Demonstration of Covalent Binding of Lipoprotein(a) [Lp(a)] to Fibrin and Endothelial Cells†

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ABSTRACT: It has been well documented that Lp(a) binds noncovalently to fibrin or human umbilical vein endothelial cells. This binding is to lysines and is inhibited by lysine analogues such as ϵ-aminocaproic acid (EACA). In the present study, Lp(a) (0.006−0.6 μM) binding to immobilized fibrin and endothelial cells was evaluated by ELISA with an anti-Lp(a) antibody. A significant portion (≈65%) of the Lp(a) was found to resist dissociation by EACA (0.2 M). The EACA resistant binding of Lp(a) was time and concentration dependent. The addition of EDTA to the incubation mixture had no effect, thereby excluding cross-linking by transglutaminase as a mechanism. This portion of Lp(a) was also resistant to dissociation by acid (0.1 N HCl), 0.1% SDS, 1 M benzamidine, Tris-HCl (1 M, pH 12), or DTT (5 mM), but it was washed off by 0.1 N NaOH (which did not remove the immobilized fibrin). This suggested that the Lp(a) was covalently linked by an ester bond. Covalent binding was inhibited when Lp(a) was mildly oxidized by BioRad Enzymobeads, which may explain why it escaped recognition in experiments with radiolabeled Lp(a). Covalent binding was attenuated when Lp(a) was pretreated with DFP suggesting that the serine residue in the pseudo active site of Lp(a) was involved. Lp(a) also bound covalently to immobilized BSA, indicating some nonspecificity. However, binding to BSA was almost 3-fold less than to fibrin, suggesting that lysine binding may facilitate covalent binding. A similar proportion of EACA resistant binding of Lp(a) was found with endothelial cells. In conclusion, the findings demonstrate a novel, covalent binding by Lp(a) which is kringle independent and is postulated to involve the pseudo protease domain of Lp(a). This property may contribute to the deposition of Lp(a) on endothelial surfaces and its colocalization with fibrin in atheromas.

Lipoprotein(a) [Lp(a)][1] was first identified in human plasma in 1963 (*1*). It is a low density lipoprotein (LDL)-like particle made up of apolipoprotein (apo) B-100 linked by a disulfide bridge to apo(a), a glycoprotein with multiple kringle domains and a serine protease-like domain homologous to plasminogen. The apo(a) kringles consist of one copy of plasminogen kringle 5 and multiple copies of plasminogen kringle 4. Its physiological function is not known, but it is of significant clinical importance because Lp(a) is positively associated with coronary heart disease, as first reported in 1972 (*2*), and has subsequently been found to be an important, independent risk factor for atherosclerotic vascular disease in general (*3*−*7*), according to most studies (*8*). Lp(a) has also been correlated with the extent of coronary atherosclerosis when evaluated angiographically (*9*) and Lp(a) is found deposited in atherosclerotic plaque of coronary (*10*) and cerebral (*11*) vessels, where it appears to be preferentially deposited over LDL. In transgenic animals, apo(a) has been shown to colocalize with lipid deposits (*12*) and to promote the atherogenic effect of apoB (*13*).

The mechanism for the atherogenic or thrombogenic effect of Lp(a) has not been established. However, the remarkable homology of Lp(a) with plasminogen has prompted the widely held hypothesis that Lp(a) mimics and competes with plasminogen for binding to the fibrin or endothelial cell surface and thereby inhibits plasminogen activation and fibrinolysis. This could favor atherogenesis since a depressed fibrinolytic activity has been generally believed to be an important risk factor for cardiovascular disease (for review, see, ref 14).

Consistent with this pathogenic mechanism is the observation that Lp(a) shares with plasminogen the ability to interact with lysine residues and lysine analogues as evidenced by its binding to lysine-Sepharose (*15*) and fibrin (*16*), especially plasmin degraded fibrin (*17*). At the same time, Lp(a) has been shown to be highly heterogeneous with respect to lysine binding (*18*) with the proportion of Lp(a) binding to lysine-Sepharose ranging from 17 to 91% in individual healthy donors (*19*). Nevertheless, since the K_D of Lp(a) binding to fibrin (0.02−0.03 μM) is more than 10-fold lower than that of plasminogen (*16*, *17*,) pathological levels of Lp(a) could,

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[1] Abbreviations: DFP, diisopropyl fluorophosphate; EACA ϵ-aminocaproic acid; HUVEC, human umbilical vein endothelial cells; Lp(a), lipoprotein(a); SDS, sodium dodecyl sulfate; mA, mini-absorbance.

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theoretically, compete with plasminogen for lysines despite the latter's high molar concentration (≈2 μM) in plasma.

Studies in purified systems have shown that Lp(a) inhibits plasminogen activation by tissue plasminogen activator (t-PA) in the presence of fibrin (*16, 20*−*22*). However, this inhibition was of the uncompetitive type (*16, 21*−*22*), which is inconsistent with the hypothetical mechanism of a competition between plasminogen and Lp(a) for lysine residues in fibrin. In transgenic mice expressing the human apo(a) gene, t-PA-induced lysis of human platelet-rich plasma clots was found to be depressed (*23*). By contrast, in most studies of fibrinolysis in patients with elevated Lp(a) levels, no inhibition of fibrinolytic activity by Lp(a) was found (*24*−*28*). In one study, Lp(a) was shown actually to enhance plasma clot lysis in vitro (*29*), and in another study, increasing concentrations of Lp(a) were found to have no effect on t-PA-induced clot lysis in a plasma milieu (*30*).

In an attempt to reconcile some of these inconsistencies, we previously studied the effect of Lp(a) on plasminogen binding to immobilized fibrin D-dimer. At low concentrations of plasminogen, inhibition by Lp(a) was found, whereas at physiological concentrations of plasminogen, Lp(a) actually promoted binding, a finding which was related to plasminogen binding to fibrin-bound Lp(a) (*31*). This phenomenon explained the contradictory findings of competitive binding and uncompetitive inhibition. The finding was also consistent with the diphasic effect of plasminogen activation by t-PA, in which uncompetitive inhibition by Lp(a) was seen at low concentrations of fibrin, whereas at higher concentrations, Lp(a) induced a 2.4-fold promotion of plasminogen activation (*22*). The binding of plasminogen to fibrin-bound Lp(a) may also help explain the paradoxical finding that Lp(a) and plasminogen were found to be colocalized in autopsy samples of atherosclerotic plaques, rather than being inversely correlated (*32*).

The many disparate observations from the literature suggest that the effect of Lp(a) on fibrinolysis is complex and put into some question the hypothesis that the atherothrombotic effect of Lp(a) is predominantly due to its competition with plasminogen for lysine residues on fibrin or cells. An additional mechanism seems to be implicated. In the present study, evidence is presented that in addition to noncovalent lysine binding, a significant proportion of Lp(a) is bound covalently to fibrin or endothelial cells.

METHODS AND MATERIALS

Materials. Fibrin D-dimer was prepared as previously described (*31*). Fibrinogen, enzyme labeled anti-rabbit antibody, diisopropyl fluorophosphate (DFP), and BSA were purchased from Sigma (St. Louis, MO). [^{3}H]DFP was obtained from DuPont NEN (Boston, MA). Human umbilical vein endothelial cells (HUVECs) were obtained from Clonetics (San Diego, CA). The rabbit anti-Lp(a) antibody and anti-D-dimer antibody were obtained from Boehringer Mannheim (Indianapolis, IN) and Diagnostica Stago (Franconville, France), respectively.

Methods. (1) Preparation of Lp(a). Lp(a) was purified from a donor with an elevated Lp(a) concentration by ultracentrifugation and gel filtration according to the procedure previously described (17). The molecular weight of Lp(a) was 923 000. On the basis of molecular weight, this represents the isoform containing 37 kringle IV-like copies and one kringle V-like copy plus the protease domain.

(2) ELISA Assay for Immobilized Fibrin D-Dimer Bound Lp(a). Lp(a) was previously found to bind to the fibrin D-domain rather than E-domain (*22*). Therefore, Lp(a) binding to fibrin was measured by using immobilized fibrin D-dimer. Fibrin D-dimer (coating concentration, 0.02 mg/mL) was immobilized on a plastic plate (Dynatech Immulon) by an overnight incubation at 4 °C. The extra protein binding sites were blocked by 2% BSA. A range from 6 to 600 nM Lp(a) in 0.05 M Tris-HCl, 0.15 M NaCl, 0.01% NaN_3, 0.01% Tween 80, and 0.25% BSA (pH 7.4) was then incubated in the plate at 37 °C for 2 h. EACA, 0.2 M, was included either during the incubation or in the after-incubation washes. The bound Lp(a) or immobilized D-dimer was measured by ELISA with anti-Lp(a) or anti-D-dimer antibody as follows: 100 μL per well of rabbit antibodies specific for Lp(a) or D-dimer (1:1000 dilution) in 0.05 M Tris-HCl, 0.15 M NaCl, 0.01% NaN_3, 0.01% Tween 80, and 0.25% BSA (pH 7.4) were incubated at 37 °C for 1 h, followed by a 5-time wash with 200 μL per well of the same buffer. After that, 100 μL per well of goat anti-rabbit IgG antibody labeled with alkaline phosphatase (Sigma, St. Louis, MO) (1:1000 dilution) was added and incubated at 37 °C for another hour, followed by the same washing procedure. Finally, 150 μL per well of *p*-nitrophenyl phosphate (1.4 mg/mL) in 1.0 M diethanolamine with 0.01% of $MgCl_2$ was added and incubated at room temperature. The OD increase was measured over time at 410 nm against a reference wavelength of 490 nm (410/490 nm) on the microtiter plate reader. Approximately 1.56 ± 0.28 pmol of D-dimer, as determined by ELISA, was present in each well.

To characterize the nature of the binding, 0.1 N HCl, 0.1 N NaOH, 5 mM DTT, 1 M benzamidine, 0.1% SDS, 0.1% Tween 80, or Tris-HCl (1 M, pH 12) was added to determine if they removed the EACA resistant, fibrin-bound Lp(a).

(3) ELISA Assay for HUVECs Bound Lp(a). HUVECs were seeded in a gelatin-coated P6-well plate and cultured with 2% fetal calf serum (FCS) and Debacco modified eagle medium (DEME) in a 5% CO_2 incubator at 37 °C until confluent. The confluent cells were then briefly washed with 50 mM glycine-HCl (pH 3.0) containing 0.10 M NaCl for 2 min at room temperature, to generally remove cell-bound proteins. The cells were washed with FCS-free DEME 4 times and then incubated with or without 0.06 μM Lp(a) in FCS-free DEME at 37 °C for 2 h. EACA (0.2 M) was added to certain Lp(a) wells for an additional $^1/_2$-h incubation. Some other wells with or without Lp(a) were treated with 0.05% trypsin for 3 min to remove the cells, to differentiate cell-binding from binding to the cell matrix. The wells were washed again with FCS-free DEME 4 times and then incubated with FCS-free DEME containing rabbit anti-Lp(a) antibody (1:1000 dilution) at 37 °C for 0.5 h. After washing away the first antibody, the enzyme labeled anti-rabbit antibody was added and incubated in FCS-free DEME at 37 °C for 0.5 h, followed by the same washing process. Finally, Lp(a) binding was measured with the ELISA substrate as described above.

(4) [^{3}H]DFP Incorporation by Lp(a). Purified Lp(a) (0.5 μM) was incubated with 0.1 mM [^{3}H]DFP (the maximal concentration obtainable) at 37 °C for 6 h in the presence or absence of 1 μM fibrin D-dimer in a buffer containing 0.05

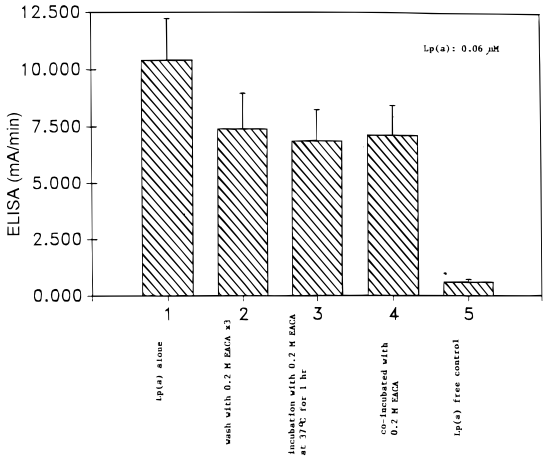

FIGURE 1: ELISA of Lp(a) binding to immobilized fibrin D-dimer alone (1), after washing with 0.2 M EACA 3x (2), after incubation (37 °C) 1 h with EACA (3), co-incubated with EACA (4), and Lp(a) free control (5). Mean ± SD from four duplicate experiments.

M Tris-HCl, 0.15 M NaCl, 0.01% NaN_3, 0.01% Tween 80, and 0.1% BSA (pH 7.4). The samples were then boiled immediately in reducing SDS sample buffer or boiled after exhaustive dialysis. After 5% SDS−PAGE (reducing conditions) and autoradiography, the radioactive band [Apo(a), MW ≈500 kDa] was cut out for counting.

RESULTS

1. Characterization of the EACA Resistant Binding of Lp(a) to Fibrin. After incubation (37 °C) of the fibrin-coated walls with 0.06 μM Lp(a) for 2 h, washing, and ELISA assay, the wells were washed (3x) with 0.2 M EACA and reassayed. About 65% of the Lp(a) remained bound even after an additional 1 h (37 °C) incubation with 0.2 M EACA. When EACA (0.2 M) and Lp(a) (0.06 μM) were co-incubated with immobilized fibrin D-dimer for 2 h or overnight, the same amount of Lp(a) remained bound (Figure 1).

When 0.06 μM Lp(a) was incubated in the presence of 0.2 M EACA for up to 90 min, the binding of Lp(a) to immobilized fibrin was found to be time dependent and plateaud in about 30 min (Figure 2A).

When 0−0.6 μM Lp(a) was incubated in the presence of 0.2 M EACA for 90 min, binding to the immobilized fibrin was found to be concentration dependent (Figure 2B).

When the EACA-resistant portion of fibrin-bound Lp(a) was incubated (37 °C) for 1 h with HCl (0.1 N), SDS (0.1%), Tween 80 (0.1%), Tris-HCl (1 M, pH 12), DTT (5 mM), or benzamidine (1 M), no appreciable dissociation occurred. By contrast, the Lp(a) dissociated completely in the presence of NaOH (0.1 N) (Figure 3). Co-incubation of EDTA (5 mM) with 0.06 μM Lp(a) and 0.2 M EACA was not found to significantly affect the amount of Lp(a) bound to immobilized fibrin.

To verify that the fibrin remained attached to the wells during the above studies, it was assayed by an ELISA for D-dimer before and after these incubations. No loss of D-dimer occurred (data not shown).

2. Attenuation of EACA-Resistant Fibrin Binding by Oxidation or DFP Treatment of Lp(a). When Lp(a) was pretreated (room temperature, 1 h) by the mild oxidizing agent, BioRad Enzymobeads, the EACA-resistant binding was reduced by about 56%, suggesting that this binding

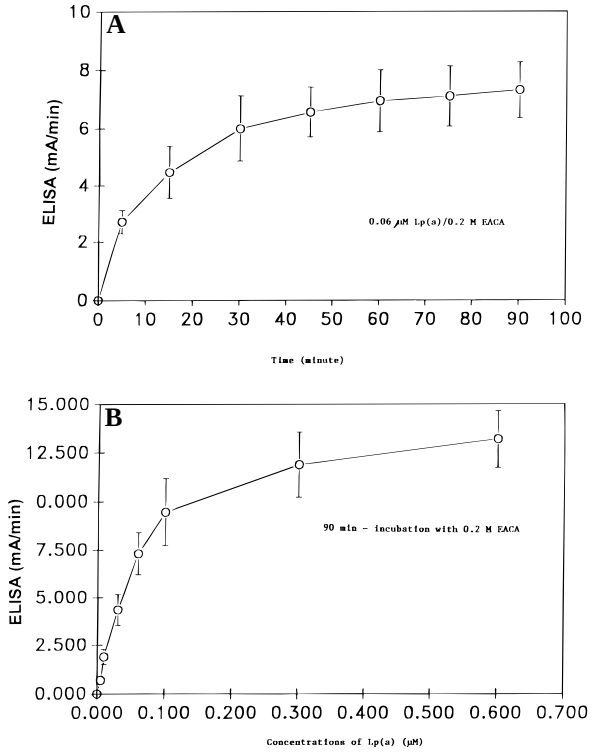

FIGURE 2: (A) Time dependence of Lp(a) (0.06 μM) binding to immobilized fibrin D-dimer in the presence of 0.2 M EACA. Mean ± SD from four duplicate experiments. (B) Lp(a) concentration dependence of binding to immobilized fibrin D-dimer in the presence of 0.2 M EACA after 90 min incubation. Mean ± SD from four duplicate experiments.

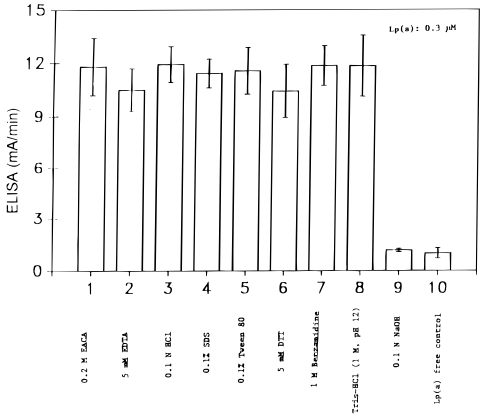

FIGURE 3: Lp(a) binding to immobilized fibrin D-dimer in the presence of 0.2 M EACA (1), 5 mM EDTA (2), 0.1 N HCl (3), 0.1% SDS (4), 0.1% Tween 80 (5), 5 mM DTT (6), 1 M benzamidine (7), Tris-HCl (1 M, pH 12) (8), 0.1 N NaOH (9), and Lp(a)-free control (10). Mean ± SD from four duplicate experiments.

would be attenuated by radiolabeling the Lp(a). Oxidation has also been shown to inhibit the lysine binding site interactions of Lp(a) (*33*). When Lp(a) was pretreated with DFP, the EACA resistant binding was reduced by ≈37% (Table 1), suggesting that the pseudo active site of the protease domain of apo(a) may be involved in this binding. Radiolabeled DFP incorporation by Lp(a) was studied. Incorporation by a small but significant portion (≈0.7%) of Apo(a) was detected on a SDS−PAGE under reducing conditions. The DFP incorporation was inhibited when soluble fibrin D-dimer was included in the incubation mixture. Exhaustive dialysis induced dissociation of the

Table 1: Effect of Oxidization and DFP Treatment on the Covalent Binding of Lp(a) to Fibrin D-Dimer

reagents added	ELISA of Lp(a) and D-dimer,* mA/min				
	anti-Lp(a)				anti-D
	Lp(a)	oxi-Lp(a)	DFP−Lp(a)	buffer	buffer
0.2 M EACA	7.75 ± 1.40	3.7 ± 0.59	5.19 ± 0.88	0.82 ± 0.10	17.69 ± 2.51
0.1 N NaOH	0.80 ± 0.09	0.72 ± 0.09	0.79 ± 0.11	0.61 ± 0.06	17.88 ± 2.14

[a] 0.06 μM of Lp(a), oxidized Lp(a) and DFP−Lp(a) were used in the experiment. Oxidized Lp(a) was obtained by incubating Lp(a) (0.5 μM) with Enzymobeads plus 0.4% $\alpha\beta$-D-glucose at room temperature for 1 h. DFP−Lp(a) was made by incubation of 0.5 μM Lp(a) with 50 mM DFP at room temperature for 1 h. Mean ± SD from four duplicate experiments.

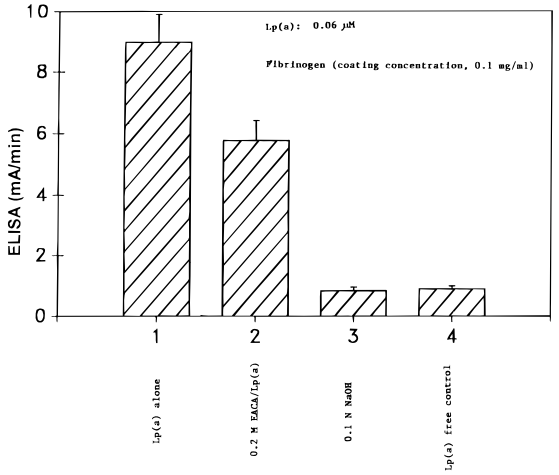

FIGURE 4: ELISA of Lp(a) bound to immobilized fibrinogen (1), co-incubated with 0.2 M EACA (2), followed by washing with 0.1 N NaOH (3), and Lp(a)-free control (4). Mean ± SD from four duplicate experiments.

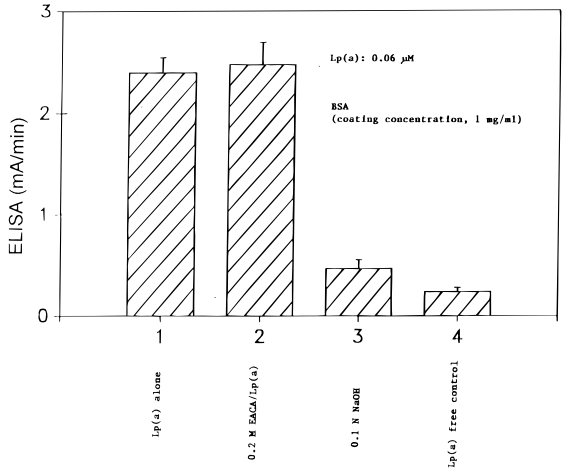

FIGURE 5: ELISA of Lp(a) binding to immobilized BSA (1), followed by washing with 0.2 M EACA (2), followed by washing with 0.1 N NaOH (3), and Lp(a) free control (6). Mean ± SD from 4 duplicate experiments.

DFP, suggesting that the binding to Lp(a) was much less stable than the covalent binding between DFP and a typical serine protease.

3. EACA-Resistant Lp(a) Binding to Other Surfaces. A similar EACA-resistant binding was observed when fibrinogen was substituted for fibrin D-dimer. The proportion of EACA-resistant binding to total binding was comparable and dissociation by 0.1 N NaOH was again observed (Figure 4). When BSA was used as the binding surface, all of the bound Lp(a) resisted dissociation by EACA, but total binding was only about 30% of that which bound to fibrin or fibrinogen. The BSA-bound Lp(a) also dissociated in the presence of 0.1 N NaOH (Figure 5). It was shown that the NaOH did not cause dissociation of the BSA from the plate.

When HUVECs were used, about 70% of the bound Lp(a) resisted dissociation by EACA (0.2 M). To ensure that this binding was to the cells, trypsin treatment was used to remove the cells. When this was done, the level was reduced to that in the Lp(a)-free control, indicating that the EACA resistant Lp(a) was associated with the HUVECs (Figure 6).

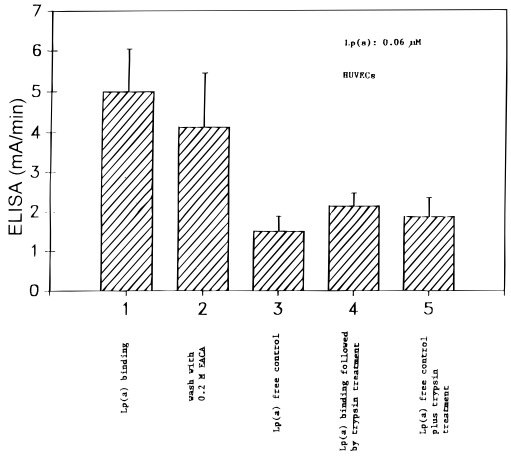

FIGURE 6: ELISA of Lp(a) binding to HUVECs (1), after washing with 0.2 M EACA (2), Lp(a)-free control (3), Lp(a) binding followed by trypsin treatment (4), Lp(a) free control plus trypsin treatment (5). Mean ± SD from four duplicate experiments.

DISCUSSION

These findings indicate that Lp(a) binds to fibrin and endothelial cells by two distinct mechanisms. The first type is a well-established reversible binding which is elutable by the lysine analogue, EACA, which interrupts the lysine-binding site interactions mediated by the kringle 4 domains of Lp(a). This binding of Lp(a) is promoted when immobilized fibrin is degraded by plasmin, which exposes new carboxyterminal lysines (*17*). The second type is an irreversible binding which occurred in the presence of EACA. This binding accounted for about 65% of the total binding to intact fibrinogen or D-dimer and has, to our knowledge, not been previously described (Figure 1). The EACA-resistant binding was time and Lp(a) concentration dependent. This portion of the fibrin-bound Lp(a) also resisted dissociation by a variety of other reagents (Figure 3), including EDTA, which excluded cross-linking by transglutaminase as a mechanism for the EACA resistance. Binding, however, was found to be dissociable by NaOH (0.1 N) but not unheated acid (0.1 N HCl), suggesting that binding was covalent (which is also consistent with its

resistance to SDS) and involved an ester bond. The NaOH was shown not to dissociate the immobilized D-dimer.

The apparent covalent binding of Lp(a) is probably mediated by apo(a) rather than LDL, since LDL has previously been shown not to bind to D-dimer (*22*). The present finding that binding was significantly attenuated by DFP treatment of apo(a) (Table 1), suggests that the active site serine residue of the protease domain of apo(a) was involved. The pseudo active site of apo(a) contains all three essential residues (Ser-His-Asp) and the substrate binding pocket of the protease domain of plasmin. The main difference is that Lp(a) has a serine in place of the cleavage site arginine of plasminogen, although additional amino acid substitutions have also been implicated in rendering apo(a) proteolytically inactive (*34*). Nevertheless, it has also been reported that Lp(a) retains a low proteolytic activity (*35, 36*). This raises the possibility that Lp(a) can form a tetrahedral/acyl intermediate which is nondissociable since it cannot complete the reaction. Such a complex would be similar to that found with all serpin−serine protease inhibitor complexes, which are either tetrahedral or acyl intermediate complexes (*37, 38*). The hypothesis that the apo(a) protease domain forms a comparable complex with fibrin would explain the covalent binding observed, in which the ester bond with the hydroxyl group of the active site serine could be broken by a nucleophilic base, such as NaOH or hydroxylamine.

The finding that 0.1 N NaOH caused dissociation suggests that the bond of Lp(a) with fibrin was weaker than that of a serpin with a serine protease, which is dissociable only by 1.0 M hydroxylamine. This explanation is consistent with Lp(a) having an immature protease domain. The absence of an effect by benzamidine, a serine protease inhibitor, also indicated that the active site of Lp(a) was incomplete.

These observations were consistent with the data from the [^{3}H]DFP incorporation experiments. A small but significant portion (≈0.7%) of Apo(a) incorporated the [^{3}H]DFP, which was prevented by the presence of fibrin. A much weaker bond than that of a typical DFP−serine protease was also evidenced by the finding that exhaustive dialysis caused dissociation of the DFP from Lp(a). These phenomena were reminiscent of [^{3}H]DFP incorporation by pro-urokinase, which is a zymogen but has a significant intrinsic activity. Although pro-urokinase incorporates DFP (*44, 45*), this reaction was similarly shown to be reversible (*45*). The reducing SDS−PAGE excluded the possibility that the [^{3}H]-DFP incorporation by Lp(a) was due to contaminant serine proteases. However, the portion of [^{3}H]DFP incorporation (≈0.7%) was much smaller than the inhibition (≈37%) of covalent binding caused by DFP. This discrepancy has not been explained. However, one possibility is that the concentration of DFP used for the inhibition (Table 1) was 500-fold higher than that used in the incorporation experiments, in which the maximal concentration of commercially available [^{3}H]DFP was used.

A major attenuation of the covalent binding to fibrin was also seen when Lp(a) was pretreated with the oxidizing agent, BioRad Enzymobeads, which is used for radiolabeling (Table 1). This observation may explain why covalent binding of Lp(a) has escaped attention in studies with radiolabeled Lp-(a) (*39*−*43*). When radiolabeling was not used, about 50% of intact fibrin-bound Lp(a) resisted EACA according to some published figures (*17*), but this was left unexplained. In other studies, only total binding of Lp(a) was measured (*42, 43*). The remarkable kringle identities between Lp(a) and plasminogen may have discouraged a search for additional mechanisms of Lp(a) binding. It is also noteworthy that plasmin-degraded fibrin, which specifically promotes lysine binding (*17*), was used in most of the binding studies published. In the present study, intact fibrin (D-dimer) was used, which makes covalent binding by Lp(a) more conspicuous, being proportionally greater under these conditions.

A lysine-binding-site independent binding by Lp(a) was apparent in a recent study of transgenic mice expressing native and mutant forms of Lp(a). A decrease by only 35−55% in vascular accumulation of Lp(a) by Lp(a) mutants missing lysine binding sites was observed (*46*). The mechanism responsible for the up to 65% remaining accumulation of the Lp(a) found in these transgenic mice was not specified in the published report but may be explained by the present study.

Binding of Lp(a) to HUVECs had a similar (≈70%) EACA resistant component, consistent with a comparable mechanism to that found with immobilized fibrin or fibrinogen. Covalent binding also occurred on immobilized albumin, indicating that the covalent binding of Lp(a) was relatively nonspecific. However, against albumin the amount of Lp(a) was only about one-third of that which bound to fibrin (Figure 5), suggesting that lysine binding facilitates the covalent binding of Lp(a). Alternatively, it is possible that a specific sequence or structure of albumin is a less optimal substrate for the protease domain of Lp(a) and thereby results in less covalent binding.

The hypothesis that the protease domain of Lp(a) may contribute to its binding properties needs verification by studies with recombinant apo(a) protease domain and site-directed mutations. However, it is relevant that this domain, even though it is located on the carboxyl end of apo(a), has remained highly conserved in Lp(a), suggesting that it has a function despite being essentially catalytically inactive. The present study suggests a function, although its physiological role, like that of the whole molecule, remains unknown.

In conclusion, the findings indicate that Lp(a) has dual binding properties. The first is well established and mediated by its lysine binding sites on the kringle domains of apo(a). The second binding resists dissociation by lysine analogues, and is consistent with a covalent interaction, which is postulated to be mediated by the pseudo protease domain of Lp(a). The reversible lysine binding of Lp(a) appears to facilitate its covalent binding, which probably contributes to the atherogenic effects of Lp(a).

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