Interaction of Lipoproteins with Heparan Sulfate Proteoglycans and with Lipoprotein Lipase. Studies by Surface Plasmon Resonance Technique†

Aivar Lookene,*,‡ Roger Savonen, and Gunilla Olivecrona*

Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden

Received October 28, 1996; Revised Manuscript Received February 13, 1997⊗

ABSTRACT: Interaction of different classes of lipoproteins with heparan sulfate, heparin, and lipoprotein lipase was studied by a surface plasmon resonance based technique on a BIAcore. The proteoglycans were covalently attached to sensor chips as previously described [Lookene, A., Chevreuil, O., Østergaard, P., & Olivecrona, G. (1996) *Biochemistry 35*, 12155−12163]. Binding of all lipoproteins, except for β-VLDL, to endothelial heparan sulfate was low. Binding of chylomicrons (from rat lymph) and of human VLDL was much increased by the presence of lipoprotein lipase. With human LDL, binding was low in the absence of lipase or at low lipase concentrations. For efficient binding, 2−4 lipase dimers per LDL particle were necessary, indicating cooperativity in the interaction. In contrast, HDL did not bind under any conditions. Heparin had higher binding capacity for lipoproteins than heparan sulfate. This was due to a higher number of binding sites on the heparin chains. Binding of LDL, VLDL, and chylomicrons to heparan sulfate-covered surfaces, both in the presence and in the absence of lipoprotein lipase, was characterized by high values for association rate constants (10^4−10^5 M^{-1} s^{-1}) and low values for dissociation rate constants (10^{-4}-10^{-5} M^{-1} s^{-1}). In some experiments, rabbit β-VLDL were directly immobilized to the sensor chips. Binding of lipoprotein lipase to these surfaces was characterized by a very high association rate constant (10^6 M^{-1} s^{-1}). The dissociation of triacylglycerol-rich lipoproteins was more rapid with catalytically active lipase than with active site-inhibited lipase. It was also markedly increased in the presence of free heparin, suggesting fast exchange kinetics at the surface. Based on that, we propose that lipoproteins are relatively mobile at heparan sulfate covered surfaces. Our study emphasizes the important role of lipoprotein lipase, or molecules with similar properties (apolipoprotein E, hepatic lipase), as mediators for binding of lipoproteins to proteoglycans. It also demonstrates the great potential for the use of biosensors for studies of lipoprotein interactions.

Plasma lipoproteins are spherical molecular aggregates composed of a core of insoluble lipids (triacylglycerols and cholesteryl esters) surrounded by a surface layer of more polar lipids (phospholipids and cholesterol) and specific, lipid-binding proteins (apolipoproteins) (Segrest et al., 1994). Chylomicrons are made from dietary lipids in the intestinal mucosal cells, while the smaller very low density lipoproteins (VLDL)[1] are made in the liver from endogenous lipids. The major step in catabolism of these lipoproteins is hydrolysis of their triacylglycerols by lipoprotein lipase (LPL) (Olivecrona & Bengtsson-Olivecrona, 1987). This enzyme is thought to be bound to the vascular endothelium via interaction with heparan sulfate proteoglycans (Goldberg, 1996; Bensadoun, 1991; Olivecrona & Bengtsson-Olivecrona, 1989). The action of LPL reduces the size of lipoproteins and converts them to remnant particles with a lower content of triacylglycerols. The remnant particles are eventually taken up in cells by receptor-mediated endocytosis (Havel, 1994). Some surface material is shed from the particles during lipolysis (phospholipids, cholesterol, and certain apolipoproteins). This material associates with, or forms, high-density lipoproteins (HDL), which are the smallest lipoprotein particles in plasma (Eisenberg, 1990).

Since most LPL is attached to the vessel walls, and stays attached while acting on lipoproteins, lipoprotein conversion is a surface phenomenon. In addition, some apolipoproteins (apolipoproteins B and E) have heparin-binding motifs and bind both to heparin and to other glycosaminoglycans (Weisgraber, 1994; Camejo et al., 1993; Jackson et al., 1991). In recent years, it has become evident that binding of lipoproteins to heparan sulfate on cell surfaces is an important factor both for lipolysis and for receptor-mediated uptake of the remnants (Goldberg, 1996; Olivecrona & Olivecrona, 1995; Mulder et al., 1992; Eisenberg et al., 1992). LPL mediates binding of lipoproteins both to proteoglycans and to receptors like the LDL receptor-related protein (LRP), a candidate receptor for chylomicron remnants (Strickland et al., 1995; Krieger & Herz, 1994; Beisiegel et al., 1991). The structurally and functionally related hepatic lipase has similar effects (Nykjær et al., 1994; Krapp et al., 1996), and the

†This study was supported by grants from the Swedish Medical Research Council (B95-03X-00727-30C) and from the Bank of Sweden Tercentenary Foundation (92-0275:03) and by a grant from the Commission of the European Communities, Science, Research, and Development Section.

* Correspondence may be addressed to either author at the Department of Medical Biochemistry and Biophysics, Umeå University, S-901 87 Umeå, Sweden. Telephone: +46-90-165234. FAX: +46-90-167840. E-mail: Gunilla.Olivecrona@medchem.umu.se.

‡ Present address: Institute of Chemical Physics and Biophysics, Estonian Academy of Sciences, Akadeemia tee 23, Tallinn E0026, Estonia.

⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1997.

[1] Abbreviations: LPL, lipoprotein lipase; NHS, *N*-hydroxysuccimide; EDC, 1-ethyl-3-[3-dimethylamino)propyl]carbodiimide; HDS, hexadecylsulfonyl fluoride; SDS, sodium dodecyl sulfate; VLDL, very low density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apoB, apolipoprotein B; apo E, apolipoprotein E; SPR, surface plasmon resonance.

S0006-2960(96)02699-2 CCC: $14.00

content of apolipoprotein E in the lipoproteins also influences their interaction with proteoglycans and receptors (Takahashi et al., 1995; Ji et al., 1993).

In the present study, we have used the surface plasmon resonance technique to study the interaction of lipoproteins with heparan sulfate- and with heparin-covered surfaces in the absence or presence of LPL. It is shown that even though the aplipoprotein B (apoB)-containing lipoproteins (chylomicrons, VLDL, LDL) bind to proteoglycans on their own, binding is much amplified by the presence of LPL.

MATERIALS AND METHODS

Materials. LPL was purified from bovine milk as described (Bengtsson-Olivecrona & Olivecrona, 1991). Catalytically inactive LPL was made using the active site inhibitor hexadecylsulfonyl fluoride (HDS). Inhibited LPL (HDS-LPL) was prepared and isolated as previously described (Skottova et al., 1995). Endothelial heparan sulfate was isolated from cultured human umbilical endothelial cells (Lindblom & Fransson, 1990) and was a kind gift from Prof. Lars-Åke Fransson, Department of Physiological Chemistry, University of Lund, Sweden. An amino-coupling kit containing *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-[(diethylamino)propyl]carbodiimide (EDC), and 1 M ethanolamine and CM5 sensor chips were obtained from Pharmacia Biosensor AB, Uppsala, Sweden. Streptavidin was purchased from Sigma. LPL activity was determined using an assay containing tributyrylglycerol (Lookene & Bengtsson-Olivecrona, 1993).

Lipoproteins. VLDL, LDL, and HDL were isolated from fresh human plasma by sequential flotation in the ultracentrifuge using KBr to increase the density of the medium (Havel et al., 1955). β-VLDL, isolated from plasma of cholesterol-fed rabbits (Beisiegel et al., 1991), were a kind gift from Dr. Ulrike Beisiegel, University Hospital Eppendorf, Hamburg, Germany. Chylomicrons were obtained from rat lymph, using the procedure previously described (Skottova et al., 1995). Cholesterol and triacylglycerols were determined with enzymatic colorimetric kits (Boehringer Mannheim) and protein by the Bicinchonic acid assay (Pierce) (standard deviations were less than 5%). Molar concentrations of HDL, VLDL, β-VLDL, and LDL were calculated using the protein content and the average molecular masses of the different classes of lipoproteins. The values used for calculations were 21% and 2.3×10^6 Da for LDL (Shen et al., 1977), 41% and 0.36×10^6 Da for HDL (Shen et al., 1977), 8.0% and 20×10^6 Da for VLDL (Shen et al., 1977), and 9.9% and 20×10^6 Da for β-VLDL (Gudmundsen et al., 1994). The concentrations of chylomicrons were calculated from their content of triacylglycerols (90%) (Kalogeris & Story, 1992).

Surface Plasmon Resonance Analysis. All measurements were performed using a BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden). Its detection is based on surface plasmon resonance (SPR), a quantum mechanical phenomenon which detects changes in the refractive index close to the surface of thin gold film on a glass support (i.e., sensor chip). The chip surface is coated with a carboxymethylated dextran polymer to which one of the reactants can be covalently linked, while the other is injected in flow over the surface. Binding of a soluble component to the immobilized one leads to an increase of the refractive index. This change in refractive index alters the SPR, which can be detected optically. Binding is measured in arbitrary response units (RU).

Heparin or heparan sulfate chains were biotinylated as described (Lee & Conrad, 1984). A solution of 5−10 mg/mL heparin or heparan sulfate in 0.2 M $NaCO_3$, pH 8.6, was incubated with a 10-fold molar excess of sulfo-NHS-biotin (Pierce) for 3 h at room temperature. Excess of nonreacted biotin was removed on a NAP-5 column (Pharmacia Biotechnology). The same procedure was used for biotinylation of LPL and of β-VLDL. The nonreacted biotin was removed by chromatography on heparin−Sepharose in the case of LPL, or by dialysis in the case of β-VLDL.

Immobilization of streptavidin to sensor chip CM5 was performed as previously described (Johnsson et al., 1991). The surface was activated by injecting 30 μL of a mixture of EDC/NHS (0.2 M/0.05 M) at a flow rate of 5 μL/min. Then streptavidin (0.1 mg/mL in 10 mM acetate, pH 4.5) was injected. Nonreacted activated groups were blocked by injection of 30 μL of 1 M ethanolamine, pH 8.5. Biotin-labeled heparan sulfate was then coupled to the surface which had previously been derivatized by streptavidin.

Measurements of binding kinetics were performed in 20 mM Hepes, 3.4 mM EDTA, pH 7.4, and 0.15 M NaCl. In some experiments with chylomicrons, the running buffer also contained 1 mg of bovine albumin/mL (Sigma, fraction V). The isolated lipoproteins were dialyzed against running buffer (20 mM Hepes, 0.15 M NaCl, and 3.4 mM EDTA, pH 7.4), and dilutions were made just before injection. The surfaces of the sensorchips were in all cases regenerated by injection of 4 mM deoxycholate or 0.1% SDS. The chips were used for about 2 weeks in repetitive experiments. Regeneration was not possible with sensor chips to which lipoproteins (β-VLDL) had been directly immobilized via biotin/streptavidin. All of the constants were determined at a flow rate of 5 μL/min.

Determination of Kinetic Parameters. Calculation of Rate Constants. An integrated form of the rate equation was used for analysis of association phases (O'Shannessy et al., 1993):

$$\Delta R = R_t - R_o = \left\{ \frac{Lk_{ass}\Delta R_{max}[1 - e^{-(Lk_{ass}+k_{ass})t}]}{Lk_{ass} + k_{diss}} \right\} \quad (1)$$

where R_t is the signal observed during the association phase. R_t is proportional to the concentration of lipoproteins at the surface; R_o defines the base line (response at $t = 0$); ΔR_{max} is the maximal capacity of the sensor chip to bind lipoproteins expressed in response units; L is the concentration of lipoproteins in the injected solution; k_{ass} and k_{diss} are association and dissociation rate constants, respectively.

The dissociation phases were analyzed by the equation:

$$R_t = R_a e^{-k_{diss}t} + R_{(t\to\infty)} \quad (2)$$

where R_t is the response at time t in the dissociation phase, $R_{(t\to\infty)}$ is the response value after complete dissociation, and R_a is the response at $t = 0$. Because of bulk effects, the first 5 s of both the association and the dissociation phases was not used for calculations.

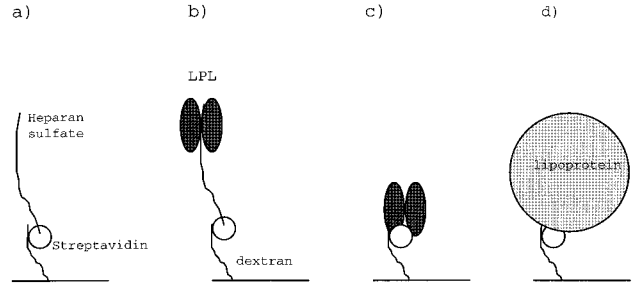

FIGURE 1: Four different experimental conditions for studies of interactions with lipoproteins. (a**)** Biotinylated heparan sulfate or heparin was immobilized to streptavidin (open circles), which was in turn covalently bound to the dextran matrix of the sensor chip. (b) Same as in (a), but with LPL dimers (solid ovals) bound to the heparan sulfate chains. (c) Biotinylated LPL was bound to the streptavidin. (d) Biotinylated β-VLDL were bound to streptavidin.

Determination of Equilibrium Dissociation Constants (K_d). K_d was calculated using the equation:

$$\Delta R = \frac{\Delta R_{max} L}{K_d + L} + bL \quad (3)$$

where ΔR is the increase of the response values at equilibrium, L is the concentration of lipoproteins, and b is a proportionality constant representing nonspecific binding. ΔR_{max} is the maximal capacity for specific binding.

Data Analysis. The data were analyzed by nonlinear regression using the FIG.P program (Biosoft, Cambridge, U.K.) and by the SAAM program (Resource Facility Analysis, University of Washington, Seattle). To calculate the mean values and standard deviations for the constants, results from experiments with different preparations of lipoproteins were used.

RESULTS

Experimental Conditions. Lipoprotein interactions were studied under four different conditions (Figure 1). In the first system, biotinylated heparan sulfate or heparin was coupled to the matrix-bound streptavidin (Figure 1a). In the second system, LPL was noncovalently attached to the streptavidin-immobilized heparan sulfate (Figure 1b). We had previously studied binding of the lipase to heparan sulfate or to heparin under these conditions (Lookene et al., 1996). The third system contained biotinylated LPL directly bound to streptavidin (Figure 1c). In the fourth condition (Figure 1d), biotinylated lipoproteins (β-VLDL) were directly bound to the steptavidin. Binding of LPL was then studied to the immobilized lipoproteins. In the association phase (on-phase), the solutions of lipoproteins (or lipase) were injected over the sensor chips, and binding was monitored. In the dissociation phase (off-phase), the sensor chips, were washed with buffer without lipoproteins (or lipase). Response values at steady state were used as a measure of an increased binding at equilibrium (ΔR_{eq}).

Effect of LPL on Binding of Lipoproteins to Heparan Sulfate Covered Sensor Chips. Binding of VLDL to a heparan sulfate-covered sensor chip was barely detectable (Figure 2A). The ΔR_{eq} increased about 200-fold in the presence of LPL. The increase in ΔR_{eq} was smaller after a second and a third sequential injection of the same solution of VLDL (Figure 2B). This suggested that the amount of LPL at the surface had decreased by the flow of VLDL. The

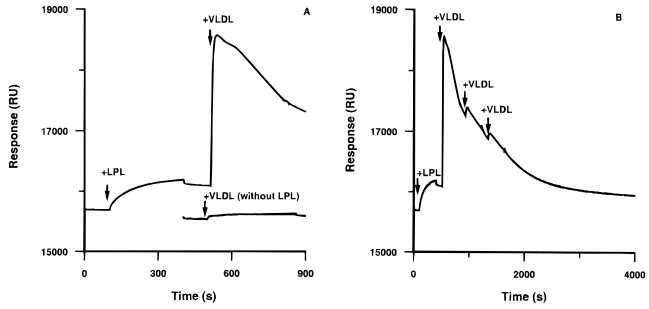

FIGURE 2: Binding of VLDL to LPL−heparan sulfate complexes. Conditions were as in Figure 1a. All experiments were performed in 10 mM Hepes, 0.15 M NaCl, and 3.4 mM EDTA, pH 7.4. The VLDL concentration was 10 μM in the solution which was passed over the sensor chip. (A) Binding of VLDL to heparan sulfate without LPL was almost not detectable (lower curve). When LPL was first bound to the layer (injection is shown by +LPL, 4.1 μg of LPL/mL in the mobile phase) followed by injection of the same amount of VLDL as in the first case, there was a large increase of the binding. (B) During the dissociation phase after binding of LPL, when buffer only was passed over the layer, a solution of VLDL was sequentially injected at 3 times (shown by +VLDL).

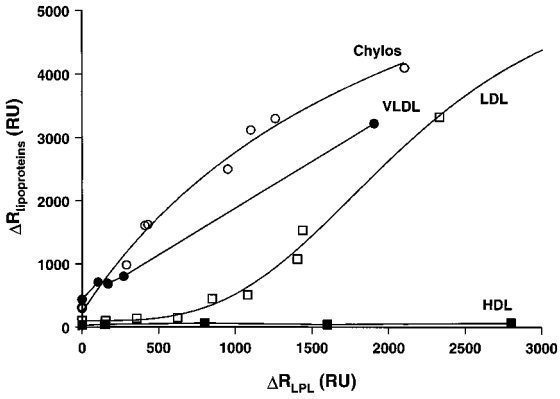

FIGURE 3: Effect of the amount of LPL bound to heparan sulfate on the amount of lipoproteins bound at equilibrium. LPL was bound to the heparan sulfate covered sensor chip (condition as in Figure 1b). The amount of bound LPL was expressed in response units (ΔR_{LPL}). Chylomicrons (○), VLDL (●), LDL (□), and HDL (■) were injected over the surface with LPL−heparan sulfate complexes. The concentration of lipoproteins was around 50 nM in all cases. The amount of bound lipoproteins was determined at steady states ($\Delta R_{lipoprotein}$).

dissociation of LPL itself from heparan sulfate-covered sensor chips is very slow [$k_{diss} = 10^{-5} - 10^{-6}$ s^{-1} (Lookene et al., 1996)] and cannot be the reason for the decreased binding. It is therefore likely that passing of VLDL increased the dissociation rate of LPL. The washing-out effect by lipoproteins was, however, only found when very high concentrations of lipoproteins were used (>10 μM) in combination with low densities of immobilized heparan sulfate (ΔR_{eq} < 100 after immobilization). At higher densities of immobilized heparan sulfate and at lower concentrations of VLDL, the washing-out effect was negligible (not shown). Such conditions were used for most of the following experiments.

A markedly increased binding to the sensor chips due to the presence of LPL was found also for chylomicrons and LDL (Figure 3). In contrast, with HDL, binding was low both in the absence and in the presence of LPL (Figure 3). With the apoB-containing lipoproteins, the amount bound at steady state, expressed in response units ($\Delta R_{lipoproteins}$), depended on the amount of LPL attached to the immobilized

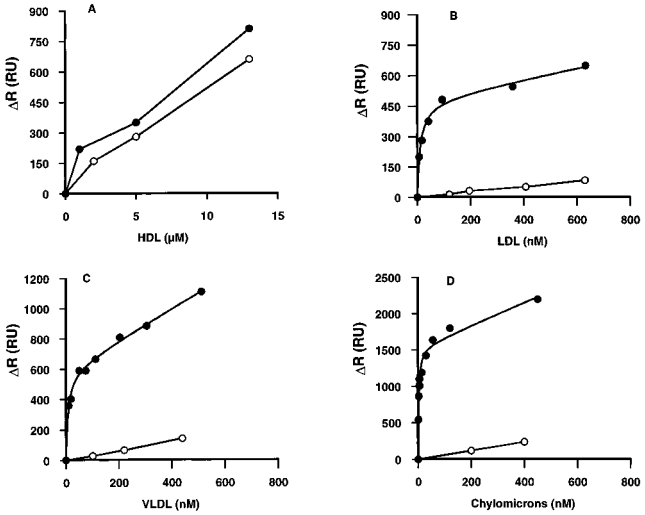

FIGURE 4: Binding of lipoproteins to biotinylated LPL. Biotinylated LPL was directly attached to the sensor chip via the covalently bound streptavidin (condition as in Figure 1c). The increase of response units after binding of LPL was 5400 units. Solutions of lipoproteins were injected over the LPL-derivatized sensor chip, and response values were determined at steady states (ΔR). Specific binding of lipoproteins is shown by solid circles. Nonspecific binding of lipoproteins to the streptavidin-covered sensor chip without LPL is presented by open circles. Panels A, B, C, and D present the results for HDL, LDL, VLDL, and chylomicrons, respectively.

Table 1: Equilibrium Dissociation Constants (K_d) for LPL−Lipoprotein Interactions[a]

lipoprotein	LPL (M)	LPL + heparan sulfate (M)
chylomicrons	$(5.0 \pm 0.4) \times 10^{-9}$	$(5.6 \pm 1.1) \times 10^{-9}$
VLDL	$(8.1 \pm 1.6) \times 10^{-9}$	$(7.4 \pm 2.3) \times 10^{-9}$
β-VLDL	$(8.8 \pm 0.6) \times 10^{-9}$	$(2.5 \pm 0.2) \times 10^{-9}$
LDL	$(11 \pm 2) \times 10^{-9}$	$(3.9 \pm 0.1) \times 10^{-9}$

[a] The K_d values were determined for the interaction of lipoproteins with biotinylated LPL bound to streptavidin (LPL, condition as in Figure 1c) and for binding of lipoproteins to LPL−heparan sulfate complexes (LPL + heparan sulfate, condition as in Figure 1b). The calculations were performed as shown under Materials and Methods. Constants represented are mean values ± standard deviation of three different determinations.

heparan sulfate, i.e., on the surface density of LPL (expressed in ΔR_{LPL}). The binding of chylomicrons and VLDL was almost linear with respect to the amount of bound LPL. Binding of LDL was low at low surface density of LPL, expressed in ΔR_{LPL}, but it increased at higher LPL concentrations, suggesting that a certain surface density of the enzyme was needed for efficient binding of a LDL particle (Figure 3). The increase of the binding started when ΔR_{LPL} was in the region 600−700 response units, which corresponded to a surface density of (5.4−6.4) × 10^{-15} mol of LPL dimer/mm^2 (110 kDa/dimer). Assuming that one LDL particle covered a region of the surface equal to its largest cross-sectional area, $S = \pi d^2/4$, where d is the diameter of LDL [d = 26 nm (Schumaker et al., 1994)], then the number of LPL dimers needed for binding was 2−4.

Analysis of Steady States for Binding of Lipoproteins to LPL in the Presence or Absence of Heparan Sulfate. The plots of ΔR_{eq} versus concentration of lipoprotein in the injection buffer at constant concentration of streptavidin-bound biotinylated LPL (conditions corresponding to Figure 1c) are presented in Figure 4. Binding of HDL was not distinguishable from the nonspecific binding (Figure 4A). The binding curves for LDL (Figure 4B), VLDL (Figure 4C), and chylomicrons (Figure 4D) started steeply, but reached a linear part already at low concentrations of lipoproteins. The slopes of the linear parts were, however, larger than for the corresponding nonspecific binding. The binding curves for the apoB-containing lipoprotein classes had very similar shapes. The curves were fitted using eq 3, which included a hyperbolic dependency for a high-affinity saturable interaction and a linear part, which describes low-affinity binding together with nonspecific effects. The equilibrium dissociation constants for the high-affinity interactions are shown in Table 1. There was a tendency toward lower affinities for the biotinylated LPL as compared to corresponding affinities for LPL−heparan sulfate complexes (conditions corresponding to Figure 1b). The differences were, however, not large. All K_d values were between 1 and 20 nM.

Analysis of Binding Kinetics. All classes of lipoproteins had lower affinity for heparan sulfate-covered surfaces than for heparin-covered surfaces. Under the conditions used (with 0.15 M NaCl), binding of HDL and of LDL to heparan sulfate was so poor that it was impossible to distinguish it from nonspecific binding. Therefore, for these lipoproteins, we present data for the interaction with heparin only (Table 2). The calculated kinetic constants for the interaction of chylomicrons, β-VLDL, and VLDL with heparin were not much different from corresponding values calculated for the interaction with heparan sulfate. The lower binding efficiency was caused by a lower number of binding sites on the heparan sulfate chains than on heparin as indicated by lower values for ΔR_{max} (Table 2). As an example of primary data for analysis of kinetics, binding of β-VLDL to heparan sulfate is shown in Figure 5A.

The association of chylomicrons, VLDL, β-VLDL, and LDL with LPL and with LPL−heparan sulfate complexes followed apparently first-order kinetics only at low LPL concentrations, when the binding was far from saturation. At higher concentrations, the dissociation kinetics were better described by two exponents than by one, suggesting a complex binding mechanism. The apparent rate constants

Table 2: Apparent Rate Constants for the Interaction of Lipoproteins with Heparin and with Heparan Sulfate[a]

	heparin		heparan sulfate		
lipoprotein	k_{ass} (M^{-1} s^{-1})	k_{diss} (s^{-1})	ΔR_{max} (RU)	k_{ass} (M^{-1} s^{-1})	k_{diss} (s^{-1})
chylomicrons	$(1.1 \pm 0.3) \times 10^5$	$(5.1 \pm 0.0) \times 10^{-5}$	>6000	$(6.4 \pm 0.4) \times 10^4$	$(1.1 \pm 0.1) \times 10^{-4}$
VLDL	$(1.3 \pm 0.2) \times 10^6$	$(8.5 \pm 0.1) \times 10^{-5}$	5200	$(4.1 \pm 0.2) \times 10^5$	$(2.1 \pm 0.1) \times 10^{-4}$
β-VLDL	$(2.2 \pm 0.2) \times 10^6$	$(3.2 \pm 0.1) \times 10^{-5}$	5700	$(1.0 \pm 0.1) \times 10^5$	$(7.1 \pm 0.0) \times 10^{-4}$
LDL	$(1.4 \pm 0.4) \times 10^4$	$(8.4 \pm 0.1) \times 10^{-5}$			
HDL	580 ± 27	$(1.4 \pm 0.2) \times 10^{-4}$			

[a] In this experiment, no LPL was present. ΔR_{max} represents the maximal binding capacity for lipoproteins expressed in response units (RU). Constants represented are mean values ± standard deviation of 3−7 determinations.

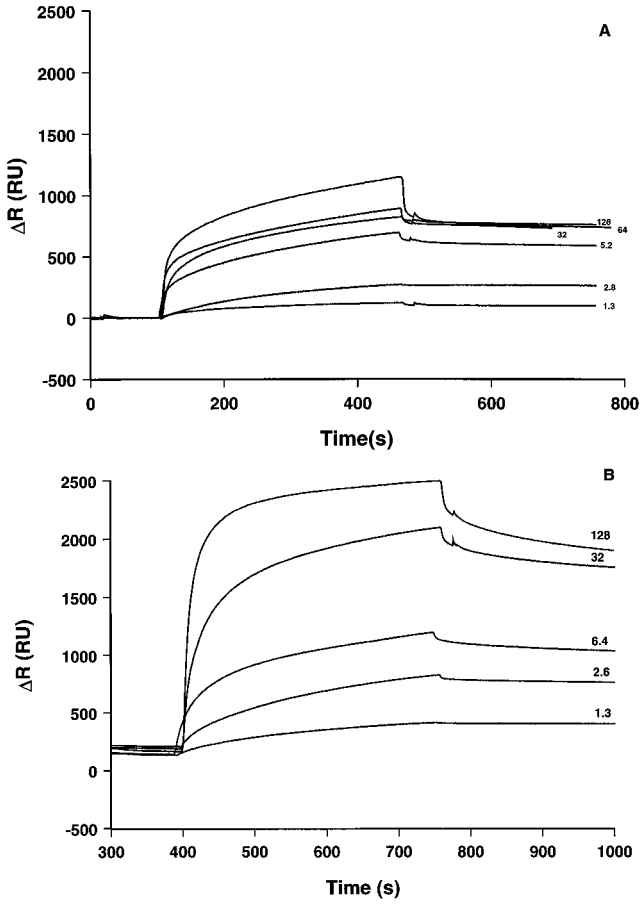

FIGURE 5: Sensorgrams for binding of β-VLDL to heparan sulfate (A) and to LPL−heparan sulfate (B). Different concentrations of β-VLDL were injected over the sensor chip with heparan sulfate (A) or with LPL−heparan sulfate (B). The increase in response units after binding of LPL was 350 ± 40 RU. The concentrations of β-VLDL (nM) are given to the right of each tracing.

calculated at low saturation are shown in Table 3. Primary data for binding of β-VLDL to LPL−heparan sulfate complexes are shown in Figure 5B. The lipoprotein−LPL interactions were characterized by fast association and by a very slow dissociation. The association rate constants were about 10-fold higher for LPL−heparan sulfate complexes than for biotinylated LPL without proteoglycans (Table 3), demonstrating a contribution of heparan sulfate to the interaction.

Comparison of data in Tables 2 and 3 illustrates that the presence of LPL did not increase the association rate of lipoproteins to heparan sulfate-covered layers. Furthermore, the dissociation rate constants were not affected by LPL. Thus, the increased binding of lipoproteins in the presence of LPL appears to be due only to an increase of the number of binding sites at the surface.

When lipoproteins were bound to LPL−heparan sulfate complexes or directly to heparan sulfate, injection of free heparin during the dissociation phase dramatically increased the dissociation rate and led to close to complete dissociation of the LPL−lipoprotein complexes from the immobilized heparan sulfate. Estimated dissociation rate constants were higher than 0.1 s^{-1}. This increase of the dissociation was similar to what was previously found for LPL alone in its interaction with heparan sulfate-covered sensor chips (Lookene et al., 1996).

Comparison of Binding to Active Site-Inhibited LPL with Binding to Catalytically Active LPL. The association rate for chylomicrons to active site-blocked HDS-LPL was only slightly slower than to catalytically active LPL (Figure 6). The dissociation rate was, however, much slower for the inhibited enzyme, indicating that the catalytic activity and generation of lipolytic products may increase the dissociation. The calculated dissociation rate constant for the binding of chylomicrons to HDS-LPL was about 100-fold lower than for the active enzyme, 4.1×10^{-6} s^{-1} and 3.2×10^{-4} s^{-1}, respectively. The association rate constant for the binding of LDL to HDS-LPL was 2.8 times lower than to the active enzyme, but with LDL there was no difference in the dissociation rates. These results, compared to those with chylomicrons, suggest that production of lipolysis products such as fatty acids from triacylglycerols in the large lipoproteins might be important for the dissociation. The presence of bovine serum albumin in the running buffer did, however, not affect the dissociation rate of chylomicrons from catalytically active LPL (data not shown).

Binding of LPL to Immobilized β-VLDL. To investigate whether lipoproteins could be directly immobilized to the sensor chips for interaction studies, β-VLDL were biotinylated and were then immobilized via binding to streptavidin. The increase in response units after addition of the lipoproteins was 2600. Binding of LPL to the immobilized lipoproteins was then studied. Figure 7A shows that the association kinetics for LPL were sufficiently well described by a one binding site model. The association rate constant was high, 10^6 M^{-1} s^{-1} , demonstrating very fast binding. The dissociation rate constant was 10^{-5} s^{-1} (Figure 7B). A disadvantage with this type of experimental system was that the sensor chips could not be regenerated after the experiments. The lipoproteins are changed by interactions with the catalytically active lipase, and the agent used to dissociate the binding would presumably also dissociate the lipoprotein particle.

DISCUSSION

Analysis of association and dissociation kinetics for binding of lipoproteins to different ligands has previously

Table 3: Apparent Rate Constants for LPL−Lipoprotein Interactions

	LPL		LPL + heparan sulfate	
lipoprotein	k_{ass} (M^{-1} s^{-1})	k_{diss} (s^{-1})	k_{ass} (M^{-1} s^{-1})	k_{diss} (s^{-1})
chylomicrons	$(0.5 \pm 0.1) \times 10^4$	$(2.8 \pm 0.1) \times 10^{-5}$	$(0.7 \pm 0.2) \times 10^5$	$(3.2 \pm 0.1) \times 10^{-4}$
VLDL	$(3.0 \pm 0.4) \times 10^4$	$(4.5 \pm 0.2) \times 10^{-5}$	$(2.3 \pm 0.3) \times 10^5$	$(1.9 \pm 0.2) \times 10^{-4}$
β-VLDL	$(2.1 \pm 0.2) \times 10^4$	$(1.2 \pm 0.1) \times 10^{-5}$	$(1.3 \pm 0.2) \times 10^5$	$(2.2 \pm 0.2) \times 10^{-5}$
LDL	$(5.1 \pm 0.1) \times 10^4$	$(6.0 \pm 0.1) \times 10^{-5}$	$(4.7 \pm 0.3) \times 10^5$	$(1.2 \pm 0.3) \times 10^{-4}$

[a] The association rate constants (k_{ass}) and dissociation rate constants (k_{diss}) were determined for the interaction of lipoproteins with biotinylated LPL bound to streptavidin (LPL, condition as in Figure 1c) and for binding of lipoproteins to LPL−heparan sulfate complexes (LPL + heparan sulfate, condition as in Figure 1b). Constants represented are mean values ± standard deviation of 5−8 determinations.

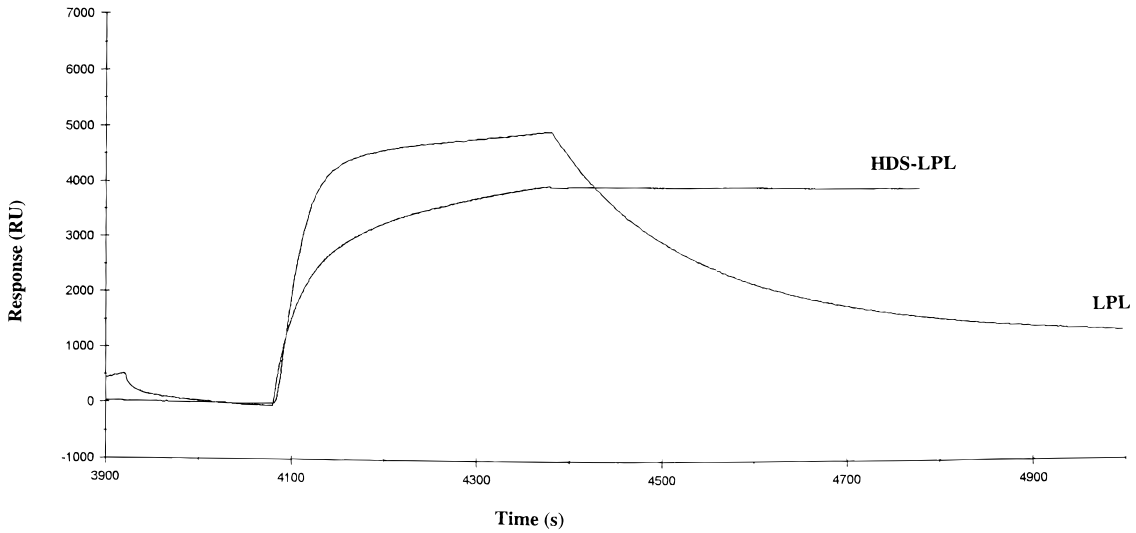

FIGURE 6: Binding of chylomicrons to catalytically active LPL and to active site-blocked HDS-LPL. LPL and HDS-LPL were bound to immobilized heparan sulfate. The increase of response units after binding of LPL was 1800 and after binding of HDS-LPL was 1920. A solution of chylomicrons (0.6 mg of triacylglycerols/mL) was injected over the layers with the bound enzymes. The increase of response units indicates binding. The dissociation was then studied by passing buffer without lipoproteins over the layers (start at 4370 s).

been virtually impossible. The BIAcore Biosensor system allows determination of kinetics of macromolecular interactions in real time (O'Shannessy et al., 1994). The present investigation represents the first effort to derive kinetic constants for lipoprotein−heparan sulfate (heparin) and lipoprotein−LPL interactions. A flow of lipoproteins over the sensor layer with immobilized heparan sulfate and with LPL mimics the situation *in vivo*, where circulating lipoproteins associate with LPL−heparan sulfate complexes at the vascular endothelium (Olivecrona & Olivecrona, 1995).

Our data demonstrate that LPL was the main factor for efficient binding of chylomicrons, VLDL, and LDL to heparan sulfate-covered surfaces. This is in good accordance with previous studies in cell culture systems, which show that LPL markedly increases the binding of the same classes of lipoproteins to cell surfaces (Fernández-Borja et al. 1996; Obunike et al., 1994; Mulder et al., 1992; Eisenberg et al., 1992). Our data enabled us to quantify the effect of LPL and to compare it between different classes of lipoproteins. HDL did not bind specifically to the sensor chips under any condition. For binding of LDL, higher surface densities of LPL were needed than for binding of chylomicrons or VLDL. This can probably be explained by the different sizes of the lipoproteins: the areas covered by chylomicrons and VLDL contain more LPL molecules than in the case of LDL. Thus, the larger lipoproteins can simultaneously bind to several LPL molecules. The absence of cooperativity in the binding of chylomicrons and VLDL might be apparent and can be caused by a lack of sensitivity in the method. We propose that a certain surface density of LPL is needed to form a high-affinity binding site for a lipoprotein particle. If this concentration is exceeded, a further increase of the surface density of LPL will only increase the number of possible binding sites. This may explain the apparently linear second parts of the plots.

There were no marked differences in the apparent equilibrium dissociation constants (K_d) for the different apoB-containing lipoproteins (chylomicrons, VLDL, β-VLDL, and LDL). The range was 2.5−11 nM, indicating high affinity. The high-affinity binding which we find here for LDL is different from previous data obtained in other systems where affinities between proteoglycans and LDL have been in the micromolar range (Gigli et al., 1992; Takahashi et al., 1996). The calculated kinetic and equilibrium constants represented here were derived for an idealized situation assuming that all density classes of lipoproteins contain only one type of defined particle. However, there is variability in size, density, and composition in all lipoprotein fractions. Therefore, the constants represented here are approximations calculated for the size and composition of average particles.

The association rate of lipoproteins for binding to heparan sulfate−LPL complexes was extremely fast. An increase in the density of LPL at the surface had only small effects on the association rate constant. This suggests that the association is therefore, at least partly, diffusion-limited and that the real values for the association rate constants might be even higher. On other hand, an increase of the flow rate from 5 to 500 μL/min (data not shown) did not influence the dissociation kinetics, indicating that the interactions are not diffusion-determined. Further investigations are needed to solve this discrepancy.

According to the simple binding model ($A + B \rightleftharpoons AB$), the equilibrium dissociation constant (K_d) calculated from the dependency of ΔR_{eq} on lipoprotein concentration should be equal to the value of the ratio of the rate constants (k_{diss}/k_{ass}). In our case, the values calculated from k_{diss}/k_{ass} were up to 15 times lower than those determined from the plot of ΔR_{eq} versus lipoprotein concentration. This discrepancy might be explained by overlapping of binding sites for large ligands (Sild et al., 1996; Stankowski, 1983). At low saturation, the ligands interact almost independently with the surface component, and the interaction follows the simple binding model. This was the case for the binding of lipoproteins at low saturation, where the rate constants were determined. At higher saturation, steric hindrances between adsorbing and already bound ligands lead to apparent negative cooperativity. This might be the reason for the complex kinetics at higher lipoprotein concentrations and for non-Michaelis−Menten-type adsorption curves even after subtraction of nonspecific binding. Simulated adsorption curves for such cases start steeply but become flat already at moderate saturation (Sild et al., 1996; Stankowski, 1983).

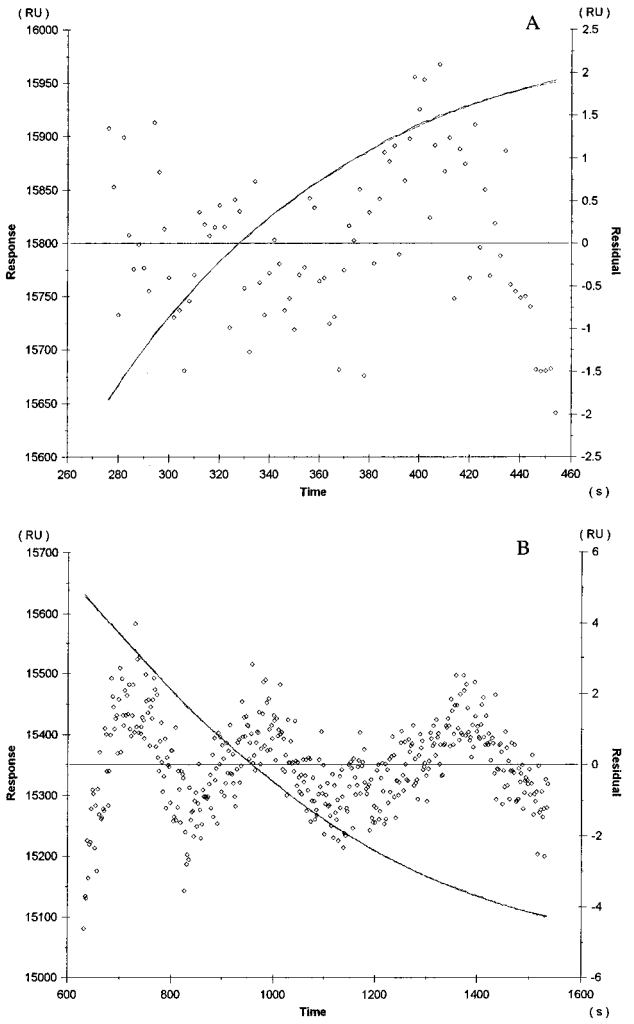

FIGURE 7: Kinetics of binding of LPL to immobilized β-VLDL. Biotinylated β-VLDL were immobilized to the sensor chip via streptavidin. Then LPL (7 nM) in 10 mM Hepes, 0.15 M NaCl, and 4 mM EDTA, pH 7.4, was injected, and association (A) as well as dissociation (B) kinetics were measured. In these figures, both experimental curves and theoretical curves are shown. The dots represent the deviation of the experimental values from the calculated values.

This is similar to our experimentally determined binding curves for lipoproteins. The determination of the K_d from the dependency of ΔR_{eq} on lipoprotein concentration took into consideration also the binding at high lipoprotein concentrations, where steric hindrances might influence the interaction. This can be the reason for the apparent lower affinity than determined from the k_{diss}/k_{ass} ratio. According to Morton et al., (1995), a two-step binding process might also be a reason for complex binding kinetics.

The association rate constants were about 10-fold lower for binding of lipoproteins to LPL which was directly bound to the sensor chip via streptavidin than with LPL−heparan sulfate complexes. Thus, a direct positive contribution of the heparan sulfate to the association is implicated, though it did not much influence the equilibrium binding constants. In addition, heparan sulfate may orient the LPL molecules in a more favorable way for interaction with lipoproteins than what is obtained with direct binding of the lipase to streptavidin.

When a solution with high concentration of VLDL or chylomicrons was injected several times over a sensor chip covered with LPL−heparan sulfate complexes, the amount of bound lipoprotein at steady state decreased after each injection. This indicated that some of the heparan sulfate-bound LPL was washed out by the flow of lipoproteins. Dissociation of LPL in the absence of lipoproteins is very slow (Lookene et al., 1996). According to our earlier study, this slow dissociation is only apparent and due to rapid rebinding of LPL to the negatively charged heparan sulfate-covered surface. It is possible that temporarily detached LPL could be shifted to the flow phase by the presence of very high concentrations of lipoproteins. In the postprandial state, after a lipid-rich meal or after an intravenous lipid infusion, the level of circulating LPL in blood increases severalfold (Peterson et al., 1990). A 10-fold increase in the LPL activity level in plasma was detected after infusion of the synthetic lipid emulsion Intralipid. Thus, the results obtained by the biosensor technique with high amounts of lipoproteins are similar to results obtained *in vivo* in humans.

Lipolysis due to the action of LPL might complicate the binding kinetics. The bound lipoproteins decrease in size, and their compositions are continuously changed. In addition, the products of lipolysis, in particular fatty acids, might influence the interaction (Olivecrona & Bengtsson-Olivecrona, 1987; Bengtsson & Olivecrona, 1980). Thus, the apparent rate constants cannot be used as true thermodynamic parameters. Since the detected response is proportional to the mass of the adsorbed molecules, the constants can be used to characterize total mass kinetics. According to our data, lipolysis increased the dissociation rate of triacylglycerol-rich lipoproteins. The triacylglycerol-poor β-VLDL had lower dissociation rate constants for LPL−heparan sulfate complexes than the chylomicrons and VLDL had, indicating that lipolysis could modulate the kinetics by increasing the dissociation rate. To study this directly, we compared catalytically active LPL with the active site-blocked HDS-LPL. The rate constant was more than 100-fold lower for the dissociation of chylomicrons from the inactive HDS-LPL than from active LPL, demonstrating that lipolysis promoted dissociation. Free fatty acids have been shown to decrease the affinity between LPL and lipoproteins (Bengtsson & Olivecrona, 1980a) and between LPL and heparin (Olivecrona & Bengtsson-Olivecrona, 1987). In the BIAcore system, the presence of bovine serum albumin, a fatty acid acceptor, at concentration of 1 mg/mL did not influence the kinetics. This is puzzling, but it is possible that fatty acids, which are locally produced by the action of LPL at surface-bound lipoproteins, are not immediately available for sequestration by bovine serum albumin. Although we cannot directly measure lipolysis in the present BIAcore system, the data convincingly demonstrate that the bound lipase is active.

It was possible to immobilize lipoproteins (β-VLDL) directly to the sensor chip for studies of binding of LPL. The association rate constant was extremely high, 10^6 M^{-1} s^{-1}, which was 10−100-fold higher than in the reverse system with binding of lipoproteins to immobilized LPL. This indicated that diffusion of the large lipoprotein particles might be more restricted than diffusion of LPL. We did not attempt to immobilize any of the other lipoprotein classes.

Immobilized heparin had much higher binding capacity for lipoproteins than heparan sulfate had. This was the case with chylomicrons, VLDL, and β-VLDL while with LDL and HDL the binding capacity was low for both types of glycan chains. The difference between heparin and heparan

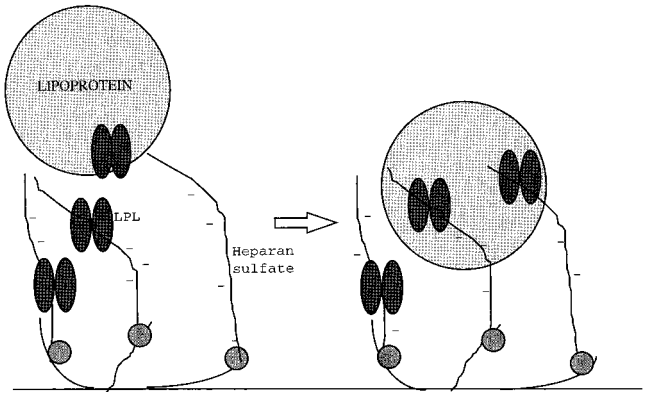

FIGURE 8: Interactions of a lipoprotein particle and LPL molecules attached to a heparan sulfate-covered surface. The figure illustrates the experimental setup used in this study (see legend to Figure 1b). LPL dimers (dark ovals) are bound to the heparan chains. LPL is the main factor for the initial interaction with the lipoprotein. Additional interactions may occur involving more LPL molecules and direct binding between lipoprotein constituents (apolipoproteins) and heparan sulfate. Our data suggest that the adsorbed lipoproteins and LPL are mobile in the plane of the surface (illustrated by the arrow).

sulfate could be explained by a higher number of binding sites on heparin, while the apparent affinities were similar for both glycosaminoglycans. The main difference between heparan sulfate and heparin is a higher degree of sulfation on the latter. Heparan sulfate from liver contains more heparin-like regions than endothelial heparan sulfate (Lyon et al., 1994). It may therefore contribute more to the binding of lipoproteins than endothelial heparan sulfate at extrahepatic sites. Interestingly, when we used hepatic lipase in the present BIAcore system, we could not detect any binding of this lipase to the heparan sulfate-covered sensor chips. This indicated that hepatic lipase had too low affinity for this type of endothelial heparan sulfate and we could therefore not do comparative studies with LPL. It is possible that hepatic lipase would bind to heparan sulfate from hepatic endothelial cells.

Only lipoproteins containing apoB bound to the sensor chips with either heparan sulfate or LPL−heparan sulfate complexes. HDL did not bind under any conditions. This is similar to what was found in experiments with lipoproteins bound to the plastic surface of microtiter plates (Choi et al., 1995). Goldberg et al., have previously demonstrated that the N-terminal end of apoB binds to LPL (Sivaram et al., 1994). It will be interesting to investigate whether the interactions seen in the BIAcore system are dependent on LPL−apoB interaction, or whether the main interaction between LPL and the lipoproteins is with the lipid. In bulk systems, LPL usually binds well to synthetic lipid emulsions without any apolipoproteins (Bengtsson & Olivecrona, 1980b). Preliminary studies with the synthetic lipid emulsion Intralipid indicate that binding to LPL in the BIAcore system is not dependent on the protein part of the lipoproteins (Lookene, Makoveichuk, and Olivecrona, unpublished results). It is, however, expected that both apoB and apoE of the lipoproteins interact directly with the heparan sulfate proteoglycans and in that way strengthen the interaction after the initial attachment to LPL (Figure 8). We did not add any extra apoE to the system, but it is likely that apoE would increase the binding of lipoproteins similarly to what was previously shown in cell culture systems (Takahashi et al., 1995; Ji et al., 1993). In support of this, the relatively apoE-rich β-VLDL bound better to the heparan sulfate-covered sensor chips than any of the other lipoproteins.

Dissociation of the lipoprotein particles from heparan sulfate-covered sensor chips was slow both in the absence and in the presence of LPL. Injection of free heparin caused a large increase in the dissociation rates. As in the case with binding of LPL alone, this demonstrates that the reason for the slow apparent dissociation is most likely fast reassociation (Lookene et al., 1996). Thus, it is more likely for the dissociated lipoprotein particle to bind again without leaving the surface. This suggests that the bound lipoproteins are rather mobile, which may simplify interactions with lipases and receptors in the plane of the cell surfaces. The main determinant for whether the lipoprotein stays is the presence of LPL. Decreased particle size, lipolysis products, free glycan chains, and possibly other factors could also limit the time the lipoprotein spends at the heparan sulfate-covered surface.

In conclusion, the surface plasmon resonance technique can be used to study several aspects of lipoprotein interactions. We have here indicated a nondestructive way of attaching lipoproteins to the sensor chip via LPL, or preferably via catalytically inactive LPL. We also attached β-VLDL directly to the sensor chips via biotin/streptavidin. With glycosaminoglycans, these biosensor chips provide a model of cell surfaces. Several different agents could be added, like membrane lipids and protein receptors, to make the model even better.

ACKNOWLEDGMENT

We are grateful to Prof. Lars-Åke Fransson, Lund University, Sweden, for providing the endothelial heparan sulfate proteoglycans, to Dr. Ulrike Beisiegel, University of Hamburg, Germany, for providing the β-VLDL, and to Prof. Thomas Olivecrona, Umeå University, Sweden, for critical reading of the manuscript.

REFERENCES

Beisiegel, U., Weber, W., & Bengtsson-Olivecrona, G. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 8342.
Bengtsson, G., & Olivecrona, T. (1980a) *Eur. J. Biochem. 106*, 557.
Bengtsson, G., & Olivecrona, T. (1980b) *Eur. J. Biochem. 106*, 549.
Bengtsson-Olivecrona, G., & Olivecrona, T. (1991) *Methods Enzymol. 197*, 345.
Bensadoun, A. (1991) *Annu. Rev. Nutr. 11*, 217.
Camejo, G., Hurt-Camejo, E., Olsson, U., & Bondjers, G. (1993) *Curr. Opin. Lipid 31*, 443.
Choi, S. Y., Sivaram, P., Walker, D. E., Curtiss, L. K., Gretch, D. G., Sturley, S. L., Attie, A. D., Deckelbaum, R. J., & Goldberg, I. J. (1995) *J. Biol. Chem. 270*, 8081.
Eisenberg, S. (1990) *Curr. Opin. Lipidol. 1*, 205.
Eisenberg, S., Sehayek, E., Olivecrona, T., & Vlodavsky, I. (1992) *J. Clin. Invest. 90*, 2013.
Fernández-Borja, M., Bellido, D., Vilella, E., Olivecrona, G., & Vilaró, S. (1996) *J. Lipid Res. 37*, 464.
Gigli, M., Consonni, A., Ghiselli, G., Rizzo, V., Naggi, A., & Torri, G. (1992) *Biochemistry 31*, 5996.
Goldberg, I. J. (1996) *J. Lipid Res. 37*, 693.
Gudmundsen, O., Tjelle, T. E., & Berg, T. (1994) *Biol. Chem. Hoppe-Seyler 375*, 305.
Havel, R. J. (1994) *Curr. Opin. Lipidol. 5*, 102.
Havel, R. J., Eder, H. A., & Bragdon, J. H. (1955) *J. Clin. Invest. 34*, 1345.
Jackson, R. L., Busch, S. J., & Cardin, A. D. (1991) *Physiol. Rev. 71*, 481.

Ji, Z.-S., Brecht, W. J., Miranda, R. D., Hussain, M. M., Innerarity, T. L., & Mahley, R. W. (1993) *J. Biol. Chem. 268*, 10160.
Johnsson, B., Löfås, S., & Lindquist, G. (1991) *Anal. Biochem. 198*, 268.
Kalogeris, T. J., & Story, J. A. (1992) *J. Nutr. 122*, 1045.
Krapp, A., Ahle, S., Kersting, S., Hua, Y., Kneser, K., Nielsen, M., Gliemann, J., & Beisiegel, U. (1996) *J. Lipid Res. 37*, 926.
Krieger, M., & Herz, J. (1994) *Annu. Rev. Biochem. 63*, 601.
Lee, W. T., & Conrad, D. H. (1984) *J. Exp. Med. 159*, 1790.
Lindblom, A., & Fransson, L. (1990) *Glycoconjugate J. 7*, 545.
Lookene, A., & Bengtsson-Olivecrona, G. (1993) *Eur. J. Biochem. 213*, 185.
Lookene, A., Chevreuil, O., Østergaard, P., & Olivecrona, G. (1996) *Biochemistry 35*, 12155.
Lyon, M., Deakin, J. A., & Gallagher, J. T. (1994) *J. Biol. Chem 269*, 11208.
Morton, A. T., Myszka, D. G., & Chaiken, I. M. (1995) *Anal. Biochem. 227*, 176.
Mulder, M., Lombardi, P., Jansen, H., Van Berkel, T. J. C., Frants, R. R., & Havekes, L. M. (1992) *Biochem. Biophys. Res. Commun. 185*, 582.
Nykjaer, A., Nielsen, M., Lookene, A., Meyer, N., Roigaard, H., Etzerodt, M., Beisiegel, U., Olivecrona, G., & Gliemann, J. (1994) *J. Biol. Chem. 269*, 31747.
Obunike, J. C., Edwards, I. J., Rumsey, S. C., Curtiss, L. K., Wagner, W. D., Deckelbaum, R. J., & Goldberg, I. J. (1994) *J. Biol. Chem. 269*, 13129.
Olivecrona, G., & Olivecrona, T. (1995) *Curr. Opin. Lipidol. 6*, 291.
Olivecrona, T., & Bengtsson-Olivecrona, G. (1987) in *Lipoprotein lipase* (Borensztajn, J., Ed.) pp 15−58, Evener Publishers, Chicago.
Olivecrona, T., & Bengtsson-Olivecrona, G. (1989) in *Heparin* (Lane, D., & Lindahl, U., Eds.) pp 335−361, Edward Arnold Publishers Ltd., London.
O'Shannessy, D. J., Brigham-Burke, M., Soneson, K. K., & Brooks, I. (1993) *Anal. Biochem. 212*, 457.
O'Shannessy, D. J., Brigham-Burke, M., Soneson, K. K., Hensley, P., & Brooks, I. (1994) *Methods Enzymol. 240*, 323.
Peterson, J., Bihain, B. E., Bengtsson-Olivecrona, G., Deckelbaum, R. J., Carpentier, Y. A., & Olivecrona, T. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 909.
Schumaker, V. N., Phillips, M. L., & Chatterton, J. E. (1994) *Adv. Protein Chem. 45*, 205.
Segrest, J. P., Garber, D. W., Brouillette, C. G., Harvey, S. C., & Anantharamaiah, G. M. (1994) *Adv. Protein Chem. 45*, 303.
Shen, B. W., Scanu, A. M., & Kézdy, F. J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 837.
Sild, V., Stålberg, J., Pettersson, G., & Johansson, G. (1996) *FEBS Lett. 378*, 51.
Sivaram, P., Choi, S. Y., Curtiss, L. K., & Goldberg, I. J. (1994) *J. Biol. Chem. 269*, 9409.
Skottova, N., Savonen, R., Lookene, A., Hultin, M., & Olivecrona, G. (1995) *J. Lipid Res. 36*, 1334.
Stankowski, S. (1983a) *Biochim. Biophys. Acta 735*, 341.
Stankowski, S. (1983b) *Biochim. Biophys. Acta 735*, 352.
Strickland, D. K., Kounnas, M. Z., & Argraves, W. S. (1995) *FASEB J. 9*, 890.
Takahashi, S., Suzuki, J., Kohno, M., Oida, K., Tamai, T., Miyabo, S., Yamamoto, T., & Nakai, T. (1995) *J. Biol. Chem. 270*, 15747.
Takahashi, S., Oida, K., Ookubo, M., Suzuki, J., Kohno, M., Murase, T., Yamamoto, T., & Nakai, T. (1996) *FEBS Lett. 386*, 197.
Weisgraber, K. H. (1994) *Adv. Protein Chem. 45*, 249.

BI962699K