High Affinity Binding between Lipoprotein Lipase and Lipoproteins Involves Multiple Ionic and Hydrophobic Interactions, Does Not Require Enzyme Activity, and Is Modulated by Glycosaminoglycans*

Received for publication, June 19, 2000
Published, JBC Papers in Press, July 5, 2000, DOI 10.1074/jbc.M005317200

M. Mahmood Hussain¶¶¶, Joseph C. Obunike**, Afsar Shaheen‡, M. Jawaad Hussain‡, Gregory S. Shelness†††, and Ira J. Goldberg**

From the ¶Department of Biochemistry, School of Medicine, Medical College of Pennsylvania Hahnemann University, Philadelphia, Pennsylvania 19129, the ¶¶Department of Anatomy and Cell Biology and Pediatrics, SUNY Health Science Center at Brooklyn, Brooklyn, New York 11203, the ‡Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157, and the **Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Lipoprotein lipase (LPL) physically associates with lipoproteins and hydrolyzes triglycerides. To characterize the binding of LPL to lipoproteins, we studied the binding of low density lipoproteins (LDL), apolipoprotein (apo) B17, and various apoB-FLAG (DYKDDDDK octapeptide) chimeras to purified LPL. LDL bound to LPL with high affinity (Kd values of 10–12 M) similar to that observed for the binding of LDL to its receptors and 1D1, a monoclonal antibody to LDL, and was greater than its affinity for microsomal triglyceride transfer protein. LDL-LPL binding was sensitive to both salt and detergents, indicating the involvement of both hydrophobic and hydrophilic interactions. In contrast, the N-terminal 17% of apoB interacted with LPL mainly via ionic interactions. Binding of various apoB fusion peptides suggested that LPL bound to apoB at multiple sites within apoB17. Tetrahydrolipstatin, a potent enzyme activity inhibitor, had no effect on apoB-LPL binding, indicating that the enzyme activity was not required for apoB binding. LDL-LPL binding was inhibited by monoclonal antibodies that recognize amino acids 380–410 in the C-terminal region of LDL, a region also shown to interact with heparin and LDL receptor-related protein. The LDL-LPL binding was also inhibited by glycosaminoglycans (GAGs); heparin inhibited the interactions by ~50% and removal of trace amounts of heparin from LPL preparations increased LDL binding. Thus, we conclude that the high affinity binding between LPL and lipoproteins involves multiple ionic and hydrophobic interactions, does not require enzyme activity and is modulated by GAGs. It is proposed that LPL contains a surface exposed positively charged amino acid cluster that may be important for various physiological interactions of LPL with different biologically important molecules. Moreover, we postulate that by binding to this cluster, GAGs modulate the association between LDL and LPL and the in vivo metabolism of LDL.

Lipoprotein lipase (LPL)† is a dynamic molecule that performs several functions during the catabolism of triglyceride-rich chylomicrons and very low density lipoproteins. First, it is essential for the hydrolysis of triglycerides present in the core of these lipoproteins. The hydrolysis results in the delivery of free fatty acids to peripheral tissues. Defects in the enzyme activity lead to Type 1 hyperlipidemia characterized by accumulation of triglyceride-rich lipoproteins, defective chylomicron removal, and the development of pancreatitis (1, 2). Second, LPL anchors lipoproteins to the cell surface and matrix proteoglycans and increases their retention by subendothelial matrix and uptake by cells. Third, it acts as a ligand and binds to various members of the LDL receptor family and may play a role in the cellular endocytosis of lipoproteins (3, 4). LPL performs these various functions by physically interacting with lipoproteins, proteoglycans, and receptors. LPL favors binding to apoB-containing lipoproteins compared with apoA-I-containing lipoproteins. The molecular and biochemical basis for the binding of LPL to apoB-containing lipoproteins are not completely understood.

It has been shown that protein-protein interactions between LPL and LDL involve the N-terminal 17% of apoE (5–7). However, the LPL-binding site(s) in the N-terminal 17% of apoB and apoB-binding site(s) in LPL have not been identified. The N-terminal 17% of apoB has been postulated to exist as an α-helical domain (8–10). This region is essential for the secretion of various C-terminal lipid-binding sequences of apoB (11). Recently, this region has been shown to contain elements necessary for MTP binding (12, 13). Although the N-terminal region of apoB interacts with LPL, the physiological importance of this is unclear. To better understand these interactions, we tested the effects of salt, detergents, and glycosaminoglycans (GAGs) on LPL-apoB binding. Furthermore, we attempted to identify the binding sites responsible for LPL-apoB interactions.

**EXPERIMENTAL PROCEDURES**

*Materials—Bovine milk LPL was purified using heparin affinity chromatography as described before (14). For some experiments, LPL was purified further by Biogel-P60 column chromatography. LPL purified from heparin affinity column was applied to a 15-mL BioGel-P60 column equilibrated with five bed volumes of 10 mM Tris, pH 7.4,
Microtiter wells were coated with 0.5 μg of purified bovine LPL or MTP and incubated in triplicate with indicated amounts of human LDL for 2 h at 37 °C. The amount of apoB bound to the immobilized proteins was measured by ELISA. Averages and standard deviations are plotted as bar graphs and error bars, respectively. The data are representative of three independent experiments.

Cells—MeA-RH7777 cells stably transfected with different C-terminally truncated forms of apoB have been described (15, 17, 18). COS-7 cells were obtained from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco’s minimal essential medium containing 10% fetal bovine serum and 1% antibiotic-antimycotic (Life Technologies, Inc.).

Binding of LDL to the Immobilized LPL—The binding of LDL to the immobilized LPL was studied as described earlier (5, 19). ELISA plates were coated with 0.5 μg of purified, homodimeric LPL. Antibodies used in ELISA for the detection of apoB have been described (12, 15, 16).

Cells—MeA-RH7777 cells stably transfected with different C-terminally truncated forms of apoB have been described (15, 17, 18). COS-7 cells were obtained from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco’s minimal essential medium containing 10% fetal bovine serum and 1% antibiotic-antimycotic (Life Technologies, Inc.).

Binding of LDL to the Immobilized LPL—The binding of LDL to the immobilized LPL was studied as described earlier (5, 19). ELISA plates were coated with 0.5 μg of purified, homodimeric LPL. Antibodies used in ELISA for the detection of apoB have been described (12, 15, 16).

Cells—MeA-RH7777 cells stably transfected with different C-terminally truncated forms of apoB have been described (15, 17, 18). COS-7 cells were obtained from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco’s minimal essential medium containing 10% fetal bovine serum and 1% antibiotic-antimycotic (Life Technologies, Inc.).

Binding of LDL to the Immobilized LPL—The binding of LDL to the immobilized LPL was studied as described earlier (5, 19). ELISA plates were coated with 0.5 μg of purified, homodimeric LPL. Antibodies used in ELISA for the detection of apoB have been described (12, 15, 16).

Cells—MeA-RH7777 cells stably transfected with different C-terminally truncated forms of apoB have been described (15, 17, 18). COS-7 cells were obtained from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco’s minimal essential medium containing 10% fetal bovine serum and 1% antibiotic-antimycotic (Life Technologies, Inc.).

Other Analyses—Protein was determined using the Coomassie Plus reagent (Pierce) with BSA as a standard (20). Optical density in ELISA plates was measured using a Dynatech MRX microplate reader (Dynatech Labs, Chantilly, VA). The data were plotted as the means ± S.D., and the binding isothersms were analyzed using Prism2 (Graphpad, San Diego, CA). The molecular masses used for LDL, LPL, Triton X102, and heparin were 512, 120, 0.757, and 16.5 kDa, respectively.

RESULTS

Nature of Interactions between apoB and LPL—The binding of human plasma LDL (0–100 μg/well) to the immobilized LPL was saturable and exhibited a rectangular hyperbola (Fig. 1A). The Bmax and Kd values were 0.018 ± 0.0002 (average ± standard error) pmol and 0.057 ± 0.004 nM, respectively. The r2 value for the curve was 0.9991. The Kd values ranged between 60 and 160 pm in five independent experiments. In previous studies, we had shown that LDL binds to MTP with Kd values ranging between 10 and 30 nM (21). Thus, LDL binds to LPL with severalfold higher affinity than MTP. The differences in the binding affinities between LPL and MTP for LDL were directly compared (Fig. 1B). In the concentration range of LDL (0–32 μg/well) used, significant binding of LDL to LPL was observed. In contrast, we could not detect the binding of LDL to MTP at these concentrations. Higher concentrations of LDL are required to get measurable binding to MTP (21). Next, we compared the binding of LDL to LPL with its binding to 1D1, a monoclonal antibody that recognizes amino acids 474–539 in apoB (22). LDL bound with similar affinity to LPL and 1D1 (data not shown). These studies indicated that the LDL-LPL binding involves high affinity interactions that are greater than that found for MTP and are similar to the binding of LDL to its monoclonal antibody.

To determine whether the interactions between LDL and LPL were ionic or hydrophobic in nature, we studied the effect of different concentrations of NaCl or Triton X102 on the binding of LDL to immobilized LPL. For control, we studied the effect of these reagents on the binding of LDL to its monoclonal antibody, 1D1. Increasing salt concentrations (0.5–2.0 M) inhibited binding of LDL to LPL (Fig. 2A). The binding of LDL was inhibited 70% at 2 M NaCl, whereas the binding of LDL to 1D1 was not significantly inhibited by salt. LDL-LPL binding was also inhibited to 80% in the presence of 0.4 M Triton X102 (Fig. 2B). Again, LDL-1D1 binding was not significantly inhibited. Similar inhibition of LDL-LPL binding was observed using Tween-20 (data not shown). Thus, detergents specifically inhibit LDL-LPL binding and do not affect LDL-1D1. The inhibition of the LDL-LPL binding by both salt and detergents suggests that the binding between LDL and LPL may be complex and involves both hydrophobic and hydrophilic interactions.

apoB17, an N-terminal fragment of apoB that does not bind lipids (17, 23, 24), has been shown to interact with LPL (19). Thus, it was of interest to determine whether apoB17 also interacts with LPL by both ionic and hydrophobic interactions. The binding of apoB17 to LPL was more sensitive to salt concentrations (Fig. 3A) compared with the binding of LDL to LPL (Fig. 2A). At the lowest concentrations (0.25 M) of NaCl used, apoB17-LPL binding was inhibited by 75% (Fig. 3A), whereas LDL-LPL binding (Fig. 2A) was not inhibited. At higher concentrations of salt (2.0 M), apoB17–LPL binding was inhibited to ~85%, but apoB17–1D1 binding was not inhibited (Fig. 3A). These studies indicated that apoB17-LPL binding involves ionic interactions. Next, we studied the effect of Triton X102 to determine whether apoB17-LPL interactions also in-
either salt or detergent was calculated. The average percentage inhibition because of the presence of salt or detergent is plotted as a line graph, whereas standard deviations are plotted as error bars. The data for salt and detergent effects are representative of seven and ten experiments, respectively.

![Figure 2](image)

**FIG. 2.** Effect of NaCl and Triton X102 on the binding of LDL to LPL. Micotiter wells were coated with LPL (0.5 µg/well) or 1D1 (1 µg/well) incubated in triplicate (2 h, 37 °C) with 100 µl of LDL (100 ng/ml) in the presence of various indicated concentrations of NaCl (A) or Triton X102 (B). The apoB17 bound to LDL was quantified by ELISA. Wells incubated with LDL alone represented 100% binding, whereas wells incubated with no LDL served as blank. Blank values were subtracted from all wells. The average percentage inhibition values by the presence of salt or detergent are plotted as line graphs, whereas standard deviations are plotted as error bars. The data for salt and detergent effects are representative of two independent experiments.

![Figure 3](image)

**FIG. 3.** Effect of NaCl and Triton X102 on the binding of apoB17 to LPL. Micotiter wells were coated with LPL (0.5 µg/well) or 1D1 (1 µg/well) incubated in triplicate (2 h, 37 °C) with 100 µl of conditioned media obtained from McA-RH7777 cells stably transfected with plasmids expressing human recombinant apoB17 in the presence of various indicated concentrations of NaCl (A) or Triton X102 (B). The apoB17 bound to LPL was quantified by ELISA. Wells incubated with the conditioned media alone represented 100% binding of apoB17, whereas wells incubated with nonconditioned media served as blank. Blank values were subtracted from all wells and the inhibition because of the presence of either salt or detergent was calculated. The average percentage inhibition because of the presence of salt or detergent is plotted as a line graph, whereas standard deviations are plotted as error bars. The data are representative of two independent experiments.

Volve hydrophobic interactions (Fig. 3B). apoB17-LPL binding was not significantly inhibited by 400 µM Triton X102. Note that at this concentration of Triton X102, LDL-LPL binding was inhibited by ~80% (Fig. 2B). Taken together, these studies indicate that the binding of LPL to LDL involves both ionic and hydrophobic interactions, whereas the binding of LPL to the N-terminal 17% of apoB involves mainly ionic interactions.

**LPL Binding Site in apoB**—We next attempted to identify the amino acid sequences in apoB17 that interact with LPL. To identify the LPL-binding site within the N-terminal 17% (amino acids 1–781) of apoB, we expressed different apoB polypeptides as FLAG chimeras (25). FLAG (DYKDDDDK) is an octapeptide that is commonly used as an epitope tag. The plasmids were transiently transfected into COS-7 cells, and the conditioned media were used to examine the secretion of FLAG/apoB chimeras (25). The optical densities were determined as described before (15, 16, 21). The optical densities were determined at 405 nm. The data are representative of six independent experiments.

### Table I

| Binding of different apoB sequences to the immobilized LPL |
|------------------------------------------------------------|
| apoB binding sites (Optical densities) | M2 binding (Optical densities) | LPL binding (Optical densities) | Ratio (LPL/M2) | Binding |
|--------------------------------------|--------------------------------|--------------------------------|----------------|--------|
| B:1–781                             | 1.05 ± 0.11                  | 1.12 ± 0.04                  | 1.07           | 100    |
| B:1–300                             | 0.55 ± 0.02                  | 0.43 ± 0.16                  | 0.78           | 73     |
| B:270–570                           | 0.58 ± 0.18                  | 0.53 ± 0.19                  | 0.91           | 86     |

It is not known whether the enzyme activity and the active site of LPL are required for its binding to LDL. To study the importance of lipase activity in apoB binding, we used tetrahydrolipstatin; this compound will completely inhibit LPL activity at 0.1 mM most likely by bind-
ing to the active site of the enzyme (26–28). We specifically considered the possibility that the lipase activity was important for LDL, but not apoB17, binding. Immobilized LPL was incubated with either LDL or apoB17 in the presence of different indicated concentrations of tetrahydrolipstatin (Fig. 5). The binding of LDL and apoB17 to LPL was not inhibited by tetrahydrolipstatin. These studies indicate that the apoB-LPL binding does not require enzyme activity.

Next, attempts were made to identify a region in LPL that might be involved in apoB binding (Fig. 6). Monoclonal antibody, 5D2, is known to inhibit LPL activity and has been shown to recognize an epitope between amino acids 380–410 in LPL (29). At $\approx 4 \mu$g/ml, this antibody inhibited LDL-LPL binding by about 70–90% (Fig. 6). Another monoclonal antibody, Mab, whose binding epitope has not yet been determined and that inhibits LPL activity and is known to block LPL binding to very low density lipoproteins (30), was less effective. These studies indicate that amino acids 380–410 of LPL, other amino acids proximal to these residues, or these amino acids with other proximal residues in LPL might constitute a site for LDL binding.

**apoB and Proteoglycans May Bind to a Similar Site in LPL.**—It is known that apoB contains several binding sites for heparin (31) and that LPL binds to GAGs present on the endothelial cells. For this reason we tested whether the high affinity LDL-LPL binding might be due to the presence of small amounts of heparin co-eluted with LPL during heparin affinity chromatography. This hypothesis was ruled out using two independent approaches. First, LPL purified by heparin affinity chromatography was subjected to Biogel P60 size exclusion chromatography in 1.5 M NaCl buffer to remove traces of heparin. The enzyme purified by Biogel chromatography was then used to study LDL binding. The Biogel P60 purified LPL bound better than the LPL purified from heparin affinity chromatography (Fig. 7A). Nonlinear regression analysis revealed that LDL bound to two different preparations of LPL with similar affinity. The $K_\text{d}$ values for the binding of LDL to heparin and Biogel purified LPL were 0.14 ± 0.06 and 0.19 ± 0.07 nM (averages ± S.E.), respectively. In contrast, the maximum binding of LDL to Biogel purified LPL was 68% higher than its binding to heparin purified LPL. The $B_{\text{max}}$ values for the binding of LDL to Biogel purified and heparin purified LPL were 0.034 ± 0.004 and 0.057 ± 0.06 pmol (averages ± S.E.), respectively. Second, LPL purified by heparin affinity chromatography and Biogel chromatography was immobilized and subjected to treatment with heparinase and heparitinase (Fig. 7B). The binding of LDL to Biogel purified LPL was not altered by the treatment of the enzyme with heparinase and heparitinase. In contrast, treatment of heparin purified LPL by these enzymes resulted in ∼50% increased binding of LDL. These studies indicate that the removal of heparin from LPL preparations increases the number of binding sites for LDL and suggests that heparin might inhibit LPL-LDL binding. This was directly determined by studying the inhibition of the binding of LDL to LPL.
interactions between apoB and LPL are of high affinity with $K_d$ values in the 10$^{-12}$ m range (Fig. 1). Interestingly, these interactions were found to be much stronger than those observed for the binding of LDL to MTP (21) and were as strong as those observed between antigen-antibody (LDL-1D1) binding (data not shown) and the binding of LDL to its receptors in fibroblasts (32). The high affinity interactions between LDL and LPL imply that they are physiologically significant. These interactions may be crucial in ensuring the hydrolysis of circulating lipoproteins before their recognition by receptors for removal.

The high affinity binding between LDL and LPL is due to both hydrophobic and hydrophilic interactions between these molecules. The evidence for hydrophobic interactions was derived from studies involving the effect of detergents. In a previous study, LPL binding to LDL immobilized on microtiter plate assays failed to show hydrophobic interactions (5). Thus, the geometry of the interactions or the exposure of apoB versus lipids may differ if the LDL, rather than LPL, is in solution. In the current study, the LDL-LPL binding was very sensitive to detergents; it was completely inhibited by 0.4 mM Triton X102. It should be pointed out that 0.1% Triton (1.5 mM) is usually used for immunoprecipitation and solubilization studies (33). In addition, 0.01% of Tween-20 inhibited ≥50% of the LDL–LPL binding (data not shown). Usually, 0.05% of Tween-20 is used in ELISA assays. Thus, LDL-LPL binding is inhibited by detergent concentrations that are lower than those used in various biochemical procedures of protein characterization. Furthermore, detergents did not inhibit apoB17-LPL binding (Fig. 3). Therefore, we conclude that the effect of detergents were specific to LDL-LPL binding. These studies indicate that detergents were probably inhibiting hydrophobic interactions between LDL and LPL independent of structural modification of proteins.

The hydrophobic interactions between lipoproteins and LPL may be physiologically significant in the hydrolysis of lipoproteins. Triton WR1339 is generally used in metabolic studies to determine the production rates of lipoproteins (34) because it inhibits the hydrolysis of lipoproteins during circulation. The mechanism of inhibition of hydrolysis by Triton WR1339 is unknown, but it is generally believed that the detergent covers the lipoprotein surface and prevents lipolysis by LPL. Our studies may provide a biochemical explanation for the inhibition of lipolysis by Triton WR1339. We propose that Triton inhibits the hydrophobic interactions between lipoproteins and LPL and inhibits hydrolysis of lipids in various lipoproteins. It is expected that the hydrophobic interactions between lipoproteins and LPL would be stronger with larger lipoproteins and may also provide an explanation for greater rates of hydrolysis of larger, triglyceride-rich lipoproteins by LPL.

Evidence for ionic interactions between LDL and LPL binding comes from studies involving the use of salt (Figs. 2A and 3A), heparin (Fig. 8), and suramin (data not shown). The inhibition of the binding of LDL to LPL was less sensitive to salt than its binding to MTP. The amount of salt required for the 50% inhibition of the binding of LDL to LPL and MTP are 1.0 M (Fig. 1A) and 0.05 M (21), respectively. This may be because LPL binds to LDL involving both hydrophilic and hydrophobic interactions, whereas LDL–MTP binding involves hydrophilic interactions only.

There are significant differences with respect to the salt sensitivity of LDL-LPL and apoB17-LPL interactions. However, such differences for the effect of salt on the binding of LDL and apoB17 to MTP have not been observed (21). The apoB17-LPL binding was more sensitive to salt than the LDL-LPL interactions.
binding (compare Figs. 2 and 3), whereas the salt sensitivity of apoB17-LPL was the same as that observed for either apoB17-MTP or LDL-MTP binding (21). Different amounts of salt required for the inhibition of the binding of LDL and apoB17 to LPL supports the idea that LDL binds to LPL via both ionic and hydrophobic interactions, whereas apoB17-LPL binding is ionic. Because hydrophobic interactions are not inhibited by salt, higher amounts of salt are required to inhibit the binding of LDL to LPL. Although salt is known to alter LPL dimeric structure (35), in the current study LPL was immobilized on microtiter plates and is not expected to monomerize. Furthermore, the differences between the binding of LPL to LDL and apoB17 (Figs. 2 and 3) indicate that the effect of salt observed is not related to changes in the LPL structure. Thus, we conclude that although the tertiary structure of LPL or apoB could have been altered either by the salt or the detergent, both protein-protein and protein-lipid interactions are involved in LDL–LPL binding. Furthermore, we conclude that interactions between LDL and apoB17 are protein-protein interactions involving ionic amino acid residues.

**LPL-binding Sites in apoB**—To determine the LPL-binding site in apoB17, we used apoB-FLAG chimeras that have been successfully used in the identification of apoB-binding site in MTP (12). LPL bound to B17F, B1–300, and B270–570. The binding of these chimeras was not due to FLAG-LPL binding (Fig. 4). Thus, we conclude that LPL binds to apoB at multiple sites. Different binding sites for LPL on apoB may result in the anchoring of several LPL molecules on lipoproteins and facilitate rapid and simultaneous lipid hydrolysis at different sites.

**apoB-binding Site in LDL**—The binding of LDL to LPL does not require enzymatic activity. Tetrahydrolipstatin is a potent inhibitor of LPL and is known to completely inhibit the enzyme activity. It did not inhibit LDL-LPL binding (Fig. 5). This is rather surprising because of the observations that LDL-LPL binding involves hydrophobic interactions and most likely involves the binding of LDL to lipids present in the LDL. These studies indicate that the lipid-binding site in LDL may be independent of its lipid hydrolyzing activity. Thus, it is conceivable that LPL binds to LDL apolipoproteins and lipids and subsequently accesses triglyceride molecules for hydrolysis.

The binding of LDL was inhibited by a monoclonal antibody 5D2, indicating that amino acids 380–410 or a region close to these residues might be involved in LDL binding. It is quite possible that the inhibition of LDL-LPL binding by antibodies may be due to steric exclusion of LDL by the antibodies and may not be due to competition for the LDL-binding site. Thus, the suggestion that amino acids 380–410 are involved in apoB binding should be confirmed by independent methods such as site-directed mutagenesis. Nonetheless, it is interesting to note that a similar region in the C terminus of LDL has been implicated for its binding to the LDL receptor-related protein and Sortilin/neuromedin receptor-3 (4, 36, 37). Nielsen et al. (36) have shown that amino acids 308–384 and 404–430 in LDL bind to the LDL receptor-related protein. This region is characterized by the presence of high density of positive charges and is probably exposed on the surface of the molecule (4). It is also interesting to note that the binding of LDL to LDL receptor-related protein, neuromedin receptor-3, and LDL is inhibited by heparin. Thus, it is conceivable that the binding sites for 5D2 monoclonal antibody, apoB, heparin, LDL receptor-related protein, and the Sortilin/neuromedin receptor-3 in LDL may be the same or juxtaposed, and this site may be critical for the binding of the enzyme to different biologically important molecules.

**Modulation of LDL-LPL Binding by Glycosaminoglycans**—LPL is well known to bind GAGs. apoB has also been suggested to bind to GAGs but does so weakly in physiologic salt solutions (38). In the present study, we show that the protein-protein interactions between LDL-LPL involving ionic residues are inhibited by heparin (Fig. 8). Thus, GAGs can modulate catabilism of lipoproteins by LDL in several ways. First, by binding to both LDL and lipoproteins, GAGs may help bring substrate and enzyme together for efficient hydrolysis on the endothelium. Second, by inhibiting the binding of LDL to lipoproteins they can bring enzyme and substrate apart and modulate the extent of hydrolysis. Third, they can dislodge both lipoproteins and LDL from the endothelial cell surface for removal from vascular system. Once LDL leaves its site of physiologic action on the luminal endothelial surfaces, it circulates in the bloodstream attached to LDL, prior to its removal by the liver. If LDL occupies the GAG-binding site of LPL, then it would prevent LPL from reassociating with the endothelial surface, thus insuring its removal.

In summary, we show that LDL–LPL binding involves high affinity interactions involving hydrophilic and hydrophobic forces. The N-terminal region of apoB contains multiple binding sites for ionic interactions with LPL. The enzymatic activity of LDL is not required for LDL binding. The LDL-binding region of LDL also binds to monoclonal antibodies, heparin, and LDL receptor-related protein. We speculate that LDL may contain a surface exposed positively charged amino acid cluster that may be important for various physiological interactions of LDL with different biological molecules.

**Acknowledgment**—We gratefully appreciate the technical assistance of Neeru Nayak.

**REFERENCES**

1. Brunzell, J. D. (1995) in *The Metabolic and Molecular Bases of Inherited Disorders* (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 1913–1932, McGraw-Hill, Inc., New York
2. Bhattacharya, A. (1999) in *Lipoproteins in Health and Disease* (Betteridge, D., Illingworth, D. R., and Shepherd, J., eds) pp. 737–752, Arnold, London
3. Goldberg, I. J. (1996) *J. Lipid Res.* 37, 693–707
4. Hassan, M. M., Strickland, D. K., and Bakillah, A. (1999) *Ann. Rev. Nutr.* 19, 141–172
5. Choi, S. Y., Sivaram, P., Walker, D. E., Curtiss, L. K., Gretch, D. G., Sturley, S. L., Attie, A. D., Deckelbaum, R. J., and Goldberg, I. J. (1995) *J. Biol. Chem.* 270, 8081–8086
6. Pang, L., Sivaram, P., and Goldberg, I. J. (1996) *J. Biol. Chem.* 271, 19518–19523
7. Choi, S. Y., Pang, L., Kern, P. A., Kayden, H. J., Curtiss, L. K., Vanni-Reyes, T. M., and Goldberg, I. J. (1997) *J. Biol. Chem.* 272, 77–85
8. Segrest, J. P., Jones, M. K., Mishra, V. K., Anantharamaiah, G. M., and Garber, D. W. (1984) *Arterioscler. Thromb.* 14, 1674–1685
9. Segrest, J. P., Jones, M. K., Mishra, V. K., Skiba, Y. K., Pieretti, V. Young, S. H., Borin, J., Innerarity, T. L., and Dashi, N. (1998) *J. Biol. Chem.* 273, 85–102
10. Segrest, J. P., Jones, M. K., and Dashi, N. (1999) *J. Biol. Chem.* 274, 1401–1416
11. Gretch, D. G., Sturley, S. L., Wang, L., Lipton, B. A., Dunning, A., Grunwald, K. A. A., Wetterau, J. R., Yao, Z., Talmud, P., and Attie, A. D. (1996) *J. Biol. Chem.* 271, 8682–8691
12. Hussain, M. M., Bakillah, A., Nayak, N., and Shelnaz, G. S. (1998) *J. Biol. Chem.* 273, 25624–25631
13. Bradbury, P., Mann, C. J., Kochl, S., Anderson, T. A., Chester, S. A., Hancock, J. M., Ritchie, P. J., Amey, J., Harrison, G. B., Levitt, D. G., Banaszak, L. J., Scott, J., and Shoulders, C. C. (1999) *J. Biol. Chem.* 274, 3159–3169
14. Obukine, J. C., Edwards, I. J., Rumsey, S. C., Curtiss, L. K., Wagner, W. D., Deckelbaum, R. J., and Goldberg, I. J. (1994) *J. Biol. Chem.* 269, 13129–13135
15. Hussain, M. M., Zhou, Y., Kancha, R. K., Blackhart, B. D., and Yao, Z. (1995) *Arterioscler. Thromb. Vasc. Biol.* 15, 485–494
16. Bakillah, A., Zhou, Z., Luchoum, J., and Hussain, M. M. (1997) *Lipids* 32, 1113–1118
17. Yao, Z., Blackhart, B. D., Linton, M. F., Taylor, S. M., Young, S. G., and Scott, J. (1994) *J. Biol. Chem.* 269, 9409–9412
18. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248–254
19. Hussain, M. M., Bakillah, A., and Jamil, H. (1997) *Biochemistry* 36, 13060–13067
20. Pease, R. J., Milne, R. W., Jessup, W. W., Law, A., Provost, P., Fruchtman, J. C., Dean, R. T., Marcel, Y. L., and Scott, J. (1990) *J. Biol. Chem.* 265, 553–568
21. Du, R. Z., Kurth, J., Wang, S.-L., Humiston, P., and Davis, R. A. (1994) *J. Biol. Chem.* 269, 24169–24176
22. Hervitzov, H., Hadzopoulou-Cladaras, M., Walsh, M. T., Cladaras, C., Zannis, V. I., and Small, D. M. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 7313–7317

**LPL-apoB Binding**

29329
25. Shelness, G. S., Morris-Rogers, K. C., and Ingram, M. F. (1994) J. Biol. Chem. 269, 9310–9318
26. Looker, A., Skottova, N., and Olivecrona, G. (1994) Eur. J. Biochem. 222, 395–403
27. Hadvary, P., Sidler, W., Meister, W., Vetter, W., and Wolfer, H. (1991) J. Biol. Chem. 266, 2021–2027
28. Yin, B., Loike, J. D., Kako, Y., Weinstock, P. H., Breslow, J. L., Silverstein, S. C., and Goldberg, I. J. (1997) J. Clin. Invest. 100, 649–657
29. Chang, S. F., Reich, B., Brunzell, J. D., and Will, H. (1998) J. Lipid Res. 39, 2450–2459
30. Goldberg, I. J., Paterniti, J. R., Jr., France, D. S., Martinelli, G., and Cornicelli, J. A. (1986) Biochim. Biophys. Acta 878, 168–176
31. Wetzgraber, K. H., and Rall, S. C., Jr. (1987) J. Biol. Chem. 262, 11097–11103
32. Goldstein, J. L., Basu, S. K., and Brown, M. S. (1983) Methods Enzymol. 98, 241–260
33. Helenius, A., and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
34. Tietge, U. J. F., Bakillah, A., Maageais, C., Tsukamoto, K., Hussain, M. M., and Rader, D. J. (1999) J. Lipid Res. 40, 2134–2139
35. Bengtsson, G., and Olivecrona, T. (1983) Biochim. Biophys. Acta 751, 254–259
36. Nielsen, M. S., Breijing, J., Garcia, R., Zhang, H., Hayden, M. R., Vilarno, S., and Gliemann, J. (1997) J. Biol. Chem. 272, 5821–5827
37. Nielsen, M. S., Jacobsen, C., Olivecrona, G., Gliemann, J., and Petersen, C. M. (1999) J. Biol. Chem. 274, 8822–8826
38. Goldberg, I. J., Wagner, W. D., Pang, L., Paka, L., Curtiss, L. K., DeLezier, J. A., Shelness, G. S., Young, C. S. H., and Pillarisetti, S. (1998) J. Biol. Chem. 273, 35355–35361

LPL-apoB Binding