Lipoprotein(a) Binds to Human Platelets and Attenuates Plasminogen Binding and Activation†

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ABSTRACT: Lipoprotein(a) [Lp(a)] is a unique lipoprotein consisting of a low-density lipoprotein moiety (LDL) covalently linked to apoprotein(a). Previous work has demonstrated that Lp(a) can compete with plasminogen (PGN) for binding to endothelial and mononuclear cells and can inhibit PGN activation in cell-free systems. We have assessed the binding of Lp(a) to platelets and the influence of binding on the activation of PGN by tissue-type plasminogen activator (t-PA) in this system. In direct binding experiments, Lp(a) bound specifically, saturably, and reversibly to platelets with an estimated apparent K_d of 0.20 μM. Scatchard analysis revealed a single class of binding sites with 81 000 ± 22 000 particles of Lp(a) bound at saturation. Interestingly, Lp(a) bound to a similar extent to thromboasthenic platelets. Activation of platelets with ADP or thrombin reduced Lp(a) binding capacity by approximately 50% without changing affinity. Lp(a) also inhibited the binding of PGN to platelets with an IC_{50} of approximately 0.23 μM. Over a similar concentration range, LDL did not inhibit PGN binding to platelets. In addition, Lp(a) inhibited PGN binding to plasmin-treated platelets with an IC_{50} of approximately 0.2 μM. Kinetic experiments demonstrated that Lp(a) acted as a competitive inhibitor of PGN activation by t-PA on the platelet surface, with an estimated K_i of 0.49 μM. In the presence of platelets, Lp(a) decreased the k_{cat}/K_m for t-PA by 3-fold, owing primarily to an increase in the K_m of t-PA for PGN. In contrast, LDL did not alter the kinetics of PGN activation by t-PA on the platelet surface. Importantly, Lp(a) inhibited t-PA binding to platelets with an IC_{50} of approximately 0.1 μM. Thus, Lp(a) may impair fibrinolysis in part by attenuating the binding of PGN and t-PA to the platelet surface, thereby effectively reducing platelet surface-mediated enhancement of PGN activation.

Since its identification in 1963 by Berg, much has been learned about the structure, epidemiology, and genetics of lipoprotein(a) [Lp(a)] (Berg et al., 1974; Dahlen et al., 1986; Kostner et al., 1981; Hoff et al., 1988; Gaubatz et al., 1983; Uterman & Weber, 1983). Although it is now accepted that elevated plasma levels are strongly associated with coronary artery disease (Dahlen et al., 1986), myocardial infarction (Kostner et al., 1981), and saphenous vein graft stenosis after coronary artery bypass surgery (Hoff et al., 1988), the mechanism by which Lp(a) confers an increased risk of atherosclerosis remains incompletely understood. The cDNA sequence of Lp(a) (Mclean et al., 1987; Eaton et al., 1987) suggests that one such mechanism may relate to the structural homology of apoprotein(a) [apo(a)] with the zymogen plasminogen.

Lp(a) comprises a low-density lipoprotein (LDL) moiety covalently linked via a disulfide bridge to apo(a). Apo(a) contains multiple "kringle" domains that are approximately 80% homologous with kringle 4 of plasminogen (PGN), a homologous kringle 5-like domain, and a serine protease domain that is devoid of plasmin activity owing to an amino acid substitution at the analogous activation site.

Recent studies have demonstrated that Lp(a) can impair PGN activation by tissue-type plasminogen activator (t-PA) in cell-free systems (Loscalzo et al., 1990; Edelberg & Pizzo, 1990). Lp(a) binds to both fibrin (Loscalzo et al., 1990) and t-PA (Simon et al., 1991) and thereby attenuates plasminogen activation. In addition, Lp(a) can bind directly to endothelial and mononuclear cells, inhibit PGN binding to these cells (Miles et al., 1989; Hajjar et al., 1989), and attenuate the enhancement of plasminogen activation that occurs on the surface of these cells. Incubation of Lp(a) with endothelial cells has also been shown to increase the expression of mRNA for plasminogen activator inhibitor type 1 (PAI-1) and to increase release of this serpin into the extracellular milieu (Etingin et al., 1991).

Thus, Lp(a) may play an important role in altering the balance between hemostasis and fibrinolysis since it can interact with many of the requisite regulatory elements found in the local vascular milieu.

The specific relationship of Lp(a) to platelets, important cellular determinants of primary hemostatic and fibrinolytic responses, and the functional consequences of this interaction have not been characterized. In this report, we demonstrate that Lp(a) binds directly to platelets, inhibits PGN binding to platelets, and competitively inhibits the enhancement (Shuwei et al., 1990) of PGN activation by t-PA that occurs on the platelet surface. We provide evidence that the binding of Lp(a) to platelets occurs independent of glycoprotein IIb/IIIa and is not significantly altered by ADP or thrombin. Furthermore, we demonstrate that Lp(a) inhibits t-PA binding

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to platelets, providing another mechanism for the reduction of surface-bound plasmin generation.

MATERIALS AND METHODS

Lysine–Sepharose, Sephadex G-25, and Sepharose 2B were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Bovine serum albumin (BSA), tranexamic acid, and gangliosides (the monosialoganglioside GM_3, the disialoganglioside GD_{1a}, and the trisialoganglioside GT_{1b}) were purchased from Sigma Chemical Co., St. Louis, MO. $Na^{125}I$ was obtained from Amersham Corp., Arlington Heights, IL, and Iodobeads were purchased from Pierce Chemical Co., Rockford, IL. Tissue-type plasminogen activator (t-PA) was kindly provided by Genentech, Inc., South San Francisco, CA. S-2251 was purchased from Kabi Vitrum, Stockholm, Sweden. All other chemicals were reagent grade or better. Deionized water was used throughout.

Plasminogen Preparation and Iodination. Glu-plasminogen (PGN) was purified from fresh-frozen plasma thawed at 37 °C using a modification of the method of Deutsch and Mertz (1970), as described previously (Simon et al., 1991). SDS–polyacrylamide gel electrophoresis was performed as described by Laemmli (1970) using the Phast System (Pharmacia, Uppsala, Sweden). The gels were prepared using high and low molecular weight standards (Bio-Rad, Richmond, CA) and were stained with Coomassie Brilliant Blue. Protein concentrations were determined by the methods of Lowry et al. (1951) and of Bradford (1976). PGN was concentrated using Centricones and was radioiodinated using Iodobeads as described previously (Loscalzo et al., 1986). Two Iodobeads were preincubated with 1.0 μCi of $Na^{125}I$ for 10 min at 25 °C, after which 1.25 mL of 0.88 mg/mL PGN was added. After incubation for 15 min, the solution was removed from the Iodobeads (stopping the iodination reaction) and passed over a Sephadex G-25 column that had been developed first with 10 mM Tris, pH 7.8, and 0.15 M NaCl (Tris-buffered saline, TBS) with 1% BSA, followed by TBS. Fifteen 0.15-mL fractions were collected and assayed for total and 100% trichloroacetic acid-precipitable radioactivity. Peak fractions routinely contained at least 94% precipitable radioactivity and had a specific activity of 10 000–15 000 cpm/μg of protein. t-PA was iodinated in a similar fashion and contained 94% precipitable radioactivity with a specific activity of 86 000 cpm/μg.

Isolation of LDL and Lp(a). LDL was prepared from the plasma of fasting normolipemic volunteers by sequential ultracentrifugation as described by Havel et al. (1955). SDS–polyacrylamide gel electrophoresis was used to ensure the purity of LDL. Apo(a)-free LDL was prepared as described by Fless et al. (1986). Lp(a) was obtained from Drs. Angelo Scanu and Gunther Fless (University of Chicago, Chicago, IL) and was prepared from blood drawn into sterile bottles immersed in ice that contained a final concentration of 0.15% EDTA, 0.01% NaN_3, and 0.4 μM soybean trypsin inhibitor. Plasma was then separated by low-speed centrifugation at 4 °C, and diisopropyl fluorophosphate was added to a final concentration of 1 mM to minimize proteolysis. Total lipoproteins were prepared by adjusting the plasma density to 1.21 g/mL with solid NaBr and centrifuging the sample in a 60 Ti rotor at 59 000 rpm for 20 h at 15 °C. Lp(a) was isolated from the total lipoprotein fraction using rate zonal ultracentrifugation (Fless et al., 1986). To ensure the purity of Lp(a) preparations, SDS–polyacrylamide gel electrophoresis was performed and, when necessary, further purification was conducted using FPLC ion-exchange chromatography on a Mono-Q column (Pharmacia, Uppsala, Sweden) (Armstrong, et al., 1985). Lp(a) or LDL was eluted with a 0.0–1.0 M NaCl gradient superimposed on 10 mM Tris, pH 7.4, at a flow rate of 1 mL/min at 8 °C. Lp(a) eluted at 0.41 M NaCl and LDL at 0.29 M NaCl. Purity of isolated Lp(a) and LDL was checked electrophoretically. The molecular weight of Lp(a) used in these experiments was 914 000, while that of LDL was 514 000. Lp(a) was radioiodinated by the iodine monochloride method of McFarlane (1958). Lp(a) radioiodine was 97% precipitable with trichloroacetic acid, and the particle had a specific activity of 600–900 cpm/ng.

Platelet Gel Filtration. Blood was collected from normal human volunteers into citrate–phosphate–dextrose buffer (CPD), at a ratio of 1 mL of CPD/10 mL of blood, and centrifuged for 10 min at 1000 rpm to obtain platelet-rich plasma (PRP). Blood from a patient with Glanzmann's thrombasthenia, characterized by an absence of detectable glycoprotein IIb/IIIa, was obtained with the assistance of Dr. Alan Michelson (University of Masschusetts Medical Center, Worcester, MA) and was treated similarly. PRP was passed over a column of Sepharose 2B (5 mL of Sepharose/mL of plasma) that had been washed first with 10 mL of distilled H_2O and then with 100 mL of HEPES balanced salt buffer containing 5.8 mM *N*-(2-hydroxyethyl)piperazine-*N*′-1-ethanesulfonic acid (HEPES), 140 mM NaCl, 6.11 mM KCl, 2.53 mM $MgSO_4$, 2.44 mM Na_2SO_4, 5.9 μM bovine serum albumin, and 5.64 mM dextrose, pH 7.4. Platelet counts were determined using a Coulter Counter Model ZM (Coulter Electronics, Hialeah, FL) and adjusted to 150 000/μL.

Direct Binding Assay. One hundred microliters of gel-filtered, resting platelets (normal or deficient in glycoprotein IIb/IIIa) were incubated with varying concentrations of ^{125}I-Lp(a) in 10 mM HEPES, pH 7.4, and 0.15 M NaCl (HEPES-buffered saline, HBS) (final volume = 250 μL) for 60 min at 25 °C. After 1 h, 100 μL of this suspension was layered onto an oil mixture consisting of one part # 556 fluid AC and two parts # 550 fluid AE (Dow Corning, New Bedford, MA) and then centrifuged in Eppendorf microfuge tubes for 3.5 min. The Eppendorf tubes were then inverted and the tips amputated, after which the platelet pellets were counted using a Cap RIA 16 γ counter (Capintec, Inc., Ramsey, NJ). Counts were expressed in terms of the number of particles of Lp(a) bound per platelet. Specific binding was defined as that which was inhibited with 10 mM tranexamic acid or a 20-fold excess of cold Lp(a). Similar assays were performed using platelets stimulated with ADP (10 μM) or thrombin (0.5 unit/mL). Identical methodology was used in studies of PGN binding to platelets, in which case ^{125}I-PGN was used as the labeled ligand.

Reversibility of Lp(a) Binding. Gel-filtered resting platelets (570 mL) were incubated with ^{125}I-Lp(a) at 0.4 μM in HBS for 60 min at 25 °C. After 1 h, tranexamic acid (10 mM final) was added to this suspension (final volume = 1000 μL). At successive 5-min intervals, 100-μL aliquots of this suspension were removed, layered onto an oil mixture, and counted as described above. Counts were expressed in terms of the number of particles of Lp(a) bound per platelet.

Time Course of Lp(a) Binding. Gel-filtered, resting platelets (100 μL) were incubated with ^{125}I-Lp(a) at 0.2 mM in HBS for varying time intervals (0–60 min) at 25 °C (final volume = 250 mL). At determined time points, 100 μL of this suspension was layered onto an oil mixture and counted as previously described. Similar time course experiments were performed for ^{125}I-PGN at a final concentration of 2 μM.

Competitive Binding Assay. ^{125}I-PGN at 3.4 μM was incubated with varying concentrations of Lp(a) and HBS for

30 min at 25 °C. Gel-filtered resting platelets (100 μL) were then added and the mixture was incubated for 60 min at 25 °C (final volume = 250 μL). After 1 h, 100 μL of this suspension was layered onto the oil mixture (vide supra), and the platelet pellet was counted in a γ counter as described for direct binding assays. Counts were converted into the number of molecules of PGN bound per platelet. Results were expressed as the ratio of the number of molecules of PGN bound in the presence of inhibitor (B) to the number bound in the absence of inhibitor (B_0). The ability of PGN to inhibit the binding of radiolabeled Lp(a) to platelets was tested using identical methods. In addition, the effect of plasmin on the ability of Lp(a) to inhibit PGN binding to platelets was assessed using these methods. Plasmin-treated platelets were obtained by incubating PRP with plasmin (1 caseinolytic unit/mL) for 60 min at 37 °C. After 1 h, plasmin was inactivated with aprotinin (350 000 IU). PRP was then passed over a column of Sepharose 2B as described in the gel-filtration section, and the above binding methods were employed.

The ability of apo(a)-free LDL as well as gangliosides (GM_3, GD_{1a}, and GT_{1b}) to inhibit the binding of PGN or LP(a) to platelets was also examined using identical methods. All data points shown represent the results of three or more experiments, each performed in duplicate.

Kinetic Assay. Gel-filtered resting platelets (100 μL, adjusted platelet count of 150 000/μL) were incubated with varying concentrations of Lp(a) (0.06–0.3 μM) in phosphate-buffered saline (PBS; 10 mM sodium phosphate, pH 7.4, 0.15 M NaCl) for 30 min at 37 °C. PGN was then added in varying concentrations (0.65–2.3 μM) and incubated for 15 min at 37 °C. The plasmin-specific chromogenic substrate S-2251 was added to a final concentration of 0.8 mM, and 100-μL portions of this suspension were aliquoted into microtiter wells. t-PA was added to a final concentration of 100 nM and the optical density was read every 30 s for 5 min using a Dynatech MR 5000 card reader (Dynatech, Chantilly, VA). In all cases, the identical platelet suspension containing all protein components except t-PA served as a blank to which the study wells were compared. These experiments were repeated with apo(a)-free LDL and gangliosides, with or without Lp(a). In addition, similar kinetic assays were performed in the absence of gel-filtered platelets (or inhibitor) to determine whether or not the activation of PGN was increased on the platelet surface compared to its rate of activation in solution. The initial reaction velocity was determined from the slope of the plot of the change in absorbance at 405 nm over time versus time as previously described (Ranby et al., 1982). Double-reciprocal plots of initial reaction velocity versus plasminogen concentration were generated to estimate the K_m and V_{max} of t-PA for PGN in the presence of the different concentrations of Lp(a). A Dixon plot (Dixon, 1972) of the inverse of the initial reaction velocity versus the concentration of inhibitor was generated to estimate the K_i of Lp(a) for PGN and to characterize the inhibitory mechanism.

In order to determine the direct effect of Lp(a) and/or platelets on the enzymatic activity of t-PA, 100 nM t-PA was added to a suspension of the chromogenic substrate S-2288 at 0.8 mM in PBS, pH 7.4, in the presence and absence of Lp(a) at 0.2 μM and/or gel-filtered resting platelets. The optical density was recorded every 30 s for 5 min using a card reader. A plot of the absorbance at 405 nm versus time was generated to evaluate any change in the activity of t-PA. To evaluate the effect of Lp(a) on t-PA binding to the platelet surface, competitive binding curves using ^{125}I-t-PA and cold Lp(a), as well as ^{125}I-Lp(a) and cold t-PA, were performed using the methods described above (vide supra).

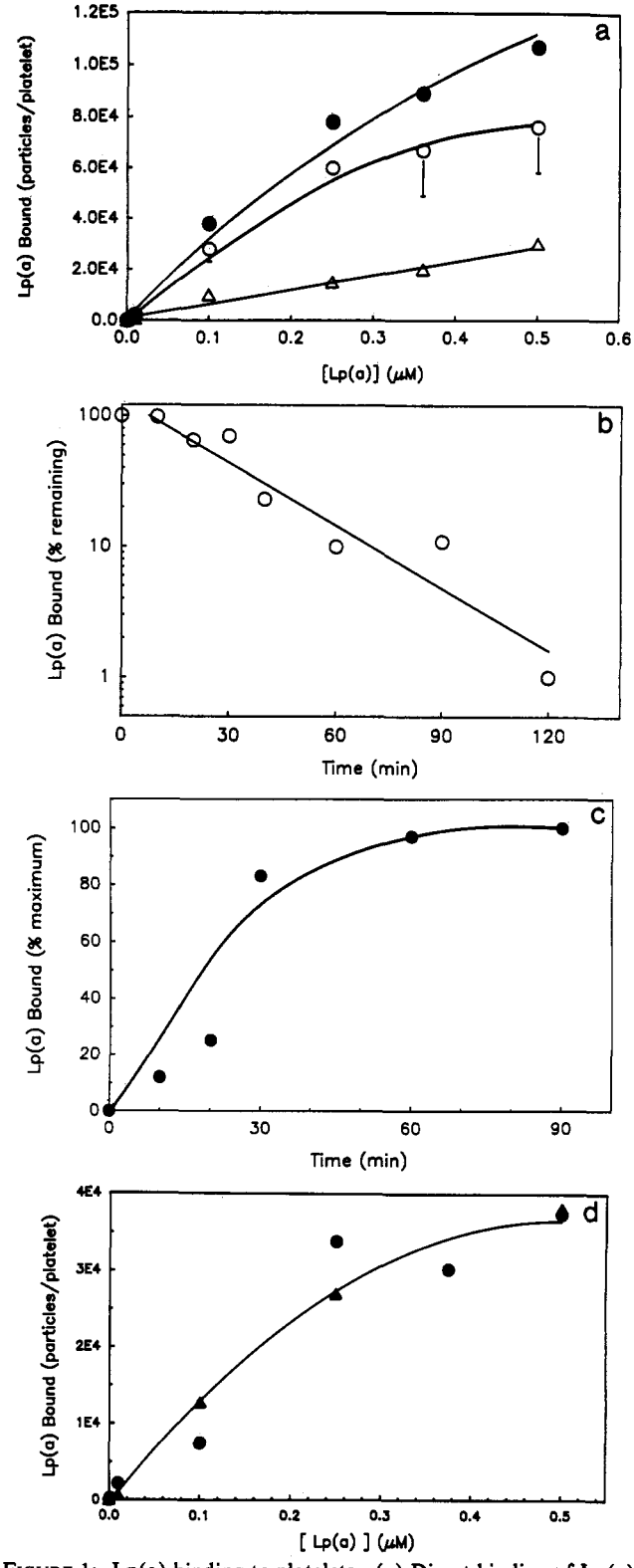

FIGURE 1: Lp(a) binding to platelets. (a) Direct binding of Lp(a) to platelets. Specific binding was defined as that which was inhibited by 10 mM tranexamic acid or a 20-fold excess of cold Lp(a). Results represent the mean ± SEM of 3–6 experiments using different platelets on different days from different donors. Filled circles, total binding; open circles, specific binding; open triangles, nonspecific binding. Error bars are shown only for direct binding for purposes of clarity of presentation. (b) Reversibility of Lp(a) binding to platelets. (c) Time course of Lp(a) binding to platelets. (d) Direct binding of Lp(a) to ADP- or thrombin-stimulated platelets. Results represent the mean of two experiments, each performed in duplicate. Filled circles, ADP-stimulated; filled triangles, thrombin-stimulated.

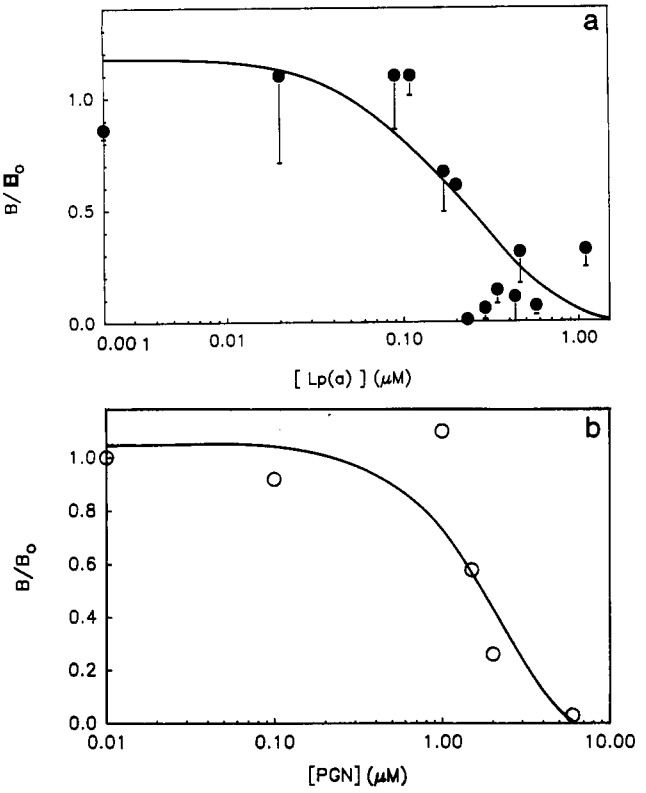

FIGURE 2: Inhibition of Lp(a) and PGN binding to platelets. (a) Lp(a) inhibits PGN binding to platelets. PGN (3.4 μM) was incubated with increasing concentrations of Lp(a) and platelets in HBS as described in Materials and Methods. Results are expressed as the mean ± SEM of 3–6 experiments. (b) PGN inhibits Lp(a) binding to platelets. Lp(a) (0.2 μM) was incubated with increasing concentrations of PGN and platelets in HBS as described in Materials and Methods.

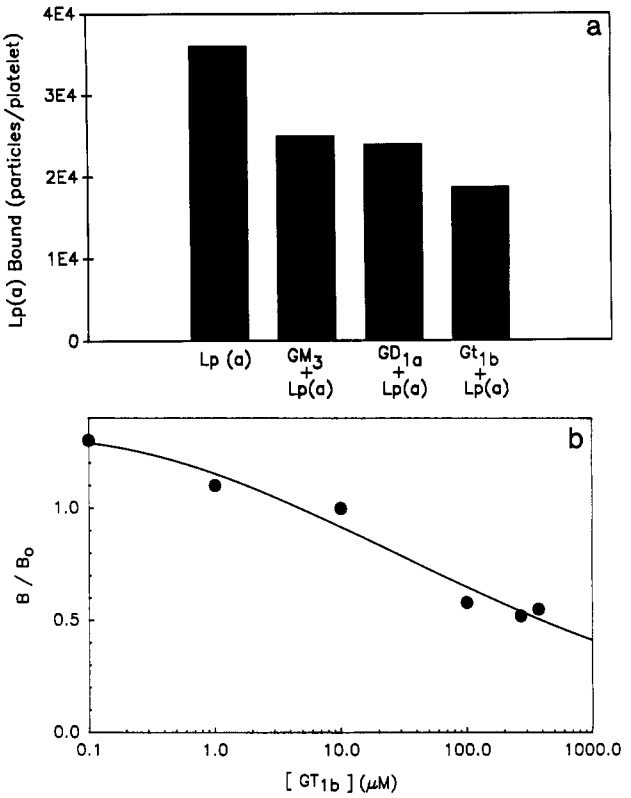

FIGURE 3: Gangliosides and Lp(a) binding to platelets. (a) Gangliosides inhibit specific binding of Lp(a) to platelets. Lp(a) (0.20 μM) was incubated with gangliosides (250 μM) (GM_3, GD_{1a}, and GT_{1b}) and platelets in HBS and binding was determined as described in Materials and Methods. (b) Lp(a) (0.20 μM) was incubated with platelets in HBS, and competitive binding was determined for GT_{1b} over a range of concentrations of the ganglioside. Binding was determined as described in Materials and Methods.

RESULTS

Direct binding experiments demonstrated that Lp(a) bound saturably (Figure 1a) and reversibly (Figure 1b) to platelets with an estimated apparent K_d of 0.20 μM. At saturation, approximately 81 000 ± 22 000 particles of Lp(a) bound specifically, and Scatchard analysis provided evidence for a single class of binding sites. Time course experiments indicated that Lp(a) binding reached apparent equilibrium within 30–40 min of incubation (Figure 1c). Importantly, Lp(a) bound to gel-filtered thromboasthenic platelets similarly with no significant difference in number of particles bound at physiological concentrations of lipoprotein. Lp(a) bound equally to ADP- and thrombin-stimulated platelets with an estimated apparent K_d of 0.15 μM and a B_{max} of approximately 40 000 particles/platelet (Figure 1d).

Lp(a) inhibited the specific binding of ^{125}I-PGN to platelets (Figure 2a). The IC_{50} for Lp(a) was approximately 0.23 μM. Apo(a)-free LDL did not affect the binding of PGN to platelets over a similar range of concentrations (0.002–0.5 μM). In addition, PGN inhibited the specific binding of ^{125}I-Lp(a) to platelets with an IC_{50} of approximately 1.6 μM (Figure 2b). Lp(a) also inhibited the specific binding of PGN to plasmin-treated platelets with an IC_{50} of approximately 0.2 μM (data not shown).

A recent report has demonstrated that gangliosides can inhibit PGN binding to cells (Miles et al., 1989a). Although the role of these glycolipids in thrombosis and fibrinolysis is uncertain, they may in part modulate PGN binding site function. In competitive inhibition experiments, gangliosides GM_3, GD_{1a}, and GT_{1b} all inhibited significantly the specific binding of Lp(a) to platelets (Figure 3a). GT_{1b} inhibited the specific binding of Lp(a) to platelets with an IC_{50} of approximately 300 μM (Figure 3b).

Table I: Kinetic Constants for Plasminogen Activation by t-PA: Effect of Platelets and Lp(a)[a]

	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} μM^{-1})
–GFP	4.0	0.17	0.043
+GFP	1.1	0.13	0.12
+GFP + Lp(a) 260 nM	2.5	0.17	0.068

[a] GFP = gel-filtered platelets at 150 000/μL. The concentration of t-PA used was 100 nM. Plasminogen activation was tested over a range of plasminogen concentrations from 0.65 to 3.4 μM, as detailed in Materials and Methods.

In order to examine the functional consequences of the ability of Lp(a) to inhibit PGN binding to platelets, PGN activation on the platelet surface was assessed in the presence and absence of Lp(a). A plot of the change in absorbance over time versus time was used to determine the initial reaction velocity of the activation of PGN by t-PA, as described in Materials and Methods. As shown in Table I and Figure 4, the K_m of t-PA for PGN was increased 4-fold in the absence of platelets compared to the presence of platelets, and the catalytic efficiency (k_{cat}/K_m) was commensurately reduced approximately 3-fold. Increasing concentrations of Lp(a) (Table I and Figure 4) over a range of values from 0.06 to 0.30 μM led to a progressive reduction of the catalytic efficiency in the presence of platelets to the limiting value observed in the absence of platelets. This effect of Lp(a) was principally the result of an increase in K_m, with an accompanying increase in k_{cat} at the highest concentrations of Lp(a)

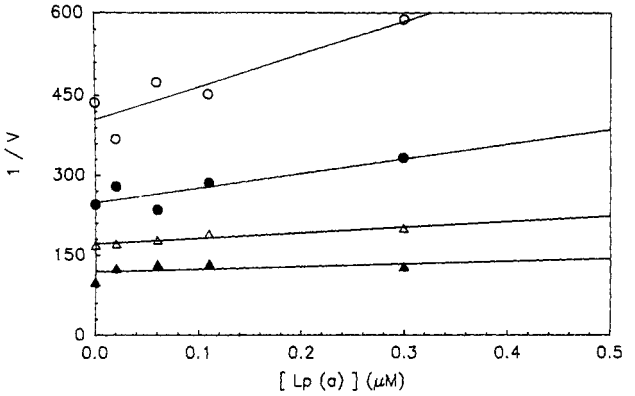

FIGURE 4: Competitive inhibition of plasminogen activation by t-PA with Lp(a). Open circles, [PGN] = 0.65 μM; filled circles, [PGN] = 0.9 μM; open triangles, [PGN] = 1.3 μM; filled triangles, [PGN] = 2.3 μM. The K_i for Lp(a) derived from these data is 0.49 μM.

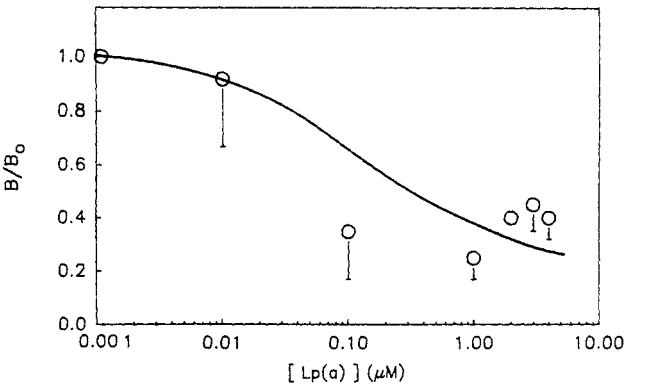

FIGURE 5: Lp(a) inhibits t-PA binding to platelets. t-PA (600 nM) was incubated with varying concentrations of Lp(a) and platelets in HBS as described in Materials and Methods.

tested that was insufficient to offset the increased Michaelis constant. Importantly, apo(a)-free LDL had no effect on the kinetics of PGN activation by t-PA in the presence or absence of platelets, but the ganglioside GT_{1b} was able to reduce the catalytic efficiency for t-PA in the presence of platelets to values seen in their absence. A Dixon plot generated from the data of Figure 4 revealed an estimated apparent (competitive) inhibition constant (K_i) for Lp(a) of 0.49 μM.

In order to evaluate the effect of Lp(a) on the enzymatic activity of t-PA and the binding of t-PA to the platelet surface, assays of t-PA activity and competitive binding experiments with t-PA and Lp(a) were performed. Kinetic analysis revealed no difference in t-PA activity in the presence or absence of Lp(a) and/or gel-filtered resting platelets. However, Lp(a) inhibited the binding of ^{125}I-t-PA to platelets with an IC_{50} of approximately 0.1 μM (Figure 5).

DISCUSSION

In this study we have attempted to characterize the interaction of Lp(a) with platelets and the functional consequences of this interaction. We have demonstrated that Lp(a) binds directly, saturably, and reversibly to resting platelets with an estimated apparent K_d of 0.20 μM over a time course of approximately 30–40 min. Lp(a) also binds to thromboasthenic platelets, characterized by a deficiency of platelet glycoprotein IIb/IIIa. In addition, ADP or thrombin stimulation of normal platelets does not significantly alter the affinity but modestly reduces the binding capacity for Lp(a). Taken together, these data suggest that Lp(a) does not bind to glycoprotein IIb/IIIa but, given the reversibility of binding by lysine analogues, to a cell-surface protein containing an accessible lysyl residue, the availability of which is reduced with platelet activation.

In addition and concordantly, Lp(a) inhibits the binding of PGN to platelets with an estimated IC_{50} of 0.23 μM. Again, this effect can be attributed to the kringle domains of the apo(a) moiety of Lp(a) since binding was inhibited by a lysine analogue, and no such effects were demonstrable with apo(a)-free LDL. Interestingly, the IC_{50} value of 0.2 μM observed with plasmin-treated platelets argues against a significant contribution of a plasmin-induced conformational change in platelets to the observed effects of Lp(a) on PGN binding. These IC_{50} values are in agreement with those that have been previously reported for Lp(a) by Miles and colleagues (Hajjar et al. 1989) for endothelial and mononuclear cells (0.13 and 0.69 μM, respectively). In addition, however, our estimated apparent K_d of Lp(a) for platelets (0.20 μM) is significantly lower than the previously reported K_d of PGN for platelets (1.9 μM) (Miles & Plow, 1985), (1.6 mM in our lab), and points to a potential pathophysiologic role for Lp(a) at plasma concentrations that are known to convey an increased risk of atherothrombotic vascular events [e.g., >30 mg/dL, or >0.625 μM for a 480-kDa isoform (Gavish et al., 1989)].

We have also shown that gangliosides can partially inhibit the specific binding of Lp(a) to platelets with an estimated IC_{50} of 300 μM for GT_{1b}. Gangliosides are glycolipids containing residues of sialic acid that are present in high density on eukaryotic plasma membranes and can influence the binding of specific ligands to the cell surface. Culturing monocytes in the presence of gangliosides results in increased incorporation of these molecules into cell membranes and increased specific PGN binding to these cells (Moss et al., 1976). Miles and colleagues recently demonstrated that solution-phase gangliosides can inhibit PGN binding to monocytes, platelets, and endothelial cells (Miles et al., 1989a). The mechanism by which gangliosides, PGN, and its cell-surface protein receptor(s) are related is uncertain. It is possible that gangliosides may modulate PGN receptor conformation and function or, in certain circumstances, serve directly as receptors and influence plasminogen activation. The inhibition of Lp(a)'s binding to platelets by gangliosides may be dependent on the kringle domains of Lp(a), since prior studies have shown that lysine analogues inhibit the interaction of these glycolipids with PGN (Miles et al., 1989a).

To characterize further the possible physiologic importance of Lp(a)'s effect on PGN binding to platelets, we examined the kinetics of PGN activation by t-PA. Lp(a) was found to attenuate the activation of PGN by t-PA in the presence of platelets. This effect could be attributed to a competitive inhibitory mechanism by Lp(a) with PGN for t-PA. Although Lp(a) did not demonstrate any appreciable effect on t-PA activity, the inhibition of t-PA binding to the platelet surface by Lp(a) provides another mechanism for the reduction in platelet surface-bound plasmin generation. The relatively high concentration of Lp(a) required to observe the effects demonstrated here is achievable with plasma concentrations in a high-normal to pathologic range (>30 mg/dL) as described above. In addition, local concentrations at sites of atherosclerotic plaque may be higher than plasma levels, given the propensity of Lp(a) to partition preferentially into the vessel wall (Smith & Cochran, 1990). Furthermore, since the activation of PGN is enhanced on cell surfaces, the estimated competitive inhibition constant (K_i) in the presence of platelets may also be relevant pathophysiologically.

Shu-wei et al. (1990) have reported that platelets increase the catalytic efficiency of t-PA for the conversion of PGN to

plasmin by a factor of approximately 10. This effect was primarily the result of a decrease in the K_m of PGN for t-PA. Lp(a) at concentrations ranging from 0.06 to 0.3 μM decreased the k_{cat}/K_m in the presence of platelets by virtue of an effect primarily on K_m but also on k_{cat} at the highest concentrations tested. The finding that Lp(a) acts as a competitive inhibitor of PGN activation reinforces the functional relevance of the binding data, which demonstrated that Lp(a)'s inhibition of PGN binding to platelets was due to the interaction of the kringle domains of apo(a) with the PGN binding site(s). Importantly, no differences were found in the enzymatic activity of t-PA in the presence or absence of Lp(a) and/or platelets.

Evidence has accumulated to suggest that Lp(a) may impair fibrinolysis and promote thrombosis by several mechanisms. Lp(a) may inhibit plasminogen binding to cell surface receptors (Miles et al., 1989b; Hajjar et al., 1989) and to fibrin (Loscalzo et al., 1990; Edelberg et al., 1990), thereby impairing stimulation of PGN activation. Lp(a) may also bind to heparan sulfate on the cell surface and reduce the stimulation of PGN activation by t-PA provided by this glycosaminoglycan (Edelberg & Pizzo, 1990). t-PA binds to surface-bound Lp(a) through its activator site, as a result of which the enzymatic action of this PGN activator is inhibited (Simon et al., 1991). Finally, Lp(a) also induces a significant increase in secreted PAI-1 and in PAI-1 mRNA in endothelial cells (Etingin et al., 1991), providing yet another mechanism for the promotion of thrombosis.

The inhibition of PGN and t-PA binding to the platelet surface by Lp(a) reported here may also have adverse (prothrombotic) consequences. By impairing cell-surface-stimulated PGN activation by t-PA, the inhibition of platelet aggregation induced by plasmin (Schafer & Adelman, 1985) may be prevented. Inhibition of plasmin generation localized to the platelet surface may also reduce the likelihood that kinetically selective proteolysis of platelet surface-bound fibrinogen will occur, thereby inhibiting the disaggregation of platelets induced by t-PA (Loscalzo & Vaughan, 1987). The potential relevance of these theoretical mechanism, however, awaits confirmation in more complex in vivo experimental systems.

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