Lipoprotein lipase (LPL) efficiently mediates the binding of lipoprotein particles to lipoprotein receptors and to proteoglycans at cell surfaces and in the extracellular matrix. It has been proposed that LPL increases the retention of atherogenic lipoproteins in the vessel wall and mediates the uptake of lipoproteins in cells, thereby promoting lipid accumulation and plaque formation. We investigated the interaction between LPL and low density lipoproteins (LDLs) with special reference to the protein-protein interaction between LPL and apolipoprotein B (apoB). Chemical modification of lysines and arginines in apoB or mutation of its main proteoglycan binding site did not abolish the interaction of LDL with LPL as shown by surface plasmon resonance (SPR) and by experiments with THP-1 macrophages. Recombinant LDL with either apoB100 or apoB48 bound with similar affinity. In contrast, partial delipidation of LDL markedly decreased binding to LPL. In cell culture experiments, phosphatidylcholine-containing liposomes competed efficiently with LDL for binding to LPL. Each LDL particle bound several (up to 15) LPL dimers as determined by SPR and by experiments with THP-1 macrophages. A recombinant NH2-terminal fragment of apoB (apoB17) bound with low affinity to LPL as shown by SPR, but this interaction was completely abolished by partial delipidation of apoB17. We conclude that the LPL-apoB interaction is not significant in bridging LDL to cell surfaces and matrix components; the main interaction is between LPL and the LDL lipids.

The functional location of LPL is at the vascular side of endothelial cells where it is anchored through electrostatic interaction with heparan sulfate proteoglycans. LPL activity is regulated according to the nutritional state, in a tissue-specific manner, based on the needs of the tissue for fatty acids.

In addition to its lipolytic activity, LPL acts as a potent bridge between lipoproteins and cell-surface proteoglycans and lipoprotein receptors or components of the extracellular matrix (1, 3–6). The presence of LPL increases by severalfold the binding of lipoproteins to cells in culture, to perfused livers, and to matrix components deposited on culture dishes by fibroblasts. This bridging ability of LPL is independent of its catalytic activity (7).

LPL is produced by macrophages and smooth muscle cells and has been found in the arterial wall in connection with atherosclerotic lesions (8–10). Recently it has been suggested that LPL contributes to the retention of low density lipoprotein (LDL) in the vessel wall by bridging LDLs to either macrophages or the extracellular matrix (1, 3, 5, 11, 12). Specific biological responses to the retained lipoproteins lead to biochemical and cellular events that promote atherogenesis (13, 14). In vitro, LDL and other lipoproteins containing apolipoprotein (apo)B bind weakly to heparin and other proteoglycans (15). It is therefore possible that molecules such as LPL, which enhances the interaction of LDL with proteoglycans, could play an important role in atherogenesis in vivo.

Goldberg et al. proposed that protein-protein interaction between LPL and apoB, the sole protein moiety of LDL, is more important than the interaction between LDL and LDL lipids (16–18). However, LPL interacts efficiently with liposomes and lipid emulsions that do not contain any apolipoprotein (19, 20). We therefore investigated whether the interaction between LPL and LDL is mediated primarily by the LDL protein (apoB) or by LDL lipids. For these studies, we produced modified and recombinant LDL and a recombinant fragment of apoB (apoB17) for experiments with surface plasmon resonance (SPR) (21–23) and cultured THP-1 monocyte-derived macrophages.

**EXPERIMENTAL PROCEDURES**

**Materials**—LPL was isolated from bovine milk as previously described (24). THP-1 monocytes were purchased from the American Type Culture Collection (Manassas, VA). An amino-coupling kit containing N-hydroxysuccinimide, N-ethyl-N’-[diethylamino]propyl]carbodiimide, 1 x ethanolamine, and CM5 sensor chips was obtained from BIAcore (Uppsala, Sweden). Streptavidin was purchased from Sigma. RPMI 1640 medium with GlutaMAX II, TC-100 medium, SF-900 serum-free medium, and Eagle’s minimum essential medium without methionine were obtained from Life Technologies, Inc. Fetal calf serum was from Biochrom KG (Berlin, Germany). Garamycin was from Schering (Kenilworth, NJ). Methionine, fatty acid-free bovine serum albumin was purchased from PAA Laboratories (Laxenburg, Austria). The remaining chemicals were purchased from Sigma, Merck, or Roth.
min, sodium pyruvate, disodium carbonate, sodium hydrogen carbonate, acetate anhydride, cyclohexanone, phenylmethylsulfonyl fluoride, butylated hydroxytoluene, benzamide, N-\(^{\text{\textregistered}}\)-p-tosyl-\(\text{\textregistered}\)-lysine chloromethyl ketone, pepstatin A, leupeptin, and phorbol 12-myristate 13-acetate were from Sigma. Heparin was from Leo Pharma AB (Malmö, Sweden). Trypan blue-methylene blue buffered saline contained 0.2% (w/v) NaN\(_3\), and apob were from DAKO (Glostrup, Denmark). Trypsolin (aprotinin) was from Bayer (Leverkusen, Germany). N-Acetyl-Leu-Leu-norleucinal was from Roche Molecular Biochemicals. Carrier-free Na\(^{125}\)I was from Nor- dian (Kenon, Ontario, Canada). l-a-Phosphatidylcholine from egg yolk (type XI-E) and L-a-lysophosphatidylcholine (Type I) were from Sigma. Cells—Transgenic mice were cultured from mouse stock purchased from DAKO (10 ml of 1-butanol:diisopropyl ether (1:1), and the radioactivity in lipid and protein moieties was determined with a CHOD-PAP kit (Axiom, Berlin, Germany), and used for the dispersion and were used immediately. Cholesterol was produced as described by Gretch et al.

**Radiolabeling of Lipoproteins for Cell Experiments—** LDLs were delipidated with diisopropyl ether:butanol essentially as described previously (29). To selectively modify arginines or lysines in apoB100, recombinant LDLs were incubated with acetic anhydride or cyclohexanone, respectively, as described by Innerarity et al. (30).

**Chemical Modification of ApoB—** To selectively modify arginines or lysines in apoB100, recombinant LDLs were incubated with acetic anhydride or cyclohexanone, respectively, as described by Innerarity et al. (30).

**Binding Studies by SPR—** The binding studies were performed on a BIACore 2000 instrument. Biotinylaton and analysis of kinetics were performed as described previously (21, 22, 34). Briefly, avidin was immobilized on the surface of the flow cell of the BIACore 2000 using N\(^{\text{\textregistered}}\)-(diethylamino)propyl]carbodiimide/n-hydroxysuccinimide. The specific radioactivity of both preparations was about 500 cpm/ng.

**Determination of Mass/Response Relationship for LDL and LPL—** The binding experiments were carried out in 10 mM Hepes, 0.15 M NaCl, pH 7.4, at 37 °C with the same amount of \(^{125}\)I-LDL and LPL as in the binding studies. The cells were not cooled before the experiment, and all washings were performed with warm solutions.

**Determination of LDL Binding to Heparin and LPL—** To investigate, in real time, how LPL interacts with LDL we used SPR. The use of this method to study interactions of LPL and heparin/eparan sulfate proteoglycans with lipoproteins was described previ
showed that the delipidation did not result in any oligomer chromatography analysis of the partially delipidated LDL soluble after removal of even more lipids without altering the of total cholesterol and 51% of triglycerides). LDLs remain on basic residues in apoB100 or on any specific binding site in that the interaction between LPL and LDL was not dependent similar binding affinities for LPL (Table I). These results show apoB100-containing LDL and apoB48-containing LDL had

We then studied the importance of the carboxyl terminus of heparin. LPL increased the binding of all LDL variants to somewhat lower affinity than LDL purified from human (controls).

The presence of LPL on heparin or proteoglycans markedly increased their affinity for heparin but significantly lowered their affinity for LPL (Table I).

**Table I**

| LDL variant | Binding of LDL to sensor chip |
|-------------|-------------------------------|
|             | Heparin | LPL | LPL-heparin |
| LDL         | 100     | 100 | 100         |
| Ac-LDL      | 0       | 64  | 45          |
| CHD-LDL     | 0       | 156 | 74          |
| Mut-LDL     | 0       | 62  | 66          |
| RecomLDL100 | 78      | —   | 66          |
| RecomLDL48  | 120     | —   | 72          |
| Delipidated LDL | 2200 | 16  | —           |

The relative affinities are expressed as percentages of the binding of native LDL, isolated from human plasma (LDL). The concentrations of LDL, expressed in protein concentration, were 0.3 mg/ml in experiments with immobilized heparin and 0.05 mg/ml in experiments with immobilized LDL or LPL-heparin. Data shown are mean values of duplicate determinations. Affinities were calculated as described under "Experimental Procedures." Some combinations were not studied due to limited amounts of material (—). RecomLDL100, recombinant LDL containing apoB100; RecomLDL48, recombinant LDL containing apoB48.

## LPL Binding to Lipoprotein Lipase Is Dependent on Lipids

We used. In experiments performed at 37 °C, most of the LDL-derived radioactivity associated with cells was heparin-resistant and probably corresponded to LDL that had been taken up by the cells. In agreement with the results of SPR studies, recombinant LDL with apoB48 and recombinant LDL with apoB100 bound to cells with similar affinity (Table III). We conclude that LDL with decreased or abolished ability to interact with proteoglycans and certain lipoprotein receptors can still to a large extent be bound to cells if LPL is present.

**Competition between LDL and Liposomes for LPL-mediated Binding to Cells**—To investigate the role of the LDL lipids in the interaction with LDL macrophages were incubated with LDL in the presence of PC liposomes (Fig. 1). LPL-mediated binding of LDL to the cells was markedly reduced, demonstrating that liposomes competed efficiently with LDL for interaction with LPL. Similar results were obtained with liposomes made of PC only, a mixture of PC and lyso-PC, or PC and FC.

**Stoichiometry of the LPL-LDL Interaction as Studied by SPR**—LDLs contain only one copy of apoB100 per particle (41). If there were only one specific interaction site in apoB100 for LDL, a 1:1 molar ratio would be expected for the interaction between LDL and LPL. To estimate how many LPL molecules bind per LDL particle, we first determined the mass/response correlation for LPL and LDL on the Biacore sensor chip (Fig. 2B). The correlation was linear, but the slope was 1.4 times lower for LDL than for lipid-free proteins like LPL and other previously studied proteins (42). Hence, an LDL surface density of 1 ng/mm² increased the response level by 715 RU (Fig. 2B). Using this value, we determined the stoichiometry of the LPL-LDL interaction by passing three different concentrations of LPL over sensor chips on which LDL had been immobilized; the response at steady state was then used to calculate the amount of bound LPL (Fig. 2A). Saturation occurred at around 14 LPL molecules/LDL particle. At LPL concentrations >100 nM, high nonspecific binding of LPL to the dextran matrix (20–40% of the total binding) might have affected the stoichiometric determination. At LPL concentrations <50 nM (about 5 μg/ml), the association kinetics could be fitted to a single exponential function, suggesting that up to three to four LPL dimers can interact independently with LDL. The interaction was characterized by a very high association rate constant (kₐ = 2.1 × 10⁻⁶ M⁻¹ s⁻¹) and a low dissociation rate constant (k₅ = 10⁻⁴ s⁻¹). Therefore, several LPL molecules bound to each LDL particle. Simultaneous interaction with many LPL molecules may explain the strong effect of LPL on binding of LDL to heparin-covered surfaces and to cells.

**Stoichiometry of the LPL-LDL Interaction as Studied with THP-I Macrophages**—Cells were incubated at 4 °C with in-

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Table II

| LDL variant | Heparin-releasable LDL at 4 °C (binding) | Heparin-resistant LDL at 37 °C (uptake) |
|-------------|------------------------------------------|----------------------------------------|
|             | Without LPL                              | With LPL                               |
| LDL         | 1753 ± 135                               | 31 ± 2                                 |
| Ac-LDL      | 490 ± 41                                 | 48 ± 12                                |
| CHD-LDL     | 1512 ± 10                                | 25 ± 7                                 |
| Mut-LDL     | 885 ± 47                                 | 57 ± 10                                |
| RecomLDL100 | 1184 ± 103                               | 28 ± 3                                 |
| LDL variant | Without LPL                              | With LPL                               |
| LDL         | 1529 ± 41                                | 1038 ± 44                              |
| Ac-LDL      | 479 ± 28                                 | 823 ± 28                               |
| CHD-LDL     |                                          |                                        |
| Mut-LDL     |                                          |                                        |
| RecomLDL100 |                                          |                                        |

Table III

| LDL variant | Heparin-releasable LDL at 4 °C (binding) | Heparin-resistant LDL at 37 °C (uptake) |
|-------------|------------------------------------------|----------------------------------------|
|             | Without LPL                              | With LPL                               |
| RecomLDL100 | 6                                         | 61                                     |
| RecomLDL48  | 13                                        | 96                                     |

Fig. 1. Inhibition of LPL-mediated binding of LDL to THP-1 cells by liposomes. THP-I monocyte-derived macrophages were incubated for 30 min at 4 °C with a mixture of 125I-labeled LDL (5 μg/ml), bovine LPL (10 μg/ml), and liposomes (LS) prepared from PC, FC, and lyso-PC. Heparin-releasable radioactivity representing bound LDL was determined as described under “Experimental Procedures.” Data are mean values from duplicate determinations. Open bars indicate binding in the absence of liposomes. Open bars indicate binding in the presence of PC liposomes only (135 μg/ml of incubation medium in the case of nondiluted liposomes, 13.5 μg/ml in the case of 1:10 diluted liposomes). Hatched bars indicate liposomes of PC and FC (95 μg/ml and 40 μg/ml, respectively, in the case of nonordinated liposomes). Cross-hatched bars indicate liposomes of PC and lyso-PC (total concentration was 135 μg/ml of medium in the case of nonordinated liposomes).

Fig. 2. Stoichiometry of binding of LPL to immobilized LDL as determined by SPR. A, LPL at the indicated concentrations was injected into Biacore flowcells containing matrix-bound LDL. The response values (RU) at steady state were used to calculate the mass of LPL bound to LDL. These values were then used to calculate the binding stoichiometry of LPL dimers (110 kDa) to LDL on a molar basis. The stoichiometries presented are mean values of data from three different surface concentrations of matrix-bound LDL (1.5, 2.5, and 3.5 ng/mm²). The experiments were performed in 10 mM Hepes, 0.15 M NaCl, pH 7.4. B, the mass/response correlation for LDL compared with LPL. Different amounts of 125I-labeled LDL or 125I-labeled LPL were coupled to sensor chips, and the response value for each chip was determined. The bound radioactivity was measured, and the immobilized mass was calculated from the respective specific radioactivities. Relationships are shown for LDL total mass (filled circles), LDL protein mass (open circles), and LDL protein mass (squares).

This was constantly more than was bound in the absence of LDL (2.7 μg/mg of cell protein). The calculated molar ratio of LPL/LDL bound to the cells was around 15 in this experiment.

Binding of ApoB17 to LPL—To directly study the interaction between LPL and apoB, we used recombinant apoB17, which contains the first 771 amino acids of apoB and has a molecular mass of ~87 kDa (33). SPR studies showed that the affinity of apoB17 for LPL was very weak (1/1000) compared with the affinity of LDL for LPL (Table IV). However, even after purification on an anti-apoB affinity column, apoB17 con-
macrophages and calculation of stoichiometry between LPL and LDL under near saturating conditions. Binding of \(^{125}\)I-labeled LDL and LPL to THP-1 monocyte-derived macrophages was studied as described in Fig. 1. In all experiments the concentration of LDL was 5 \(\mu\)g/ml. A, inverted triangles, 1 \(\mu\)g of LPL/ml of medium; circles, 10 \(\mu\)g of LPL/ml; squares, 20 \(\mu\)g of LPL/ml; and triangles, 50 \(\mu\)g of LPL/ml. B, the concentration of LPL added was 10 \(\mu\)g/ml of medium. The standard bar (hardly visible) indicates binding of LDL without LPL. The open bar indicates binding of LDL in the presence of LPL. The hatched bar indicates binding of LPL in the presence of LDL. The crosshatched bar indicates binding of LPL without LDL.

The concentration of LPL added was 10 \(\mu\)g/ml of medium. The solid bar (hardly visible) indicates binding of LDL without LPL. The open bar indicates binding of LDL in the presence of LPL. The hatched bar indicates binding of LPL in the presence of LDL. The crosshatched bar indicates binding of LPL without LDL.

DISCUSSION

LPL has been proposed to have a role in atherosclerosis, functioning as a bridge that links atherogenic LDL with the subendothelial matrix of the arterial wall. Retention of LDL in the arterial wall may be a key initiating event in atherogenesis (12). LPL can also directly mediate binding and uptake of atherogenic lipoproteins by cells in the vessel wall, thereby promoting lipid accumulation in these cells. Recent data from animal models have provided compelling evidence that LPL located in the arterial wall is proatherogenic (10, 45–47).

To investigate the role of apoB in the LPL-LDL interaction, we first tested how modifications of LDL affect its ability to interact with LPL. In contrast to earlier studies (48), our results demonstrate that chemical modification of the arginines or lysines in apoB100 did not abolish the LPL-LDL interaction. Therefore, basic residues in apoB, which are essential for normal LDL-proteoglycan interaction (49, 50), are not important for the interaction with LPL. Thus, LPL must interact with another site or sites in LDL. This explains why oxidized LDL can have higher affinity for LPL than native LDL has for LPL even though the basic residues in apoB lose their positive charges during LDL oxidation (19, 22, 36, 51, 52).

Eight regions in delipidated apoB100 bind heparin (46–48). We previously showed that one of these sequences (residues 3359–3369) is the main proteoglycan binding site in apoB100-containing LDL (34) and is also the heparin binding site in LDL in vivo. This finding is in agreement with recent data by Gaus et al. (23) showing that only one or two heparin molecules are involved in binding the LDL. However, our data imply that partial delipidation exposes several of the remaining heparin binding sequences, resulting in an enhanced binding of heparin. In the current study, partially delipidated LDL had decreased binding affinity for LPL, indicating that lipid is required for proper LPL-LDL interaction.

Because it had been suggested that apoB interacts specifically with LPL (17, 18), a major emphasis in this study was to determine whether a LDL particle can bind more than one LPL dimer. In SPR experiments, the average molar ratio of LPL molecules per LDL particle at saturation is around 14. Because the saturation of LDL by LPL was achieved at relatively high LPL concentrations, one might question the physiological significance of the interaction. However, even at nanomolar concentrations of LPL, the molar ratio was clearly greater than 1. In experiments with cultured macrophages, the calculated stoichiometry in the binding of LDL to LPL on the cell surface also indicated that several (up to 15) LPL dimers could bind per LDL particle. In these experiments a consistent finding was that more LPL bound to the cells in the presence of LDL than in the absence of LDL, indicating secondary attachment of some LPL to already bound LDL.

These results are incompatible with the notion that apoB has only one specific LDL binding site; instead, they imply that there are many LDL-binding sites on apoB or, more likely, that LPL mainly binds to the exposed lipid surface. ApoB covers 40–60% of the surface area of LDL (53). The remaining surface should be exposed for interaction with other proteins. The hydrated radius of dimeric LDL is 4.4 nm (54). Thus, packed together with apoB, 20–25 LPL dimers could be accommodated on a LDL particle. The finding that partially delipidated LDL had markedly decreased binding affinity for LPL strongly in-
indicates that the majority of LPL molecules interact directly with the LDL lipid. This was further supported by the results of competition assays of the LPL-LDL interaction with liposomes.

In vivo, the situation is different: LPL is bound to cell-surface heparan sulfates, and LDLs are passed over the cell surfaces by blood circulation. A similar situation was analyzed by SPR in a previous study (21). At that time, we did not have values for the mass/response relationship for LDL. Therefore the effect of the amount of LPL on the amount of bound LDL was expressed in RU. Our previous study indicated that detectable LDL binding starts when three or four heparan sulfate-bound LPL dimers interact simultaneously with the LDL particle and that the amount of bound LDL is proportional to the concentration of LPL at the surface. The slope of this relationship indicates how much LPL is needed to form a binding site for LDL. Knowing the mass/response relationship for LDL, we can now calculate that six or seven LPL dimers were needed for optimal binding of LDL to artery wall proteoglycans and to cells of the vessel wall although the amounts of LPL at these sites may not be high enough to allow the highest affinity interaction between one LDL particle and many LPL dimers.

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