Lipoprotein(a) Binding to Other Apolipoprotein B Containing Lipoproteins†

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ABSTRACT: A method combining ligand dot blotting and digital imaging was used to determine the apparent dissociation constant (K_D) for the binding of lipoprotein(a) to low-density lipoproteins$_2$ (Lp(a)–LDL$_2$). By use of this approach, the K_D for the Lp(a)–LDL$_2$ complex was shown to be in the nanomolar range [$(1.05 \pm 0.21) \times 10^{-8}$ M, $n = 4$]. The Lp(a)–LDL$_2$ interaction was both hydrophobic and ionic; however, hydrophobic forces predominated because the interaction was demonstrable at high salt concentration (>2 M NaCl), while no complex was detectable at low salt concentration (<0.08 M NaCl). Consistent with the hydrophobic nature of this interaction, the Lp(a)–LDL$_2$ complex was stable over a wide pH range (4–10). Plasminogen did not compete with Lp(a) binding to LDL$_2$ even at a 2.2×10^3 molar excess of plasminogen over the LDL$_2$ concentration. The only component identified in plasma and serum that inhibited the binding of LDL$_2$ to Lp(a) was apolipoprotein B containing lipoproteins (apoB-Lp). These studies indicate that the Lp(a)–LDL$_2$ complex could exist in plasma. In fact, up to 72% of purified Lp(a) added to an Lp(a)-negative hypertriglyceridemic plasma floated with apoB-Lp ($d < 1.063$ g/mL) following ultracentrifugation, whereas only 9% of the purified Lp(a) added to the apoB-Lp-free 1.12 g/mL infranate floated at $d < 1.063$ g/mL. The formation of a complex of Lp(a) with apoB-Lp could increase the amount of cholesterol ester bound per cellular receptor, e.g., LDL receptor, and thus potentially accelerate cholesterol removal from the vascular compartment.

Increased levels of lipoprotein(a) [Lp(a)][1] in the plasma compartment are associated with increased risk of coronary artery disease (Kostner et al., 1981; Albers et al., 1977; Dahlen et al., 1986; Rhoads et al., 1986). However, no striking correlations with other serum lipoprotein parameters have been noted (Albers et al., 1977) and the role of this lipoprotein in plasma lipid transport and metabolism remains unknown. Recently human apolipoprotein(a) [apo(a)] has been shown to have a striking sequence homology to plasminogen (Pg) (McLean et al., 1987). Partial amino acid sequence analyses of apo(a) and the deduced amino acid sequence of apo(a) show that apo(a) has many repeats of kringle-4-like domains, one kringle-5-like domain, and a domain analogous to the protease domain of plasminogen but lacking the activation site (arginine replaced by serine) (McLean et al., 1987). As discussed by Patthy et al. (1984), the kringle domains correspond to autonomous, structural, functional, and folding units that serve as versatile binding modules. Thus, the homologous kringle-4 domain found in both plasminogen and apo(a) (McLean et al., 1987) should serve an analogous role in their interactions with other proteins. Recently several reports have shown that the kringle-4-like domains of apo(a) can participate in many reactions involving plasminogen (Harpel et al., 1989; Gonzalez-Gronow et al., 1989; Edelberg et al., 1989; Hajjar et al., 1989; Miles et al., 1989).

The kringle-4 domain has been reported to have an affinity for the lysine-rich portion of fibrin (Banyai & Patthy, 1984) as well as for lysine–Sepharose (Lerch & Rickii, 1980). The interaction of Lp(a) with apoB-containing lipoproteins (apoB-Lp) depends in part on lysine residues of apoB (Ye et al., 1988), shown by both modification of the lysine residues on apoB and the use of a lysine analogue. It is of note that the putative binding domain of apoB is lysine rich in analogy with the apoE receptor binding domain (Knott et al., 1985). Monoclonal antibodies that block receptor-mediated uptake of apoB bind to a domain of apoB near this putative site (Marcel et al., 1987). To assess whether the Lp(a)–LDL interaction is of potential physiological relevance, we determined some of the physical properties of the Lp(a)–apoB-Lp complex.

Materials and Methods

Bovine serum albumin (essentially fatty acid and globulin free), dithiothreitol (DTT) and peroxidase substrate were purchased from Sigma Chemical Co., St. Louis, MO; Sepharose CL-6B from Pharmacia, Uppsala, Sweden; streptavidin–biotinylated-peroxidase complex from Amersham Corporation, Arlington Heights, IL. All other chemicals were of reagent grade.

Plasma Collection. Human plasma samples from a local blood bank were collected by plasmapheresis from healthy subjects. Type V plasma was provided through the courtesy of C. N. Corder. To prevent degradation of LDL$_2$ (d = 1.030–1.063 g/mL) and Lp(a) by endogenous enzymes, the plasma was adjusted with PPACK, leupeptin, NaN_3, and phenylmethylsulfonyl fluoride to final concentrations of 1 μM, 40 μg/mL, 0.1%, and 0.5 μM, respectively (Cardin et al., 1984).

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[1] Abbreviations: K_D, dissociation constant; Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); apo, apolipoprotein; apoB, the principal protein moiety of LDL$_2$; apoB-Lp, apoB-containing lipoproteins; LpB, lipoprotein B, the principal lipoprotein of LDL; VLDL, very low density lipoproteins (d < 1.006 g/mL); LDL, low-density lipoproteins (d = 1.006–1.063 g/mL); LDL$_2$, subclass of low-density lipoproteins (d = 1.030–1.063 g/mL); HDL$_2$, subclass of high-density lipoproteins (d = 1.063–1.12 g/mL); Lp, lipoprotein; BSA, bovine serum albumin; DTT, dithiothreitol; Na_2EDTA, disodium ethylenediaminetetraacetate; NaN_3, sodium azide; Pg, plasminogen.

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Isolation of Lipoprotein Density Classes. The plasma treated with inhibitors as described above was adjusted to d = 1.063 g/mL with solid KBr and centrifuged in a Beckman Ti 50.2 rotor at 40 000 rpm, 5 °C, for 22 h. The supernates removed by a tube-slicing technique were diluted to d = 1.030 g/mL and centrifuged again under identical conditions. The resulting supernates (VLDL + LDL_1 fraction, d < 1.030 g/mL) were collected separately and the infranatant fraction was recentrifuged under the same conditions at d = 1.063 g/mL to isolate LDL_2 (d = 1.030–1.063 g/mL). HDL_2 (d = 1.063–1.12 g/mL), HDL_3 (d = 1.12–1.23 g/mL), and VHDL (d > 1.23 g/mL) were isolated by ultracentrifugation, dialyzed, and used for further studies.

Lp(a) Isolation. Lp(a) was isolated from HDL_2 as previously described (Ye et al., 1988). Homogeneity of Lp(a) was assessed by nondenaturing gradient gel electrophoresis combined with immunoblotting for Lp(a) and LpB (Ye et al., 1988).

Ligand Dot Blotting. Native LDL_2 was biotinylated (Heggeness & Ash, 1977) by reaction of a 1 mg/mL solution of purified LDL_2 in 0.2 M borate buffer, pH 8.5, with 60 μL/mL of freshly made Biotin-X-NHS (Boehringer-Mannheim, Indianapolis, IN), 1.2 mg/mL in dimethyl sulfoxide, as we have recently described (Ye et al., 1988). Biotinylated LDL can be stored at −20 °C in 50% glycerol for at least a year without loss of ligand affinity. Biotinylated LDL_2 were shown to be reactive with antiserum to apoB by double-diffusion analyses and appeared to be spherical particles of 20–25 nm by electron microscopy of negatively stained specimens.

Lp(a) was spotted onto a Magna 66 nylon membrane (Fisher Scientific, Pittsburgh, PA) by using a dot-blot apparatus (Bio-Rad, Richmond, CA). The wells were washed with Tris/NaCl buffer (0.05 M Tris, 0.15 M NaCl buffer, pH 7.4). The membrane was removed from the dot-blot apparatus and the nonspecific sites were blocked by incubating the membrane at room temperature for 60 min in Tris/NaCl buffer containing 3% (w/v) powdered nonfat dry milk. The immobilized Lp(a) was reacted with different concentrations of biotinylated LDL_2 for 60 min in Tris/NaCl buffer containing 3% bovine serum albumin (BSA). The concentrations of biotinylated LDL_2 used are indicated in the text. After three washes at room temperature for 10 min each in the Tris/NaCl buffer, the membranes were incubated for another 60 min in a 1:600 dilution of streptavidin–biotinylated-peroxidase complex in Tris/NaCl buffer containing 3% BSA. The wash step was repeated as described above and the dots were visualized using a peroxidase substrate solution dissolved together with H_2O_2 in distilled water. Incubation was carried out with constant gentle agitation using the nutator rotary platform (Fisher Scientific, Pittsburgh, PA).

Other Methods. Neutral lipid components of lipoproteins were determined by gas–liquid chromatography (Kuksis et al., 1975). For protein determination, Bio-Rad dye reagent concentrate was used (Bradford, 1976); alternatively, protein content was determined from the amino acid composition. Antibodies used in this study have been previously described (Ye et al., 1988). Quantitation of Lp(a) was performed by using the electroimmunoassay for Lp(a) as described (Ye et al., 1988a). The number of moles of Lp(a) was determined by assuming a 1:1 molar ratio of apoB/apo(a) for Lp(a), and the molecular weight of apoB was 500K (Knott et al., 1985) and that of apo(a) was approximated at 500K as assessed by SDS–PAGE. The number of moles of Lp(a) is based on the conversion of Lp(a) protein to Lp(a) by a factor of 3.7 as previously described by Albers et al. (1977). This estimated molecular weight of 3700K is in reasonable agreement with those previously reported by Eigner et al. (1979) and Fless et al. (1986) of 4660K and 3780K, respectively. Plasminogen was purified from plasma by the procedure of Deutsch and Mertz (1970) as recently described (Rapacz, Jr., et al., 1989).

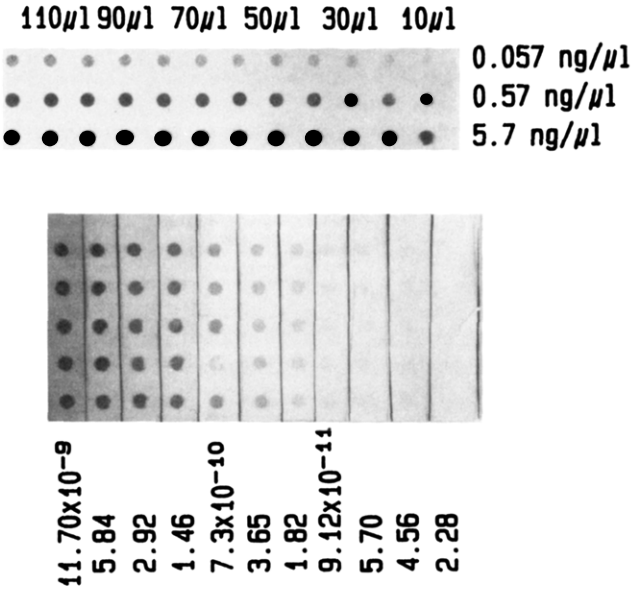

FIGURE 1: Dot-blot assay for the determination of the K_D of the Lp(a)–LDL_2 complex. The top panel is the biotinylated LDL_2 standard at different concentrations. Different amounts (10 to 120 μL at 10-μL increments) of three different biotinylated LDL_2 concentrations (5.7, 0.57, and 0.057 ng/μL) were spotted on a nylon sheet and then visualized with a streptavidin–peroxidase system. On the bottom panel, 83 ng of Lp(a) was spotted per well and ligand blotted with different concentrations of biotinylated LDL_2. The concentrations of the biotinylated LDL_2 (ng) used are listed below each strip. The standard and the ligand blots were performed at the same time on the same sheet of nylon for each K_D determination.

Results

The K_D of the Lp(a)–LDL_2 Complex. To determine the dissociation constant (K_D) of the Lp(a)–LDL_2 complex, we used an imaging system and JAVA software (Jandel Scientific, Corte Madera, CA) to quantitate the amount of LDL_2 bound to a known amount of Lp(a) immobilized on a nylon membrane. Double-reciprocal plot of the amount of LDL_2 bound on the ordinate and the concentration of LDL_2 used on the abscissa using the method of least squares gave an intercept on the X-axis equal to $-1/K_D$. This approach has been used previously to determine the K_D of the antigen–antibody complex by using a solid-phase system (Kennel, 1982).

In a typical experiment different amounts of biotinylated LDL_2 were spotted on the nylon membrane by using a dot-blot apparatus and visualized with the streptavidin–peroxidase system to construct a standard curve (Figure 1, upper panel). For each determination of K_D, a standard curve relating the inverse of intensity to the amount of biotinylated LDL_2 was generated. In the JAVA program, the lightest dot was assigned the largest number; therefore, the intensity of the dots was reported as the inverse of intensity. This generated a curvilinear line by the curve fitter program (Interactive Microware, Inc., College Station, PA) with a correlation coefficient of 0.997 (Figure 2a). The linear region of the standard curve was used to determine the amount of biotinylated LDL_2 bound to the immobilized Lp(a) incubated with various concentrations of biotinylated LDL_2 (Figure 1, lower panel). The plot of the amount of LDL_2 bound versus the concentration of LDL_2 used (Figure 2b) exhibited a saturation curve for

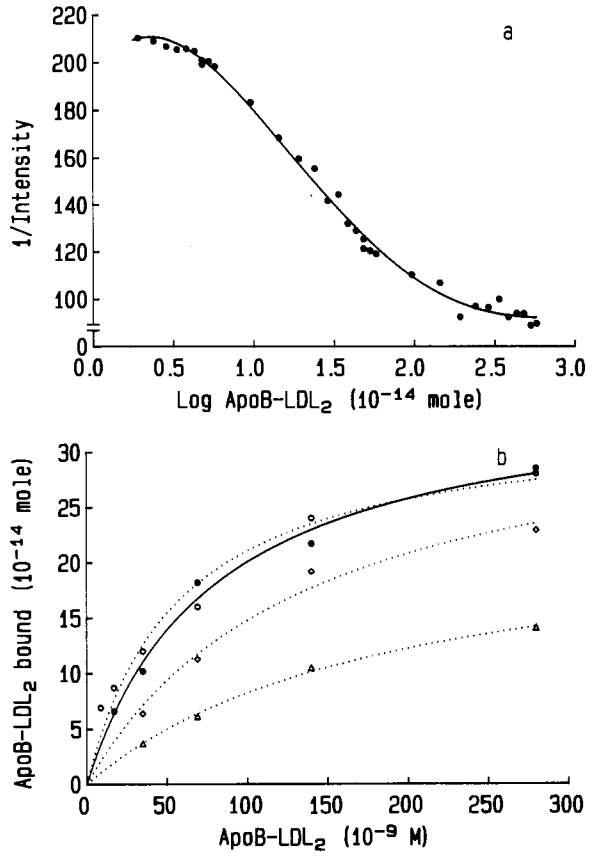

FIGURE 2: Determination of the K_D of the Lp(a)–LDL_2 complex. (a) The standard curve relating the inverse of intensity to the log of the amounts of biotinylated LDL_2 immobilized on the nylon shown in Figure 1. (b) The binding curve of the Lp(a)–LDL_2 complex. Lp(a), 8×10^{-14} mol per dot, was immobilized and ligand blotted with different concentrations of biotinylated LDL_2 for 90 min (open circle), 60 min (filled circle), 30 min (diamond), and 10 min (triangle).

ligand binding. Binding was maximal after a 1-h incubation and was evident after a 10-min incubation with the ligand. Previously we have shown that the binding is not due to modification of LDL_2 during the biotinylation process because nonbiotinylated LDL competes with the biotinylated LDL for binding (Ye et al., 1988). Furthermore, binding is specific because biotinylated LDL_2 does not bind to immobilized LDL_2 and modification of the lysine residues of LDL_2 or addition of ϵ-aminocaproic acid inhibits binding (Ye et al., 1988). The double-reciprocal plot of the data for a 1-h incubation shown in Figure 2b gave a linear line with a correlation coefficient of 0.970. The X-intercept of the double-reciprocal plot gave a K_D of 1.0×10^{-8} M of LDL_2. Results were similar at 90 min ($K_D = 2.2 \times 10^{-8}$ M). ApoB molecular weight was approximated at 500K (Knott et al., 1985). K_D determination using four different LDL_2 preparations gave a mean K_D of $(1.05 \pm 0.21) \times 10^{-8}$ M. Similar K_Ds were obtained regardless of the Lp(a) preparation used. The number of moles of apoB-Lp bound per mole of Lp(a) at saturating binding was determined by using three different Lp(a) preparations and five different LDL_2 preparations. The results yielded a ratio of 2.1 ± 0.99 mol of apoB-Lp per mole of Lp(a). The molecular weight of Lp(a) was calculated assuming a 1:1 ratio of apoB/apo(a) in Lp(a) and the apo(a) molecular weights were similar to that of apoB as determined by SDS–PAGE and were approximately 500K.

Binding of LDL_2 to Native Lp(a). Binding experiments using an immobilized monoclonal antibody directed against apoB and various concentrations of biotinylated LDL_2 were performed. The experiments yielded a K_D of 7.6×10^{-10} M

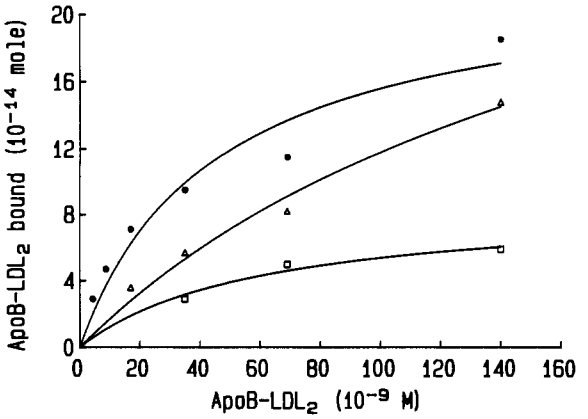

FIGURE 3: Determination of the K_I of Lp(a). The binding curve of the Lp(a)–LDL_2 complex in the presence of 0.0 M (filled circle), 1.6×10^{-8} M (triangle), and 8×10^{-8} M (square) Lp(a).

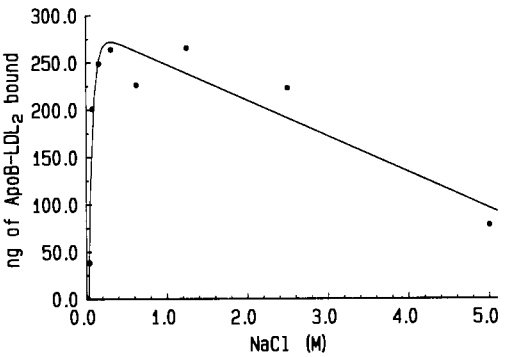

FIGURE 4: Binding of LDL_2 to immobilized Lp(a) at different NaCl concentrations. The amount of LDL_2 bound to the immobilized Lp(a) is plotted against the salt concentration used. Lp(a), 83 ng per well, was applied and ligand blotted by using a solution of 2.2×10^{-9} M biotinylated LDL_2. Bovine serum albumin (BSA) was omitted from the LDL_2 solution to prevent effects due to changes in the solubility of BSA at different salt concentrations.

of LDL_2, which was in agreement with the reported K_D of 2.9×10^{-11} M (Koren et al., 1986). Therefore we concluded that the dot-blot method described here is comparable to other methods for determining binding constants. To further show that our method measured a property of fluid-phase Lp(a), we performed a competition experiment using three different concentrations of Lp(a). As shown in Figure 3, binding was reduced when purified Lp(a) was added to the LDL_2 solution. The double-reciprocal plot of the same data was typical for competitive inhibition—the apparent K_D became progressively larger and the Y-intercept remained approximately the same as the concentration of the inhibitor [Lp(a)] increased. The plot of the apparent K_D versus the concentration of Lp(a) gave an X-intercept equal to $-K_I$ and K_I of 0.55×10^{-8} M of Lp(a). Assuming that K_I is the same as K_D of the Lp(a)–LDL_2 complex in solution, we concluded that the K_D determined by using immobilized Lp(a) and the K_D determined by using fluid-phase Lp(a) were the same and therefore the dot/ligand blotting method was not measuring a property unique to immobilized Lp(a).

The Binding Forces of the Lp(a)–LDL_2 Complex. To determine whether the Lp(a)–LDL_2 complex was held together principally by hydrophobic or hydrophilic forces, we tested binding of LDL_2 to Lp(a) at various salt concentrations. It was clear that binding of LDL_2 to Lp(a) was mediated predominantly by hydrophobic forces (Figure 4). Binding rapidly dropped as the salt concentration was lowered below the physiological salt concentration (0.15 M NaCl). Weaker hydrophilic forces were also operating, because binding de-

Table I: Effect of pH on Binding of LDL_2 to Lp(a)[a]

pH	LDL_2 bound (ng)	pH	LDL_2 bound (ng)
4.0	0	8.0	7.3
4.5	5.8	8.5	6.8
5.0	6.7	9.0	5.4
5.5	4.8	9.5	9.6
6.0	4.5	10.0	5.1
6.5	4.6	10.5	0
7.0	7.6	11.0	0
7.5	6.6		

[a] 83 ng of Lp(a) was applied per well and ligand blotted using a solution of 2.2×10^{-9} M biotinylated LDL_2 at the pH indicated. Bovine serum albumin (BSA) was omitted from the LDL_2 solution to prevent effects due to changes in the solubility of BSA at different pH. The amount of LDL_2 bound to the immobilized Lp(a) was determined as described.

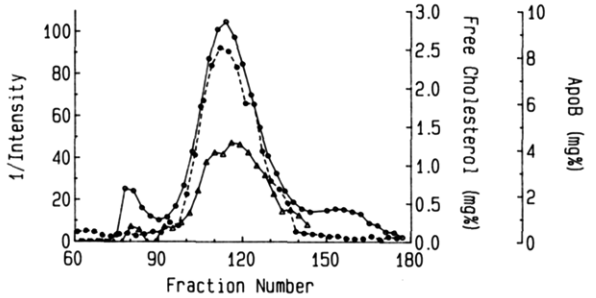

FIGURE 5: Inhibition of LDL_2 binding to Lp(a) by Lp(a)-negative plasma. Plasma from an Lp(a)-negative subject was separated on a Sepharose CL-6B column. Fractions 1–60 were discarded as the void volume and fractions 61–180 were analyzed for inhibitory activities (filled circle), free cholesterol (open circle), and apoB (open triangle). To 1 mL of each fraction, LDL_2 was added to a final concentration of 2×10^{-8} M apoB and used to ligand blot 83 ng of immobilized Lp(a).

clined as the salt concentration was raised from 1 to 5 M NaCl. As a control, the immobilized Lp(a) pretreated with different salt concentrations was ligand blotted with biotinylated LDL_2 at physiological salt concentration. No significant changes in the binding of LDL_2 to the pretreated Lp(a) were observed. Therefore, the decrease in binding was not due to the release of Lp(a) from the membrane.

If the binding forces were mainly hydrophobic, the interaction between Lp(a) and LDL_2 should be independent of pH. In support of this conclusion, we found that binding exhibited a broad optimum between the pH of 4.5 and 10.0 (Table I). Inhibition of binding at either extreme was probably due to the denaturation of Lp(a) and/or LDL_2.

ApoB-Containing Lipoproteins Inhibit the Binding of LDL_2 to Lp(a). In order for the binding of apoB-containing lipoprotein to Lp(a) to occur in vivo, there should be no other inhibitory factors in the plasma except for Lp(a)- and apoB-containing lipoproteins. As shown in Figure 5, whole plasma from an Lp(a)-negative subject has only one major inhibitory factor that exhibited the same elution profile on Sepharose CL-6B as the plasma unesterified cholesterol ($r = 0.98$) and apolipoprotein B ($r = 0.88$). There were no significant differences between the inhibitory activities of whole plasma versus those of whole serum when serum was similarly analyzed. Analyses of density classes of whole plasma from a normolipidemic and a type V subject revealed that the inhibitory activity exhibited the flotation characteristics of apoB-Lp (VLDL and LDL) (Figure 6). From these results, we concluded that Lp(a) will bind only apoB-containing lipoprotein particles.

To determine if purified Lp(a) added to Lp(a)-negative plasma would complex with apoB-Lp in the presence of plasma

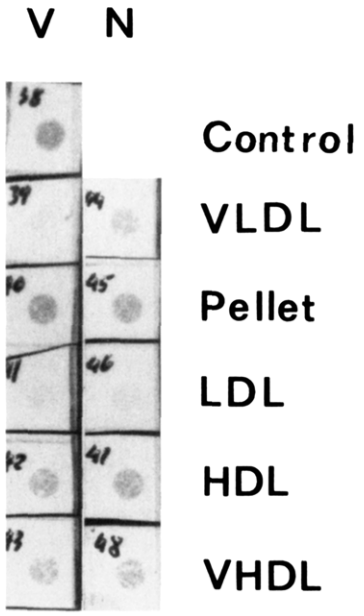

FIGURE 6: Inhibition of LDL_2 binding to Lp(a) by different density classes. Different density classes were isolated from a type V patient (V) and a normolipidemic Lp(a)-negative subject (N). Each density class was brought up to the original volume of the starting plasma, and 100 μL of each was added to 3 mL of 3% BSA in Tris/NaCl buffer containing biotinylated LDL_2 at the concentration of 2×10^{-8} M apoB and used for dot ligand blotting. The pellet was material collected from the bottom of the tube following ultracentrifugation at plasma density to obtain VLDL.

proteins, we added isolated Lp(a) to a type V plasma [Lp(a) negative by electroimmunoassay] to a final concentration of 33 mg/dL. The mixture was allowed to equilibrate at room temperature for 60 min and subjected to sequential ultracentrifugation to isolate VLDL ($d < 1.006$ g/mL), LDL ($d = 1.006$–1.063 g/mL), and HDL + VHDL ($d > 1.063$ g/mL). The isolated VLDL, LDL, and HDL + VHDL contained 17%, 55%, and 28% of the original Lp(a) ($d = 1.063$–1.12 g/mL), respectively. Recovery of the added Lp(a) was greater than 90%. A control experiment where purified Lp(a) was added to apoB free hypertriglyceridemic plasma (1.12 g/mL infranate) demonstrated that only 9% of the purified Lp(a) floated at densities <1.063 g/mL.

Plasminogen Binding to LDL_2. Because of the striking homology between apo(a) and Pg, we hypothesized that plasminogen would also bind apoB-Lp. To test this hypothesis, we attempted to measure the K_D of the Pg–LDL_2 complex using Pg immobilized on a nylon membrane. No binding was observed with biotinylated LDL_2. Furthermore, the Lp(a)–LDL_2 binding constant determined in the presence of plasminogen was the same as the binding constant determined in the absence of Pg (Table II). Binding was not diminished even at an LDL_2 concentration of 8.5×10^{-10} M and a Pg concentration of 1.8×10^{-6} M, a 2.2×10^3 M excess of Pg over LDL_2. Consistent with these results, when plasminogen was mixed with LDL_2 and electrophoresed on agarose gel, no shift in the electrophoretic mobility of either LDL_2 or plasminogen was observed as we have seen with Lp(a)–LDL_2 mixtures (Ye et al., 1988).

Discussion

Previously we have shown that Lp(a) will form complexes with other apoB-Lp. To determine whether the complex is of potential physiological significance, we analyzed the physical properties of the complex in detail. The Lp(a)–LDL_2 complex exhibited a K_D of $(1.05 \pm 0.21) \times 10^{-8}$ M. Complex formation

Table II: K_D of the Lp(a)–LDL_2 Complex in the Presence of Plasminogen[a]

	LDL_2 bound (ng) for plasminogen concn of (M)			
LDL_2 (M)	0	1.84×10^{-8}	1.84×10^{-7}	1.84×10^{-6}
1.1×10^{-8}	26.2	34.2		46
6.6×10^{-9}	34.2	22.8	18.8	15.6
3.9×10^{-9}	12.0	9.7	9.7	
2.4×10^{-9}	12.0	6.8	6.3	7.3
1.4×10^{-9}		4.8	4.4	6.3
8.5×10^{-10}	4.5	3.8	3.4	4.1
Lp(a)–LDL_2 K_D	5.1×10^{-9} M	6.4×10^{-9} M	5.1×10^{-9} M	6.2×10^{-9} M

[a] The Lp(a)–LDL_2 binding constant was determined at four different concentrations of plasminogen (0.0 M, 1.84×10^{-8} M, 1.84×10^{-7} M, and 1.84×10^{-6} M). 83 ng of Lp(a) was applied per well and ligand blotted with different concentrations of biotinylated LDL_2 as described. Plasminogen was added to the biotinylated LDL_2 solution at the concentration indicated.

is dependent on the lysine residues of apoB and the kringle-4 domain(s) of apo(a) (Ye et al., 1988). The multiple kringle-4-like domains on apo(a) may account for the high affinity observed. Tetranectin, a protein with affinity for the plasminogen kringle-4 domain, exhibited a K_D of 5×10^{-7} M for plasminogen (Kluft et al., 1989). Its affinity for Lp(a) was 38 times higher with a K_D of 1.3×10^{-8} M for Lp(a) (Kluft et al., 1989), which is similar to the binding constant of the Lp(a)–LDL_2 complex. Though apoB-Lp appears to be the only plasma component that binds Lp(a) in Lp(a)-negative plasma, we have not tested whether purified tetranectin could compete with LDL_2 for binding to Lp(a). The plasma concentration of the tetranectin tetramer is 5×10^{-8} M (Clemmensen et al., 1986), which is probably too low to effectively inhibit the binding of plasma LDL_2 to Lp(a).

Binding of lysine analogues to kringle-4 of Pg affects the aromatic resonance of kringle-4, suggesting that aromatic residues, particularly tryptophan, are involved in ligand binding (Castillino, 1981; Hochschwender et al., 1983; De Marco et al., 1989). Furthermore, modification of the Trp426 residue of kringle-4 abolishes binding (Hochschwender & Larusen, 1981). This same tryptophan residue is conserved in both the human and the rhesus monkey apo(a) (McLean et al., 1987; Tomlinson et al., 1989). These reports suggest that hydrophobic forces are important in ligand binding at kringle-4, which agrees with our finding that the binding of apo(a) to apoB is predominantly mediated by hydrophobic forces. Furthermore, we found that binding was disrupted at pH below and above 4.5 and 10.0, respectively. Kringle-4 of plasminogen denatures at the same pH boundaries (De Marco et al., 1985). However, the fact that Pg did not compete with Lp(a) for binding to LDL_2 suggested that the kringle-4-like domain or domains of apo(a) are different from the kringle-4 domain of Pg. In fact, those of apo(a) are highly glycosylated whereas that of Pg is not (McLean et al., 1987), which may also contribute to the observed binding.

The high binding constant, the absence of inhibiting activities in plasma, and the fact that binding is stable over a wide range of pH and is maximal at physiological salt concentration suggested that the Lp(a)–LDL_2 complex is of physiological significance. These studies suggest that a series of complexes between Lp(a) and differentially sized apoB-Lp could exist in plasma. In our hands, 72% of the purified Lp(a) added to an Lp(a)-negative hypertriglyceridemic plasma floated at densities less than 1.063 g/mL, indicating that a significant amount of Lp(a) could complex with the apoB-Lp in this reconstitution experiment using type V hypertriglyceridemic plasma. Since the molar stoichiometry of apoB/apo(a) (2:1, 1:1, or 1:2) in the Lp(a) molecule is uncertain (Gaubatz et al., 1987; Fless et al., 1986), several different complexes of varying apo(a) content are possible. The concept that apo(a) is linked to apoB to form Lp(a) through disulfide bonds has become generally well accepted (Armstrong et al., 1985; Fless et al., 1984; Utermann & Weber, 1983), but the fact that apo(a) cannot be removed from the apoB of reduced Lp(a) by gel filtration in either normal or high salt (Fless et al., 1985) suggests the binding of apo(a) to apoB by strong hydrophobic forces. Furthermore, some investigators have succeeded in purification of apo(a) in the absence of reducing agents (Ehnholm et al., 1972; Utermann et al., 1972; Jurgens & Kostner, 1975). Therefore, it is still unclear whether part of isolated Lp(a) is a complex of apoB with apo(a) linked by disulfide bonds and part is the Lp(a)–apoB-Lp complex bound together by noncovalent hydrophobic interactions.

At present the physiological role or roles of Lp(a) are unknown, though recently we have found a reduced incidence of Lp(a)-positive subjects among hypertriglyceridemics (McConathy et al., 1990), which suggests a relationship between Lp(a) and triglyceride metabolism. In addition, by virtue of its affinity for both apoB-containing lipoproteins and fibrin (Harpel et al., 1989), Lp(a) could be delivering lipids to the site of vascular damage and could possibly aid in sealing the vascular wall or contribute to lipid accumulation at this site. Furthermore, by competing with Pg for binding to the Pg receptor on endothelial cells (Gonzalez-Gronow et al., 1989), Lp(a) could shift the equilibrium to favor coagulation rather than fibrinolysis (Hajjar et al., 1989). This could account for Lp(a) being an independent risk factor for cardiac ischemia (Durrington et al., 1988). Alternatively, the formation of a complex between LDL_2 and Lp(a) could greatly enhance the net uptake of cholesterol esters by LDL_2 receptor mediated events by doubling the intake of cholesterol ester intake per endocytotic event. The Lp(a)–LDL_2 complex, like the LDL_2 caused to aggregate by treatment with phospholipase (Suits et al., 1989), could also be taken up by the macrophage via the LDL receptor more efficiently than uncomplexed LDL, or alternatively, the Lp(a)–LDL_2 complex may behave like aggregated LDL_2 and be taken up by the macrophage scavenger pathway (Khoo et al., 1988). These phenomena could contribute to the accumulation of cholesterol and formation of foam cells and therefore contribute to the reported atherogenicity of Lp(a).

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