.

strength buffer. Even with this protocol in which the TG concentration was severalfold greater than the usual plasma cholesterol or TG concentrations, less than 50% of the LPL activity was recovered with the lipid. Additional studies have shown that LPL associates better with apoB-containing lipoproteins than with Intralipid. In the studies of Rumsey *et al.* (17), the LPL-mediated increase in emulsion particle binding to the surface of fibroblasts was much less than that found using LDL. Thus, optimal LPL association with lipoproteins may require more than just lipid.

In the experiments presented in this paper, we studied LPL interaction with lipoproteins and lipid emulsions. Using a solid phase plate assay, we assessed the interaction between LPL and lipoproteins in a system that was free of lipoprotein receptors and cell surface proteoglycans. Because more LPL associated with LDL than HDL or lipid emulsions, we studied the roles of apoB and lipoprotein lipid in this process. Our data support a role for apoB, specifically the amino-terminal region of apoB, in LPL interaction with lipoproteins.

MATERIALS AND METHODS

Preparation of Lipoproteins—Human LDL (*d* 1.019–1.063 g/ml) and HDL (*d* 1.063–1.21 g/ml) were isolated from EDTA-containing plasma by ultracentrifugation (18). Apolipoprotein patterns of all the lipoprotein preparations were analyzed by SDS-polyacrylamide gel electrophoresis. Total protein concentration of lipoproteins was determined by the method of Lowry *et al.* (19) with bovine serum albumin (BSA) as a standard.

Preparation of ApoB17—A 6507-base pair *Eco*RI fragment encoding the amino-terminal 46% of human apoB was subcloned from a pCMV5 vector containing the apoB78 cDNA (a gift from Brian Blackhart and Brian McCarthy) into the *Eco*RI site of pBluescript M13+ (Stratagene, La Jolla, CA) to form pBS-B46. A 2569-base pair *Bam*HI fragment from this vector was then inserted into the *Bam*HI site of the baculovirus transfer vector pAcYM1, yielding pAcB17. This plasmid was cotransfected with the wild-type baculovirus genome (*Autographa californica* nuclear polyhedrosis virus) into *Spodoptera frugiperda* Sf-21 cells. Recombinant viruses were then selected, plaque-purified, and titered as described (20, 21). These viruses encode the 27-amino acid apoB signal peptide as well as the amino-terminal 782 amino acids of mature apoB.

Tissue culture supernatants from expressing and nonexpressing cells were harvested, and cells were removed by centrifugation at 1000 × *g* for 5 min. Phenylmethylsulfonyl fluoride at 0.001% and benzamidine at 0.3 mg/ml were added to the supernatant. Samples were analyzed by 10% SDS-polyacrylamide gel electrophoresis (22) followed by either Coomassie Blue staining or immunoblotting (23) with anti-human apoB monoclonal antibodies 1D1 and CC3.4 (24, 25) and alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma).

In some experiments, amino-terminal fragments of apoB were generated by thrombin digestion of human LDL as described by Cardin *et al.* (26).

Preparation of Cholesteryl Ester-rich Lipid Emulsions—Cholesteryl ester-rich lipid emulsions were prepared as reported previously (17) by adding 40 mg of triolein (Nu-Check, Elysian, MN) and 40 mg of cholesteryl oleate to 80 mg of egg yolk phosphatidylcholine (Avanti Polar Lipids, Inc., Alabaster, AL).

Purification of Bovine Milk LPL—LPL was purified from fresh bovine milk as described previously using the method of Socorro *et al.* (27). Protein was determined by the method of Lowry *et al.* (19). When analyzed by SDS-polyacrylamide gel electrophoresis, a single band with a molecular mass of approximately 55 kDa was identified on a Coomassie staining (data not shown). Enzyme activity was assayed using a method described by Nilsson-Ehle and Schotz (9). The purified enzyme was stored at −70 °C.

Radioiodination and Biotinylation of LPL—Purified LPL was radioiodinated (7) using lactoperoxidase and glucose oxidase enzymes (Sigma) as reported (7). A typical preparation had a specific activity of about 800-1000 cpm/ng LPL, and more than 90% of the counts were precipitable by 10% trichloroacetic acid.

LPL was biotinylated as described by Sivaram *et al.* (28). Briefly, *N*-hydroxysuccinimide ester of biotin (Vector Laboratories, Burlingame, CA) dissolved in dimethyl sulfoxide was added to purified LPL and incubated for 10 min at 4 °C. Biotinylated LDL was then purified from the mixture using a heparin-agarose column, and fractions were characterized by ligand blotting using streptavidin. LPL activity was also measured, and active fractions containing biotinylated LPL were pooled and stored in aliquots at −70 °C.

Monoclonal Antibodies—Either ascites containing the hybridoma antibodies or purified IgG was used. The monoclonal antibodies mAb 3 and mAb 19 have determinants in the amino-terminal region of apoB (29). mAb 47 recognizes the LDL receptor binding site of apoB close to the carboxyl terminus (24).

SDS-Polyacrylamide Gel Electrophoresis and Ligand Blotting—Samples were analyzed using polyacrylamide gels containing 0.5% SDS (22). Proteins were then transferred to a nitrocellulose membrane (Schleicher and Schuell) using a Milliblot SDE semidry blotting system (Millipore Corp., Bedford, MA) and then incubated overnight in PBS containing 3% BSA at 4 °C. The membrane was then incubated with biotinylated LPL for 2 h at 4 °C and then washed six times with PBS containing 0.3% BSA. Proteins bound to biotinylated LPL were visualized by incubation with avidin-conjugated horseradish peroxidase followed by development with 4-chloro-1-naphthol (peroxidase substrate) (7).

Microtiter Plate Assays—Solid phase plate assays were performed as described by Williams *et al.* (30). For binding studies, LDL, HDL, or VLDL, diluted in 50 mM Tris, 150 mM NaCl, pH 7.4 (TBS) containing 5 mM Ca^{2+} was added to 96-well plates and incubated at 4 °C overnight. The unbound lipoproteins were removed, and the plates were washed three times with 0.3% BSA in TBS. Then the plates were incubated with 3% BSA in TBS, 5 mM Ca^{2+} for 1 h at room temperature to block nonspecific binding sites. After washing with 0.3% BSA in TBS, ^{125}I-LPL in TBS, 5 mM Ca^{2+}, 3% BSA was added and incubated for 24 h at 4 °C. Again, the plates were washed, and 200 μl of 0.1 N NaOH was added. An aliquot of 150 μl was counted.

For competition studies, LDL (50 μg/ml protein, 91 nM) was bound to 96-well plates overnight at 4 °C. The experimental protocol used in this experiment was identical to the one described for the binding experiments except that ^{125}I-LPL (5 μg/ml, 45 nM) was added along with competitors. After incubating for 2 h at room temperature, unbound ^{125}I-LPL was removed, and radioactivity bound to the well was measured. Binding to BSA-coated wells was determined in each assay as a control. Competition studies were performed using Intralipid (20%) (Vitrum, Stockholm) and cholesteryl ester-rich emulsions. The molar concentration of Intralipid was calculated using a diameter of 300 nm/lipid particle. Cholesteryl ester-rich lipid emulsion concentration was calculated based on the total neutral lipid concentration (*i.e.* 30 μg/ul) and assuming that 1 μmol of the particle contains 5×10^6 μg. The protein molecular masses of 550 kDa for LDL, 150 kDa for HDL, and 110 kDa for LPL dimers were used to calculate the concentrations of these lipoproteins. For VLDL we estimated that the total molecular mass of VLDL was approximately 1×10^4 kDa and that 10% of this would be the protein mass; thus, we used 1×10^3 kDa as a molecular mass of VLDL.

To determine the binding of apoB17 to LPL, enzyme-linked immunosorbent assay was performed using a monoclonal antibody against the amino-terminal region of apoB (mAb 19). Briefly, LPL or BSA at 5 μg/ml was incubated at 4 °C overnight in microtiter plates. After washing the plates four times with 0.3% BSA in PBS and blocking with 3% BSA in PBS for 1 h, various concentrations of apoB17 were added. The plates were incubated for 2 h at room temperature, washed four times, and then incubated for another 2 h at room temperature with primary antibody (mAb 19 diluted to 1:500 in PBS). Unbound antibodies were removed, and horseradish peroxidase-conjugated goat anti-mouse IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) at 1:500 dilution was added. One and a half hours later, 100 μl of substrate solution containing *o*-phenylenediamine dihydrochloride (Sigma), 5 μl of 50% H_2O_2 in 0.1 M citric acid (pH 6) was added to each well. After 20 min at room temperature the absorbance at 492 nm was measured.

RESULTS

LPL binding to LDL, HDL, and VLDL—^{125}I-LPL binding to LDL-, HDL-, and VLDL-coated plates was determined. We first measured the amounts required to saturate the binding sites of the plate. To do this, ^{125}I-LDL, ^{125}I-HDL, or ^{125}I-VLDL at various protein concentrations (1–100 μg/ml) were incubated overnight at 4 °C in microtiter plate wells. After washing, labeled lipoprotein in each well was assessed. Approximately 10 μg/ml LDL or HDL and 25 μg/ml VLDL were required to saturate the plate. At this concentration, 2.3×10^{-4} nmol of LDL protein, 6.1×10^{-4} nmol of HDL protein, and 2.43×10^{-4} nmol of VLDL protein were bound to each well. Thus, several-

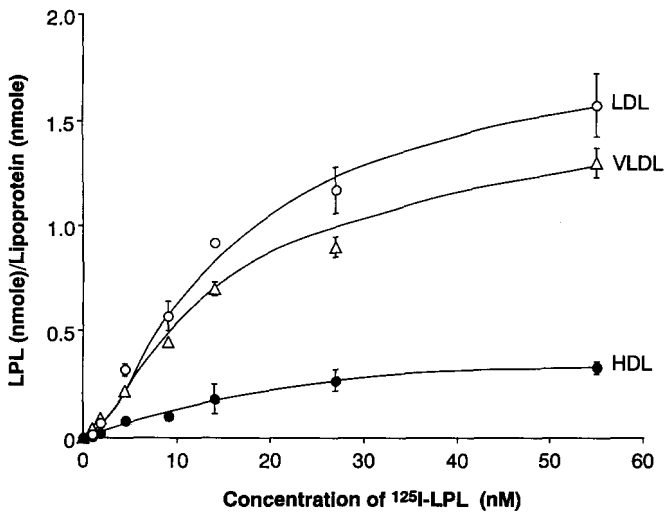

FIG. 1. **LPL binding to LDL, HDL, and VLDL.** Microtiter plates were coated with LDL, HDL, or VLDL (50 μg of protein/ml) diluted in TBS containing 5 mM Ca^{2+} by incubating overnight at 4 °C. The unbound lipoproteins were removed, and the plates were washed three times with 0.3% BSA in TBS. Then the plates were incubated with 3% BSA in TBS, 5 mM Ca^{2+} for 1 h at room temperature to block nonspecific binding sites. After washing with 0.3% BSA in TBS, plates were incubated overnight at 4 °C with ^{125}I-LPL in TBS containing 5 mM Ca^{2+} and 3% BSA. The plates were again washed, and 200 μl of 0.1 N NaOH was added. An aliquot of 150 μl was counted. Values are adjusted to nanomoles of LPL bound to each nanomole of lipoprotein on the plate and expressed as mean ± S.D. (n = 3).

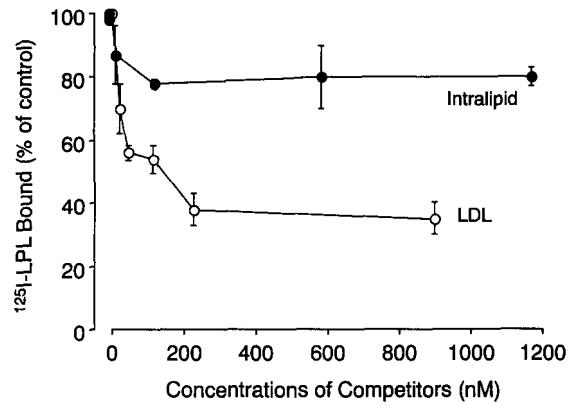

FIG. 2. **Competition for ^{125}I-LPL binding to LDL by LDL and Intralipid (20%).** The protocol used in this experiment was identical to the one described in the legend to Fig. 1 except that ^{125}I-LPL (5 μg/ml, 45 nM) was added along with competitors including unlabeled LDL and Intralipid (20%) and incubated for 2 h at room temperature. Binding to BSA-coated wells was also determined in each assay as a control and subtracted from the total binding. Values are mean ± S.D. (n = 3).

fold more HDL than LDL or VLDL bound to the plate at all concentrations of lipoproteins.

We next assessed ^{125}I-LPL binding to the lipoprotein-coated plates. First, we examined the time course of LPL binding to these lipoproteins. LPL binding reached equilibrium after overnight incubation at 4 °C (data not shown). LPL binding to albumin-coated plates was assessed and used as an estimate of nonspecific binding. Data shown in Fig. 1 were obtained after coating the plates overnight at 4 °C with 50 μg/ml HDL or LDL. This is a concentration above that required to saturate the plate. The data are expressed in terms of nanomoles of lipoprotein on the plate. ^{125}I-LPL (0.9–55 nM) binding to LDL (*open circles*) was significantly greater than to HDL (*solid circles*). Using 14 nM LPL, approximately 5-fold more LPL bound to the LDL-coated plates than to the HDL-coated plates. At 14 nM ^{125}I-LPL the molar ratio of LPL binding to LDL was 1:1. Although using greater LPL concentrations increased the molar ratio of LPL to LDL (*e.g.* to 1.6 using 55 nM ^{125}I-LPL), the rate of increase of this additional LPL binding was less steep. This suggests that a second, lower affinity process exists. Binding of LPL to VLDL was similar to that of LDL. At each concentration of ^{125}I-LPL used, nonspecific binding to BSA-coated plates was <10% of the total binding, and this was subtracted.

Competition of LDL and Intralipid (20%) for ^{125}I-LPL Binding to LDL—The experiments above suggested that LPL has a greater affinity for LDL than for HDL. We next tested whether LPL interaction with LDL was competed by excess unlabeled LDL or Intralipid. In these experiments, we used a short 2-h incubation at room temperature to minimize the hydrolysis of TG in Intralipid. Plates were coated overnight at 4 °C with LDL (50 μg/ml), and then ^{125}I-LPL at 45 nM was added alone or with various concentrations of competitors. ^{125}I-LPL binding to LDL was blocked by >95% by a 100-fold molar excess of unlabeled LPL (data not shown). This demonstrates the specificity of the interaction between ^{125}I-LPL and LDL. Unlabeled LDL at 9×10^2 nM inhibited LPL association with the plate by 65% (Fig. 2). In contrast, using a similar protocol and molar concentration of Intralipid particles, only about 20% inhibition was observed. Furthermore, inhibition of lipase activity with phenylmethylsulfonyl fluoride did not result in any difference in the competition between LDL and Intralipid for LPL binding (data not shown). These data suggested that LPL interaction with LDL involved more than a simple protein-lipid interaction.

Importance of Lipid versus Protein in LPL Interaction with LDL—To assess the role of LDL lipid in LPL-LDL interactions, we studied the effects of delipidation of LDL on LPL interaction with LDL. Plates were coated with [^{3}H]cholesteryl ester-rich emulsion particles and were delipidated by incubating with an acetone:ethanol (1:1) mixture for 1 h at −20 °C. Approximately 80% of the ^{3}H-lipid was removed by this method (data not shown). This protocol was then used to assess ^{125}I-LPL binding to delipidated LDL-coated plates. As shown in Fig. 3 (*inset*), LDL binding to control and LDL binding to delipidated plates were virtually identical. Fig. 3 shows data comparing LDL competition studies performed using control and delipidated LDL-coated plates. 181 nM unlabeled LDL inhibited the binding of ^{125}I-LPL to LDL-coated plates by approximately 50% in both control and delipidated plates. Thus, delipidation did not result in a significant reduction in ^{125}I-LPL binding to LDL nor was its binding to LPL more easily disrupted by competition with LDL in solution. These data further suggest that apoB, not LDL lipid, mediates LPL binding.

These experiments were repeated with a method described by Patton *et al.* (31) using hexane:isopropyl alcohol (3:2, v/v), which was shown to remove 95% of cholesteryl ester and TG. The results were identical to those obtained with acetone: ethanol (1:1). In a separate experiment, we examined the role of phospholipid components of LDL in LPL binding using microtiter plates coated with sphingomyelin, phosphatidylcholine, or lysolecithin. Binding of LPL to these phospholipids was less than 20% of the LPL binding to LDL (data not shown). These data suggest that LDL-LPL interaction is not mediated by interaction between phospholipid and LPL.

LPL Interaction with Apolipoproteins—To test whether LPL would bind to apoB and other apolipoproteins, ligand blotting was performed. Twenty μg of apoA1, apoE, and thrombin-digested human LDL were applied to 3–15% SDS-polyacrylamide gels under nonreducing conditions, transferred to nitrocellulose paper, and probed with biotinylated LPL. Among these proteins, only thrombin-digested fragments of apoB were recognized by LPL (Fig. 4). Furthermore, the positive reaction

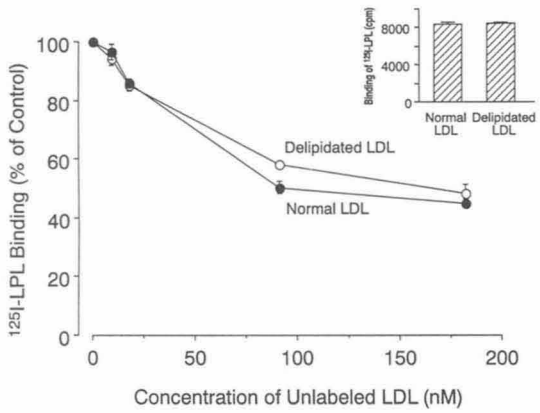

FIG. 3. **LPL binding to delipidated LDL.** Microtiter plates coated with LDL (50 μg of protein/ml) were delipidated using a mixture of acetone:ethanol (1:1) for 1 h at −20 °C followed by washing with 0.3% BSA. *Inset*, binding of ^{125}I-LPL (3 μg/ml, 27 nM) to delipidated plates was measured and compared with control non-delipidated plates using the protocol described in the legend to Fig. 1. Values are mean ± S.D. (n = 3). ^{125}I-LPL (3 μg/ml) binding to control and delipidated microtiter plates was competed by the addition of increasing amounts of LDL. Shown are the amounts of bound LPL. Values are mean ± S.D. (n = 3).

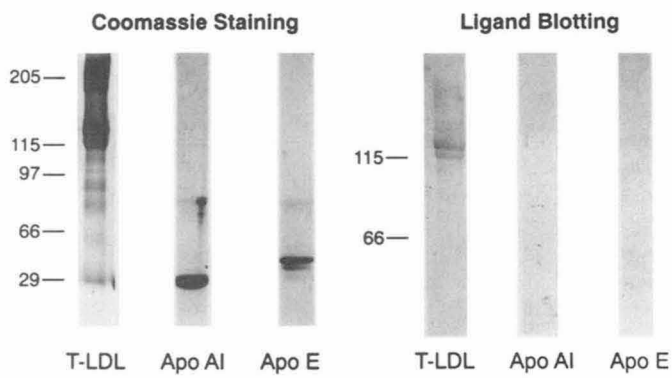

FIG. 4. **LPL interaction with apolipoproteins.** ApoA1, apoE, and thrombin-digested human LDL (20 μg) were applied to 3–15% SDS-polyacrylamide gels under nonreducing conditions. Gels were either stained with Coomassie Brilliant Blue R-250 (*right panel*) or transferred to nitrocellulose paper followed by ligand blotting with biotinylated LPL (*left panel*). Proteins bound to biotinylated LPL were visualized by incubation with avidin-conjugated horseradish peroxidase followed by development with 4-chloro-1-naphthol. *Lane 1*, thrombin-digested human LDL; *lane 2*, apoA1; *lane 3*, apoE

was confined to amino-terminal parts of apoB, in agreement with the previous data (32). Neither apoA1, apoE, nor the carboxyl-terminal regions of apoB were recognized on ligand blots.

Monoclonal Antibody Inhibition of LDL-LPL Interaction—To further characterize the interaction between LPL and apoB, we used several monoclonal antibodies against apoB. In this experiment microtiter plates were also coated with LPL (90 nM) at 4 °C. Following washing and blocking, ^{125}I-LDL (18 nM) was added with antibodies (1:50 dilution of ascites) and incubated for 2 h at 4 °C. As shown in Fig. 5, both mAb 3 and mAb 19 inhibited ^{125}I-LPL binding to LDL by 80%, whereas mAb 47 inhibited binding by only 25%. In other experiments we showed that this concentration of mAb 47 inhibited specific LDL binding to fibroblasts by >90%.

ApoB17 Binding to LPL—To further assess the role of the amino-terminal region of apoB *versus* LDL lipid in LPL-LDL interaction, we performed competition experiments using apoB17 and cholesteryl ester-rich emulsion particles. In these experiments, ^{125}I-LPL binding to LDL-coated plates was competed with apoB17 and cholesteryl ester-rich emulsions. BSA

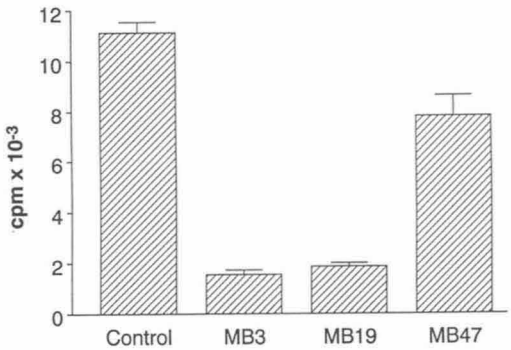

FIG. 5. **Monoclonal antibody inhibition of LPL-LDL interaction.** Microtiter plates were coated with LPL (90 nM) overnight at 4 °C. Following washing and blocking, ^{125}I-LDL (18 nM) were added with antibodies (1:50 dilution of ascites) and incubated for 2 h at 4 °C. Binding of ^{125}I-LDL was measured using the same protocol as described in the legend to Fig. 1. Values are mean ± S.D. (n = 3). *MB*, monoclonal antibody.

was also used as a negative control, and it did not inhibit the ^{125}I-LPL binding to LDL (data not shown). ApoB17 competed; apoB17 at 1.7×10^3 nM decreased LPL binding to the plates by 74%. In contrast, cholesteryl ester-rich emulsion particles at 1.8×10^3 nM inhibited ^{125}I-LPL binding to LDL-coated plates by approximately 30% (Fig. 6*A*).

We then determined the binding of apoB17 to LPL using LPL-coated plates. The apoB17 was detected by enzyme-linked immunosorbent assay as described under "Materials and Methods." Fig. 6*B* shows binding of apoB17 (0–1 μg/ml, 45 mM) to LPL- and BSA-coated plates. At 0.1 μg/ml (4.5 nM) approximately 10-fold more apoB17 bound to LPL-coated plates than to BSA-coated plates. Thus, several types of experiments support the hypothesis that LPL binding to LDL is modulated by apoB and that this process involves a specific interaction with the amino-terminal region of apoB.

DISCUSSION

The present experiments suggest a new role for the amino-terminal region of apoB as a mediator of LPL interaction with lipoproteins. Data supporting this conclusion were obtained by several different methods. When ^{125}I-LPL was added to LDL- or HDL-coated plates and incubated at 4 °C overnight to achieve equilibrium, severalfold more LPL bound to LDL- than to the HDL-coated plates (*e.g.* approximately 6-fold with 55 nM ^{125}I-LPL). Binding of LPL to VLDL was similar to that of LDL, indicating that protein-protein interaction between LPL and apoB is more important than protein-lipid interaction. In these experiments, we did not calculate the kinetic parameters using a method such as Scatchard analysis (33) for several reasons. First, LPL aggregates at higher concentrations. Second, LPL dimers dissociate over time. Thus, it is likely that monomerization and denaturation of the LPL occurred during the overnight incubation. Because active dimeric LPL is more likely to associate with LDL and inactive LPL associates with HDL (12), the K_d for active LPL binding to LDL might be lower and the K_d for HDL higher. Nonetheless, we conclude that LPL interaction with LDL is greater than with HDL and hypothesize that this is due to the presence of apoB on LDL.

Further support for this hypothesis was obtained using ligand blots. LPL bound to amino-terminal fragments of apoB generated by thrombin digestion but not to apoA1, apoE, or carboxyl-terminal fragments of apoB. Thus, the reason LPL preferentially associates with LDL in plasma and on cell surfaces might result from LPL association with apoB.

Apolipoproteins other than apoB may interact with LPL. Both apoCIII (34) and apoE (35) have been reported to decrease

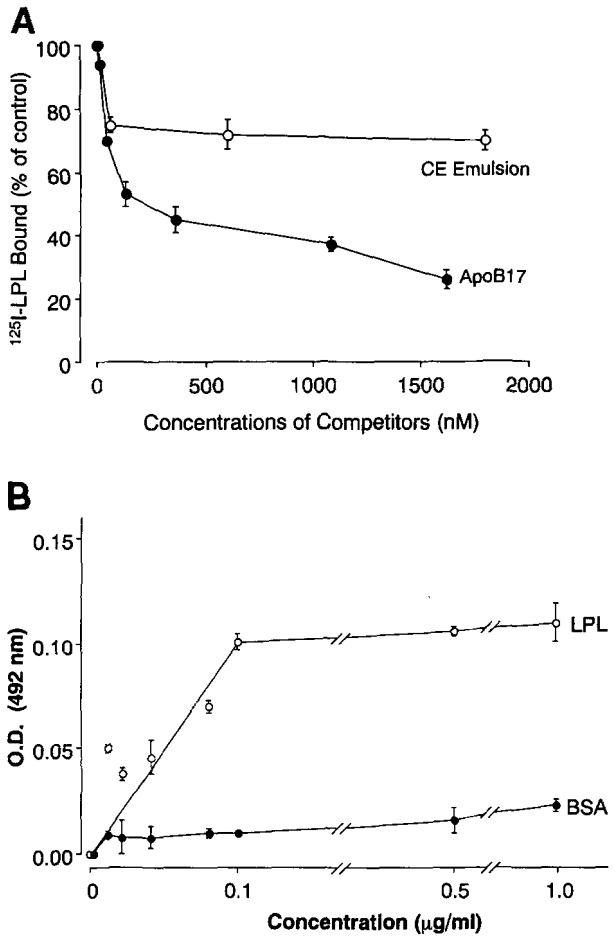

FIG. 6. **Competition of ^{125}I-LPL binding to LDL by apoB17 and cholesteryl ester-rich lipid emulsion (*CE Emulsion*).** *A*, the protocol used in this experiment was identical to that described in the legend to Fig. 2. ApoB17 and cholesteryl ester-rich lipid emulsions were added along with ^{125}I-LPL as competitors for ^{125}I-LPL binding to LDL. Values are mean ± S.D. (n = 3). *B*, binding of apoB17 to LPL. Binding of apoB17 to LPL was measured by enzyme-linked immunosorbent assay. Bovine LPL or BSA (control) at 5 μg/ml was incubated with microtiter plates overnight at 4 °C. ApoB17 was added at 0–1 μg/ml and incubated for 2 h at room temperature. After washing the plates, mAb 19 (1:500 dilution of ascites) was added and incubated for 2 h at room temperature. Unbound antibodies were removed, and horseradish peroxidase-conjugated goat anti-mouse IgG at 1:500 dilution was added. After one and a half hours of incubation, 100 μl of substrate solution containing *o*-phenylenediamine dihydrochloride, 5 μl of H_2O_2 in 0.1 M citric acid (pH 6) was added to each well. Absorbance at 492 nm was measured. Values are mean ± S.D. (n = 3).

LPL activity when added to some *in vitro* assay systems. ApoE, however, did not interact with LPL on ligand blots (Fig. 4). ApoE shares two properties with apoB that make it unique among plasma apolipoproteins; both bind to LDL receptors and both interact with a variety of glycosaminoglycans including heparin (36, 37). Because the homologous areas of apoB and apoE are near the carboxyl-terminal region of apoB (38) and LPL binds to the amino-terminal portion of apoB, it is not surprising that apoE did not directly bind to LPL on ligand blots. ApoCII is the obligatory activator for LPL hydrolysis of lipoprotein TG, but the mechanism of apoCII-LPL interaction is uncertain. Surprisingly, Shirai *et al.* (39) showed that addition of apoCII to phospholipid emulsion particles decreased LPL association with the emulsion. Thus, LPL interaction with apoB appears to differ from its interaction with other apolipoproteins.

A second lipoprotein component that might affect LPL-lipoprotein interaction is lipid. Lipoproteins contain both core and surface lipid. In addition to affecting the size and geometry of the lipoprotein surface, some hydrophobic core lipids (TG and cholesteryl ester) are thought to be exposed on the lipoprotein surface (41). To assess the role of lipids in LPL binding to particles, LPL binding to LDL was competed with Intralipid (a TG-rich, protein-deficient emulsion) and a cholesteryl ester-rich emulsion. LPL binding to LDL was inhibited by excess LDL and by the amino-terminal fragment of apoB. Less inhibition was observed with the same or greater molar concentrations of lipid emulsion particles.

Further evidence that lipid is not critical for LPL-LDL interaction was obtained by comparing LPL binding to LDL- and delipidated LDL-coated plates. Binding and competition studies using these two LDL-coated plates were indistinguishable. Thus, neither the core nor surface lipids interacted with LPL in the same manner as apoB.

Active LPL is thought to be a noncovalently linked homodimer, and each subunit molecular mass is approximately 55 kDa. This enzyme contains five functional sites (15, 42) including (*a*) a catalytic site containing an active serine residue, (*b*) an interfacial substrate recognition site consisting of hydrophobic basic residues, (*c*) a heparin binding site, (*d*) an apolipoprotein CII binding site, and (*e*) a site for subunit-subunit interaction. Although it has been suggested that LPL interaction with lipoproteins involves the hydrophobic lipid recognition sites, there are several studies that do not support this hypothesis. Hydrophobic interactions are potentiated by high ionic strength solutions; however, LPL is dissociated from lipoproteins and lipid particles in high salt solutions (11). Although LPL appears to interact with polar lipid, *i.e.* phospholipid, our studies using phospholipid-coated plates (data not shown) and previously published data (17) demonstrate that such an interaction is much weaker than LPL association with lipoproteins.

It is not clear whether LPL interaction with apoB is required for lipolysis of TG-rich lipoproteins. In our preliminary experiments, a monoclonal antibody against apoB, mAb 19, did not inhibit LPL hydrolysis of VLDL, suggesting that this interaction is not required for lipolysis (data not shown). However, Connelly *et al.* (40) reported that lipolysis of VLDL by LPL was noncompetitively inhibited by LDL and intermediate density lipoproteins. Thus, LPL may associate with LDL or intermediate density lipoproteins, and this in turn prevents the association of LPL with VLDL.

ApoB100, a large glycoprotein with a molecular mass of 550 kDa, is virtually the only protein component of LDL particles. The carboxyl-terminal region of apoB is involved in binding to LDL receptors, and this region also contains five of seven apoB heparin binding sites (43). Under physiologic ionic conditions, however, LDL binds weakly to heparin (44), demonstrating the importance of tertiary structure in apoB interaction with other molecules. ApoB also contains numerous hydrophobic domains throughout its length that are believed to be important in lipid binding (45) and multiple proline-rich sequences predicted to form amphipathic β-sheets and β-turns that are thought to have high lipid binding potential (46, 47). These sequences, however, are not found in the amino-terminal 1000 amino acids. In addition, apoB contains 25 cysteine residues of which 12, in disulfide form, are located in the first 500 amino acids. Thus, the amino-terminal region of apoB is thought to be a globular structure that extends away from the lipid core of lipoproteins (48).

The tertiary structure of apoB varies as a function of the size, lipid composition, and, perhaps, apolipoprotein content of lipoproteins. Galeano *et al.* (49) recently demonstrated that LDL particle size rather than core lipid composition was the major

determinant of LDL interaction with the LDL receptor. Kunitake *et al.* (50) reported that (*a*) the type of lipoproteins on which apoB resides, *i.e.* VLDL, intermediate density lipoprotein, or LDL, and (*b*) the core lipid composition, *i.e.* TG:cholesterol ratio, affect the conformation of apoB in the amino-terminal region. Similarly, McKeone *et al.* (51) demonstrated that TG induced structural changes in apoB remote from the receptor binding region. These changes included differences in immunoreaction with antibody mAb 3 and *Staphylococcus aureus* V8 protease cleavage at approximately the first 100 kDa of apoB. Whether such differences in the conformation of the amino-terminal region of apoB alter LPL-lipoprotein association remains to be established.

LPL-apoB interactions could play a role in efficient catabolism of circulating lipoproteins. The initial event in lipoprotein TG hydrolysis may be apoE binding to heparan sulfate proteoglycans, a process that anchors circulating lipoproteins to the endothelial cell surface. Data supporting this function of apoE have been reported by several laboratories (52, 53). We postulate that a secondary interaction that further approximates the lipoprotein and LPL is LPL interaction with the amino-terminal region of apoB.

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