Hedgehog Lipoprotein(a) Is a Modulator of Activation of Plasminogen at the Fibrin Surface

An In Vitro Study

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Lipoprotein(a) (Lp[a]), a highly atherogenic lipoprotein particle, is the prominent apolipoprotein B-containing lipoprotein in the hedgehog (Laplaud PM et al, J Lipid Res 1988;29:1157–1170). In the present work, we studied the consequences of the structural homology between the specific Lp(a) glycoprotein, apoprotein(a), and plasminogen on the generation of plasmin by fibrin-bound tissue-type plasminogen activator. The activation of plasminogen was initiated by adding either native plasma or Lp(a)-free plasma supplemented with the equivalent of 0.25 mg/ml of either purified Lp(a) or albumin to a surface of fibrin prepared on microtitration plates and to which human tissue-type plasminogen activator was specifically bound. With the Lp(a)-free plasma, an increase in the binding and activation of plasminogen as a function of time was observed. In contrast, in the presence of Lp(a) (i.e., native plasma or the reconstituted system), a significant decrease in the binding of plasminogen (–60%) was obtained. These data indicate that hedgehog Lp(a) interferes with the binding and activation of plasminogen at the fibrin surface and may thereby behave as a factor regulating the extent of fibrin deposition. These results support our previous data indicating that high levels of Lp(a) may have antifibrinolytic effects in humans (Rouy D et al, Arterioscler Thromb 1991;11:629–638), are in agreement with the observation that Lp(a) is a risk factor for atherosclerotic disease, and provide further support to the view of Lp(a) as a link between atherosclerosis and thrombosis. (Arteriosclerosis and Thrombosis 1992;12:146–154)

Lipoprotein(a) (Lp[a]) is a genetically determined lipoprotein particle that contains a specific apolipoprotein, apoprotein(a) (apo[a]), that is highly homologous to plasminogen, the precursor of the fibrinolytic enzyme plasmin.1–3 However, apo[a], unlike plasminogen, is not a substrate for plasminogen activators, and high levels of the Lp(a) particle in human plasma are considered an independent risk factor for atherosclerotic cardiovascular disease.4,5 A number of studies have therefore been recently devoted to search for a possible role for Lp(a) in both atherosclerosis and thrombosis. Recent reports have indicated that Lp(a) may bind to and displace plasminogen from the surface of endothelial cells and monocytes and from immobilized fibrinogen and fibrin and, in addition, may inhibit plasminogen activation in purified systems6–7 and in plasma.8 In subjects with high levels of Lp(a), decreased generation of plasmin at the plasma–fibrin interface was found to be associated with the binding of Lp(a) to lysine residues of fibrin.8,9 Lp(a) is the prominent apolipoprotein B-containing lipoprotein in a typical hibernator, the European hedgehog (Erinaceus europaeus).10 As described recently, apo[a] in the hedgehog shares some physicochemical and immunologic properties with its human counterpart.10 The hedgehog may therefore constitute a useful natural animal model for the in vivo investigation of the interactions between this highly atherogenic particle and the fibrinolytic system. In the present study, we have characterized the fibrinolytic system of the hedgehog and have investigated the effect of plasma Lp(a) on the binding and activation of plasminogen at the fibrin surface by tissue-type plasminogen.
activator (t-PA). We show that during ongoing plasminogen activation, both plasminogen and apo(a) (in the form of Lp[a]) bind to the fibrin surface and that the generation of plasmin depends on the concentration of these proteins. These results clearly indicate that the apo(a)-like glycoprotein of the hedgehog interacts with fibrin and may constitute a regulatory component of the fibrinolytic system of this hibernator.

Methods

Chemicals and Reagents

All the chemicals used were of the best reagent grade that were commercially available. Other products were purchased from the following sources: EDTA, bovine serum albumin, Tween 20, and polyethylene glycol (PEG) from Serva, Heidelberg, FRG; Ultrogel AcA 44 and DEAE-Trisacryl from IBB, Villeneuve-La-Garenne, France; chromogenic substrate methylmalonyldihydroxyprolylarginine-p-nitroanilide (CBS 1065) from Diagnostica Stago, Asnières, France; l-lysine- and gelatin-Sepharose 4B and PD-10 Sephadex G-25 M columns from Pharmacia Fine Chemicals, Uppsala, Sweden; polyvinyl chloride U-shaped microtitration plates and plate sealers from Dynatech, Marnes-La-Coquette, France; glutaraldehyde, 25% (vol/vol) aqueous solution from TAAB Laboratories, Reading, UK; [125I]NaI from Amersham International, Amersham, Buckinghamshire, UK; and nitrocellulose from OSI, Paris, France. Benzanidazine and 6-aminohexanoic acid were purchased from Aldrich, Strasbourg, France. Dansyl-l-glutamyl-glycyl-L-arginine chloromethyl ketone (GGACK) was obtained from Behringwerke, Marburg, FRG. Human plasmin and 4-amidinophenylmethanesulfonyl fluoride (PMSF) were obtained from France Biochem, Meudon, France; and M-Para tigen plates and d-phenylalanyl-l-prolyl-L-arginine chloromethyl ketone (PPACK) was obtained from Boehringer, Mannheim, FRG. Paranitrophenyl-p'-guanidinobenzoate was from Sigma, La Verpillière, France. Protein A was from Cappel (Flobio, Courbevoie, France). Human t-PA (>95% single-chain, 680,000 IU/mg) was purchased from Biopool (Umeå, Sweden) and was calibrated to the international t-PA standard (preparation 517 from the National Institute for Biological Standards and Control, London, UK).

Buffers

Buffer A was 0.05 M NaH2PO4 buffer, pH 7.4, containing 0.08 M NaCl. Buffer B was 0.05 M NaH2PO4 buffer, pH 6.8, containing 0.08 M NaCl. Assay buffer was buffer A containing 0.2% (wt/vol) bovine serum albumin and 0.01% (vol/vol) Tween 20. Binding buffer was buffer B containing 0.4% (wt/vol) bovine serum albumin, 0.01% (vol/vol) Tween 20, and 2 mM EDTA. All other buffers were prepared as described in the text.
tained a mixture of Lp(a) and LDL-like particles. After concentration, these two types of lipoprotein particles were separated by means of gel-filtration chromatography on a column of Sepharose CL-4B (12x1,000 mm) operated at 4°C. Elution was performed with 0.15 M NaCl, pH 7.0, at a rate of 12 ml/hour, and 2-ml fractions were collected. Elution was monitored at 280 nm. This type of chromatography led to incomplete separation of the two lipoprotein populations; therefore, the fractions corresponding to the first peak were pooled and rechromatographed in the same system. The resulting peak was approximately gaussian. Fractions corresponding to the trailing edge of this peak were discarded, and the remainder of the peak was shown to contain pure Lp(a).  

**Purification of Apoprotein(a)**

Apo(a) was purified according to a modification of the methodology of Armstrong et al.  

Plasminogen was purified from fresh hedgehog plasma by the method of Deutsch and Mertz with some modifications. The plasma was supplemented with protease inhibitors (100 kIU/ml aprotinin, 2 mM PMSF, 10 μM p-nitrophenyl-p'-guanidinobenzoate, and 1 μM PPACK), and all steps were performed at 4°C. The plasma was incubated at 37°C for 3 hours.  

The resulting peak was approximately gaussian. Fractions corresponding to the trailing edge of this peak were discarded, and the remainder of the peak was shown to contain pure Lp(a).  

**Preparation of Hedgehog Plasminogen and Fibrinogen**

Plasminogen was purified from fresh hedgehog plasma by the method of Deutsch and Mertz with some modifications. The plasma was supplemented with protease inhibitors (100 kIU/ml aprotinin, 2 mM PMSF, 10 μM p-nitrophenyl-p'-guanidinobenzoate, and 1 μM PPACK), and all steps were performed at 4°C. The plasma was incubated at 37°C for 3 hours.  

The resulting peak was approximately gaussian. Fractions corresponding to the trailing edge of this peak were discarded, and the remainder of the peak was shown to contain pure Lp(a).  

**Polyclonal Antibody Against Plasminogen**

A polyclonal antibody directed against the protein content of the third peak obtained after gel-filtration chromatography was prepared as follows. Antigen (0.5 mg) in Freund's complete adjuvant was injected subcutaneously into sheep. The same amount, in Freund's incomplete adjuvant, was similarly injected at 3 and at 6 weeks after the initial immunization. After immunologic testing of the antiserum, the sheep was bled, and serum was separated. The immunoglobulin G fraction of the antiserum was then separated by ammonium sulfate precipitation, ion-exchange chromatography on DEAE-Trisacryl, and affinity chromatography on protein A-Sepharose.  

**Preparation of Solid-Phase Fibrin Surfaces**

The solid-phase fibrin surfaces were prepared as previously described. In brief, polyvinyl chloride-bound stable polyglutaraldehyde derivatives were first produced by treating U-shaped microtitration polyvinyl chloride plates with 2.5% (vol/vol) glutaraldehyde in 0.1 M NaHCO₃ buffer, pH 9.5, for 2 hours at 22°C. Then fibrinogen (0.3 μM in 0.1 M NaH₂PO₄ buffer, pH 7.4, containing 1 mM CaCl₂) was covalently fixed for 18 hours at 4°C. After washing, a fibrin network was generated by thrombin treatment (1 National Institutes of Health unit/ml in assay buffer containing 1 mM CaCl₂). The excess thrombin was eluted by three washes with a high-ionic-strength solution (0.5 M NaCl/8 mM CaCl₂/0.05% [vol/vol] Tween 20). A final wash with 5 mM NaH₂PO₄ buffer (pH 6.8 containing 0.05% [vol/vol] Tween 20) was performed, and 100 μl per well of buffer B, containing 0.02 M lysine, 0.2% (wt/vol) bovine serum albumin, and 0.01% NaN₃, was added. The plate was sealed and stored in this state at 4°C until further use. Fibrin surfaces were prepared with both human and hedgehog fibrinogens. The ability of thrombin to cleave human fibrinogen was monitored by use of a horseradish peroxidase-labeled mouse monoclonal antibody (Y18-HRP) kindly provided by Organon Teknika (Fresnes, France). The immunoreactivity of this antibody with the Aα stretch 1-51 of human fibrinogen disappeared on treatment with thrombin.  

**Assay Methods**

Lp(a) in hedgehog plasma was determined by an immunonephelometric method (Behring nephelometer analyzer) with a monospecific sheep antiserum to human Lp(a) (Immuno AG, Vienna, Austria) and a reference human standard in which the Lp(a) mass content had been previously determined by electrophoretic testing of the antiserum, the sheep was bled, and serum was separated. The immunoglobulin G fraction of the antiserum was then separated by ammonium sulfate precipitation, ion-exchange chromatography on DEAE-Trisacryl, and affinity chromatography on protein A-Sepharose.
with increasing concentration of the same Lp(a) preparation were then performed. In both series of experiments, results showed that the method in use was satisfactory for hedgehog Lp(a) measurements. More specifically, the range of results obtained for a given hedgehog Lp(a) concentration was within 89–106% of those obtained for the same concentration of human Lp(a); in addition, a linear response to supplementation in Lp(a) was obtained throughout the range of concentrations of this lipoprotein encountered in our animals according to season, that is, 0–100 mg/dl.

Plasminogen antigen concentration in hedgehog plasma was determined by radial immunodiffusion according to Mancini et al. 18 by using the sheep antibody described above and an aliquot of the content of the third peak obtained during gel-filtration subtraction of the lysine-absorbable proteins of hedgehog plasma as a standard.

The activatable plasminogen was determined in plasma by spectrophotometric assay consisting of fibrin-bound t-PA (see below), a volume dilution (final, 1:80) of plasma in binding buffer, and 1.5 mM CBS 1065. Human plasminogen was used as a reference, and the initial rate of activation under first-order conditions was determined as indicated below.

The antiplasmin activity of hedgehog plasma was determined by titration of added human plasmin in a spectrophotometric assay as described previously,19 except that residual plasmin activity was detected with 1.5 mM of the chromogenic substrate CBS 1065. Human plasminogen was used as a reference, and the initial rate of activation under first-order conditions was determined as indicated below.

Radioiodination of Proteins

Fraker and Speck25 with the following modifications:

Hedgehog plasminogen and protein A were radioiodinated with 125I using the lodogen method of Fraker and Speck25 with the following modifications: an iodination time of 4 minutes at 4°C and removal of free 125I by molecular sieving on a PD-10 Sephadex
G-25 column. The specific activity obtained was 9-11 nCi/ng protein for plasminogen and 40 nCi/ng protein for protein A.

**Fibrin Autography**

Slab-gel/fibrin-agar autography was performed as follows. Samples were electrophoresed under nonreducing conditions. A portion of the gel was stained with 2.5% (wt/vol) Coomassie blue. The remaining part of the gel was soaked for 1 hour in 2.5% (vol/vol) Triton X-100 to remove SDS and then in distilled water for 5 minutes. The gel was then overlaid on a fibrin-agar indicator gel prepared in a moist chamber as described previously. Autographs were allowed to develop at 37°C for 6-48 hours and then photographed with dark-ground illumination.

**Results**

**Hedgehog Lysine-Adsorbable Proteins**

Proteins eluted from the lysine-Sepharose 4B column were concentrated and then chromatographed on a column of Ultrogel AcA 44. Typically, three peaks were obtained. In each series of experiments, proteins in each fraction were analyzed by SDS-PAGE. In one experiment, proteins in each peak were examined equally by gradient-gel electrophoresis, thus demonstrating that the first peak contained a protein of apparent Mr of ~620,000. Such a value was comparable to that already reported by us for certain forms of hedgehog apo(a). This molecule exhibited cross-immunoreactivity with the sheep antibody against hedgehog plasminogen, as determined by immunoblotting (see below). Proteins in the third peak were resolved into three protein bands by SDS-PAGE. Plasminogen was identified by fibrin autography in the protein band migrating with an apparent Mr of ~93,000 (Figure 1), a molecular weight similar to that of human plasminogen. Two other bands migrating in a more anodic position and exhibiting relative molecular weights of 79,000 and 72,000 did not produce fibrinolytic activity, either spontaneously or in the presence of activators. Although a trace of plasminogen was also detected in the last fractions of the peak, only the third peak was considered to contain plasminogen for the purpose of the experiments described here. Although proteins in the second chromatographic peak were eluted with an apparent molecular weight corresponding to that of 7S gamma globulins, they were resolved by SDS-PAGE into two protein bands identical to those with Mr, 79,000 and 72,000 found as contaminants of plasminogen in the third peak. Fibrinogen purified from hedgehog plasma migrated as human fibrinogen purified and electrophoresed under similar conditions (not shown).

**Activation of Plasminogen by Fibrin-Bound Tissue-Type Plasminogen Activator**

Activatable plasminogen in hedgehog plasmas (from eight animals) was equivalent to 226±97 nM human plasminogen. The corresponding value in humans is 721±98 nM (10 donors). The difference is most probably related to the higher level of antiplasmin activity observed in hedgehog plasma (145-200% compared with 80-125% for normal human plasmas) and to the presence of a substantial amount of nonactivatable lysine-binding proteins. The higher plasminogen antigen value (350-500 mg/l) found in these animal plasmas relative to the value in humans (150-200 mg/l) is in agreement with the latter observation and with the existence of a cross-reaction between the lysine-adsorbable proteins and the antibodies raised against plasminogen present in the third chromatographic peak.

Plasminogen activation experiments were performed using surfaces of fibrin prepared from monolayers of human or hedgehog fibrinogen covalently bound through polyglutaraldehyde to a solid support. The validity for use of such a surface of fibrin for plasminogen activation experiments at the plasma-fibrin interface has been previously demonstrated.

In these experiments, human t-PA was first bound to the fibrin surface, unbound proteins were eliminated by washing, and hedgehog plasma was added to initiate the activation. The extent of plasmin generation at the surface of fibrin was measured with a plasmin-selective chromogenic substrate. The possible hydrolysis of the chromogenic substrate by Lp(a) was investigated before these experiments were performed; even at high Lp(a) concentrations and after 3-4 hours of incubation at 37°C, we were unable to obtain an increase in the absorbance at 405 nm relative to that of the substrate alone. The results
of plasminogen activation (Figure 2A) show that plasmin activity was progressively detected at the surface of fibrin as a function of the activation time. The mass of protein bound to fibrin as a function of time was detected by counting the radioactivity in the wells when hedgehog 125I-plasminogen was added to plasma as a tracer and is represented in Figure 2B as a percentage with reference to the maximum bound at 4 hours. It is noteworthy that identical results were obtained with human and hedgehog fibrin surfaces. Proteins present on the surface of fibrin were eluted with 6-aminohexanoic acid and analyzed by SDS-PAGE and immunoblotting. Results after 2 and 4 hours of activation are represented in Figure 3 (lanes A–C). As shown, one protein band of high molecular weight and at least three other protein bands of low molecular weight were detected with a sheep immunoglobulin G directed against hedgehog plasminogen and radiolabeled protein A for tagging. In the low-molecular-weight zone, fibrin-bound proteins were represented mostly by a band of Mr 56,000, corresponding to that of plasmin heavy chain, and two bands with a more cathodic migration corresponding to an Mr of 65,000 and 72,000, respectively. The latter band was similar to that of the third protein band present in peak III of the AcA 44 chromatogram (Figure 3, lane D), whereas the band with an Mr of 65,000 had a migration identical to that of a protein band present in the second chromatographic peak (not shown). A faint band with a relative molecular mass (Mr 79,000) similar to that of the second protein band of peak III was also present. In no case was plasminogen detected at the surface of fibrin, indicating that all fibrin-bound zymogen molecules were transformed into plasmin. At 4 hours of activation, a band of high molecular weight (equally observed as a trace at 2 hours, lane C), probably related to the material present in the first chromatographic peak, was clearly detected. Indeed, according to the particular experiment considered, this latter material migrated in SDS-PAGE gels at a position indicative of an Mr close or identical to that of purified hedgehog apo(a).

Role of Lipoprotein(a) in the Activation of Plasminogen

To determine whether Lp(a) bound to the fibrin surface was interfering with the binding of plasminogen, comparative activation experiments were performed with native hedgehog plasma, Lp(a)-free plasma, and Lp(a)-free plasma supplemented with the equivalent of 0.25 g/l protein mass of either Lp(a) or albumin. Typical activation profiles obtained under these conditions are shown in Figure 4. The amount of plasminogen derivatives bound to fibrin as a function of time was markedly decreased when the activation was performed with native plasma (80% decrease at 4 hours; Figure 4A) and with Lp(a)-free plasma supplemented with 0.25 g/l Lp(a) (60% decrease at 4 hours; Figure 4B) compared with the activation obtained in the absence of Lp(a). It is of note that the respective antiplasmin activities of Lp(a)-free and native plasmas were similar, thus indicating that the increased generation of plasmin was actually related to the content in Lp(a).

Discussion

In the human species, the generation of plasmin at the plasma–fibrin interface is finely regulated at the level of both t-PA and plasminogen. Plasminogen interactions with two plasma proteins, histidine-rich
glycoprotein and α2-antiplasmin, and with fibrin are mediated through lysine-binding sites present in the kringle domains of plasminogen.20,28,29 As a consequence, the amount of plasminogen available for fibrin and its transformation into plasmin are lowered by 50% in plasma (activatable plasminogen was 721±98 nM in 10 normal volunteers). Exposure of new plasminogen-binding sites on plasmin-degraded fibrin is considered to be a potent effector function that increases the rate of plasmin formation by t-PA.30,31 A mechanism that would modulate the extent of fibrinolysis by decreasing the number of such new binding sites has not yet been described. In particular, under physiological conditions, no molecule or circulating protein has been shown to behave as plasminogen vis-a-vis its fibrin-binding properties. The demonstration that apo(a) is composed mainly of structural domains homologous to kringle 4 and 5 of human plasminogen suggests that this protein may be a likely ligand for plasminogen-binding sites in fibrin. Indeed, we have recently shown that in human subjects with high levels of Lp(a), a decreased generation of plasmin at the plasma–fibrin interface was associated with the binding of Lp(a) to lysine residues of fibrin.8,9 In the present work, we have further investigated the possible role of apo(a) as a modulator of plasminogen activation. For this purpose, we have studied an animal model, the hedgehog, which has been shown to possess Lp(a) as its prominent apolipoprotein B–containing plasma lipoprotein.10 Except for the human source of t-PA, these studies were performed with hedgehog plasma or its derived products, including Lp(a), apo(a), fibrinogen, and plasminogen. From the results reported in the present study, two main aspects should be stressed. First, a group of lysine-adsorbable proteins was separated from plasma by lysine affinity chromatography and subsequently fractionated by gel filtration. One of these proteins had an unusually high Mr (~620,000), resembling that of purified hedgehog apo(a) and reacting with antibodies against hedgehog plasminogen and against human Lp(a) on immunoblotting. Another protein had the same molecular mass as human plasminogen (Mr 93,000) and was efficiently transformed into a fibrinolytic enzyme by plasminogen activators; it was therefore considered to be plasminogen. Two other protein bands migrating with a molecular mass (Mr 79,000 and 72,000) lower than that of plasminogen had no proteolytic activity and were not activated by plasminogen activators. These proteins were the main components of the second chromatographic peak of the lysine-adsorbable proteins, and their presence in the peak of plasminogen was due to incomplete chromatographic resolution. It is of note that their relative molecular weights determined by SDS-PAGE were lower than expected from their chromatographic behavior, suggesting the existence in plasma of a molecular form of relatively higher molecular weight. Because the isolation of these proteins was performed under conditions that avoided proteolytic degradation immediately after sampling and during the purification procedure, we suggest that such forms are probably present in circulating plasma. Furthermore, electrophoresis of the purified products under nonnaturating conditions also showed the presence of such molecular forms. Further biochemical and functional characterization of these lysine-adsorbable proteins is in progress in our laboratory.

Second, the preparation of a hedgehog fibrin surface was feasible and proved to be useful for the binding of human t-PA and the activation of hedgehog plasminogen either in a reconstituted system or in native plasma. The generation of plasmin by fibrin-bound t-PA was markedly decreased when the activation was performed with Lp(a)-free plasma supplemented with purified Lp(a). In addition, we have positively documented the binding of the Mr ~620,000 apo(a)-like protein and, to a lesser extent, of other lysine-absorbable proteins to fibrin during ongoing plasminogen activation. Considered together, these data indicate that in the hedgehog the activation of plasminogen at the plasma–fibrin interface is modulated by proteins containing kringle structures, mainly apo(a), that bind to fibrin. Because this phenomenon was documented in intact animals bred under natural conditions, our data strongly sug-
gest that, at least in the hedgehog, Lp(a) may constitute an important regulatory factor of fibrinolysis.

The European hedgehog exhibits naturally occurring seasonal variations in the plasma concentration of Lp(a). These variations are reproducible from one year to another and are, at least in part, under endocrine control. In the human species in contrast, the plasma level of Lp(a) appears to be genetically determined and is relatively constant, being insensitive to most dietary changes and hypocholesterolemic drugs. The modulation of plasminogen activation by Lp(a) in the hedgehog, as demonstrated in the present report, is obviously a seasonal physiological phenomenon. Therefore, such seasonal variations may help to provide a clue to the observation that high levels of Lp(a) in humans are a risk factor for atherosclerotic cardiovascular disease and to support the view of Lp(a) as a link between atherosclerosis and thrombosis.

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**References**

10. Laplaud M, Beaubatie L, Rall SC, Luc G, Sabourreau M: Lipoprotein(a) is the major apoB-containing lipoprotein in the plasma of a hibernator, the hedgehog (Erinaceus europaeus). *J Lipid Res* 1988;29:1157-1170
15. Masson C, Angles-Cano E: Kinetic analysis of the interaction between plasminogen activator inhibitor-1 and tissue-type plasminogen activator. *Biochem J* 1988;256:237-244
16. Angles-Cano E: A spectrophotometric solid-phase-fibrin-tissue plasminogen activator activity assay (SOFIA-tPA) for

**Figure 4.** Line plots of effect of hedgehog lipoprotein(a) (Lp[a]) on activation of plasminogen. Activation experiments were performed as indicated in Figure 2 with activation mixtures containing a trace amount of hedgehog 123I-plasminogen. Fibrin-bound activation products were detected by counting the radioactivity in the wells with a gamma radiation counter. Amount of plasminogen derivatives bound to fibrin as a function of activation time is expressed as a percentage relative to the maximum bound at 4 hours. Panel A: Activation with hedgehog native plasma containing 0.3 g/l Lp(a) (○) and with the d>1.21 g/ml fraction (●) of the same plasma, that is, Lp(a)-free plasma. Final plasminogen concentration was similar in the two samples. Panel B: Activation with Lp(a)-free plasma supplemented either with the equivalent concentration of purified hedgehog Lp(a) (○) similar to that of original plasma (0.25 g/l) or with a solution of albumin (●). Concentration of plasminogen was kept constant by adjusting the final volumes of the plasmas.
high-fibrin-affinity tissue plasminogen activators. Anal Biochem 1986;153:201–210


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