Lipoprotein-Associated Coagulation Inhibitor (LACI) Is a Cofactor for Heparin: Synergistic Anticoagulant Action Between LACI and Sulfated Polysaccharides

By Tze-Chein Wun

Lipoprotein-associated coagulation inhibitor (LACI) is a plasma-derived protein that inhibits tissue factor (TF)/factor VIIa-induced coagulation in a factor Xa-dependent manner. The roles of endogenous plasma LACI and exogenously added LACI and heparin, in the regulation of coagulation, initiated via the intrinsic and extrinsic pathways, were studied using the activated partial thromboplastin time (APTT) and the modified prothrombin time (PT) assays, respectively. Both LACI-depleted plasma and normal plasma have identical APTTs and similar prolongations of the APTT in response to heparin; both are fully anticoagulated (arbitrarily defined as clotting times of >1 hour) at similar concentrations of heparin. These results indicate that heparin is an effective anticoagulant when coagulation is initiated by the intrinsic pathway and that endogenous LACI is not significantly involved in the regulation of this pathway. The PT of normal plasma is only marginally longer than that of LACI-depleted plasma in the absence of heparin, suggesting that endogenous plasma LACI has a very limited capacity to inhibit TF-induced clotting. However, in the presence of heparin, the PTs of LACI-depleted plasma and normal plasma are different. Prolongation of the PT occurred only moderately and linearly with increasing concentrations of heparin in LACI-depleted plasma. In contrast, normal plasma showed a greater extent of PT prolongation in response to heparin and the plasma became fully anticoagulated at a certain threshold concentration of heparin. These results suggest that LACI serves as a cofactor for heparin and thus greatly enhances the inhibition of TF-induced coagulation. LACI-depleted plasma was supplemented with purified recombinant LACI and/or heparin and the effects on TF-induced clotting were studied. A combination of LACI and heparin greatly enhanced anticoagulation compared with LACI or heparin alone. Many sulfated polysaccharides were also found to enhance the LACI-dependent inhibition of TF-induced clotting. By weight, the relative potencies of these compounds are: low molecular weight heparin (mean Mw, 5,100) > unfractionated heparin > low molecular weight heparin (mean Mw, 3,700) > pentosan polysulfate > dermatan sulfate > dextran sulfate > heparan sulfate. Based on the above results, it is concluded that LACI is a cofactor for heparin in the inhibition of TF-induced clotting and that LACI and sulfated polysaccharides act synergistically in whole plasma.

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enhancing the inhibition of TF/factor VIIa/factor Xa by LACI, the simultaneous presence of antithrombin III with heparin was found to abrogate the TF/factor VIIa inhibition in the purified system. The latter is possibly due to the effective competition of heparin/antithrombin III for factor Xa, which is a required component in the formation of the inert TF/factor VIIa/factor Xa/Ca\(^{2+}\)/LACI complex. Based on the above considerations, questions are raised concerning the net effect of the simultaneous presence of heparin and LACI on the extrinsic coagulation cascade and whether heparin interferes with LACI function in plasma.

In view of the general lack of information on the effect of heparin on the extrinsic pathway and the uncertainty of the effect of heparin on LACI function in plasma, an attempt was made to investigate the role of the heparin/LACI interaction in the regulation of coagulation. This study led to the finding that LACI is a plasma cofactor for heparin and that LACI and sulfated polysaccharides exert powerful synergistic anticoagulant action in whole plasma.

**MATERIALS AND METHODS**

**Materials.** Rabbit brain thromboplastin (tissue factor, TF) was obtained from Ortho Diagnostic (Raritan, NJ). Dade’s activated cephaloplastin reagent for the determination of activated partial thromboplastin time (APTT) was purchased from American Scientific Product (McGaw Park, IL). Unfractionated heparin (UFH, lot 038078) was obtained from Elkin-Sinn (Cherry Hill, NJ). Low molecular weight heparins with mean molecular weight of 5,100 and 3,700 were from Calbiochem (San Diego, CA). Pentosan polysulfate (PS, #P8275), bovine mucosa dermatan sulfate (DS, #C2413), and bovine intestinal mucosa heparan sulfate (HS, #H7641) were from Sigma (St Louis, MO). Dextran sulfate (DXS, mean M\(_{\text{w}}\), 7,000 to 8,000) was supplied by ICN Biochemicals (Plainview, NY). Human plasma was provided by the American Red Cross (St Louis, MO). Four units of plasma were pooled and stored frozen in aliquots at \(-80^\circ\)C until used. Bovine factor Xa and Spectrozyme Xa were obtained from American Diagnostica (Greenwich, CT).

**Expression and purification of rLACI.** rLACI was expressed in mouse C127 cells using a bovine papilloma virus vector, and the rLACI-producing cell line was grown in cell factories for harvesting of conditioned media as described previously. The serum-free conditioned medium was adjusted to 50 mmol/L (NH\(_4\))\(_2\)SO\(_4\), filtered through a 0.2-\(\mu\)m filter and concentrated 30-fold using an Amicon (Danvers, MA) YM30 radial cartridge. The concentrate was subjected to ammonium sulfate precipitation. Protein precipitated between 25\% to 90\% saturation of ammonium sulfate was collected and dialyzed against phosphate-buffered saline containing 20 mmol/L Na\(_2\)SO\(_4\), Triton X-100 was added to a final concentration of 0.05\% and the solution was clarified by centrifugation at 40,000\(\times\)g for 1 hour. The supernatant was chromatographed using an anhydrotrypsin-Sepharose 4B column (12 mL gel, prepared according to the method described by Ishii et al\(^9\)) equilibrated in phosphate-buffered saline containing 20 mmol/L Na\(_2\)SO\(_4\), 0.05\% Triton X-100 (buffer A). The column was washed with 80 mL of buffer A and 80 mL of the same buffer without Triton X-100. The bound protein was eluted with 1.5 mol/L NaSCN in three-column volumes. The eluted protein was concentrated and dialyzed against a solution containing 0.15 mol/L NaCl and 20 mmol/L Na\(_2\)SO\(_4\). The recovery of LACI was approximately 60\%.

The freshly prepared anhydrotrypsin-Sepharose 4B column had a capacity of approximately 0.6 mg LACI/mL gel. On repeated use, the capacity decreased to approximately 0.2 mg/mL gel. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the eluted protein showed a major band of M\(_{\text{r}}\) \(\sim 38,000\) corresponding to LACI with traces of high molecular weight contaminants. The contaminants were removed by adsorption with phenyl Sepharose 4B.

**Characterization of purified rLACI.** The isolated protein was substantially pure LACI by the following criteria: (1) SDS-PAGE shows essentially a single band; (2) amino acid analysis and protein sequencing match the composition and sequence deduced from the cDNA sequence of LACI; and (3) the stoichiometry of the inhibition of factor Xa in an amidolytic substrate assay is approximately 1:1 (see below).

The concentration of LACI was quantitated by amino acid analysis. The active site concentration of factor Xa was measured by titration with \(p\)-nitrophenyl-\(p\)’-guanidinobenzoate as described. The amidolytic activity of factor Xa and the anti-factor Xa activity of LACI were determined by a slight modification of the method previously described. In brief, 10 \(\mu\)L of bovine factor Xa (0.084 pmol active molecule) was mixed with 10 \(\mu\)L of TBB buffer (Tris-buffered saline containing 5 mg/mL bovine serum albumin and 2.5 mg/mL bovine \(\gamma\)-globulin) or 10 \(\mu\)L of diluted LACI in TBB buffer in a disposable cuvette for 5 minutes at room temperature. After addition of 0.22 mL of an assay buffer (0.1 mol/L Tris/HCl, pH 8.4, 0.5\% Triton X-100) and 10 \(\mu\)L of Spectrozyme Xa (12.5 mmol/L), the rate of the absorbance change at 405 nm was measured at 37°C. The control gave an absorbance change of 0.0233 at 405 nm per minute per 0.1 pmol of active factor Xa. In the reaction mixture containing LACI, the anti-Xa activity was calculated based on the decrease of factor Xa activity compared with that of the control. Using the assays described above, 2.6 ng of purified LACI (based on amino acid analysis; equivalent to 0.068 pmol assuming \(M_{\text{r}} = 38,000\)) was found to inhibit 0.065 pmol of active factor Xa. Thus, the stoichiometry of the interaction between LACI and factor Xa appears to be 1:1.

**APTT.** Dade’s activated cephaloplastin reagent was used to determine the APTT of plasma using a Fibrometer. Ninety microliters of plasma was mixed with 10 \(\mu\)L of sulfated polysaccharide or control buffer and 100 \(\mu\)L of activated cephaloplastin reagent for exactly 2 minutes at 37°C. A calcium solution (100 \(\mu\)L of 25 mmol/L CaCl\(_2\)) was added to the mixture and the time to clotting was recorded. The assay was observed for up to 1 hour. For practical purposes, the plasma is arbitrarily referred to as “fully anticoagulated” when clotting did not occur in 1 hour.

**Prothrombin time.** Rabbit brain thromboplastin (TF; Ortho Diagnostic) was diluted 1:10, 1:100, 1:1,000, or 1:10,000 in a saline solution containing 1 mg/mL bovine serum albumin for the determination of the prothrombin time (PT). One hundred microliters of plasma was mixed with 10 \(\mu\)L of control buffer, LACI solution, or sulfated polysaccharide solution, and 90 \(\mu\)L of diluted TF in the well of the Fibrometer at 37°C for 2 minutes. One hundred microliters of 25 mmol/L CaCl\(_2\) was added to the mixture and the time to clotting was determined. The concentrations of sulfated polysaccharides and LACI refer to the amounts of these compounds per milliliter of undiluted plasma (not the concentration of the final mixture). The PTs reported here are the average of two to eight determinations, depending on the length of the clotting time. When the clotting time was short (<100 seconds), the variations between determinations are small and two to three assays were made and averaged for each data point. When the clotting time was long (>100 seconds) and the variations were larger due to the use of dilute TF or high concentrations of LACI and sulfated polysaccharides, four to eight determinations were made and averaged for each data point. The assays were observed for up to 1 hour. The plasma is referred to as “fully anticoagulated” when clotting does not occur in 1 hour.
Antiserum, anti-LACI-immunoglobulin (Ig), and anti-LACI-Ig Sepharose 4B. Immunization of rabbits to raise antiserum against LACI, purification of anti-LACI-Ig by protein A-Sepharose 4B chromatography, and synthesis of anti-LACI-Ig Sepharose 4B, were performed as described previously.15

Preparation of LACI-depleted plasma. Pooled frozen plasma (100 mL) was thawed and passed through an anti-LACI-Ig Sepharose 4B column (3 mL of gel containing ~15 mg of bound Ig) five times to deplete the endogenous LACI antigen as described before.15 The immunoadsorbed plasma was essentially depleted of endogenous LACI, since an immunoassay (sensitivity of ~1 ng/mL) did not detect any LACI antigen.

RESULTS

Effect of heparin on intrinsic coagulation. In the APTT assay, the contact-phase proteins are activated, which leads to the initiation of the intrinsic coagulation cascade. To examine the effect of heparin on the intrinsic coagulation, normal plasma and the same plasma depleted of endogenous LACI were compared using the APTT assay. A moderate prolongation of clotting time (up to fivefold) was observed at heparin concentrations of 0 to 0.6 U/mL plasma. At 0.8 U heparin/mL, the plasma remained unclotted for more than 1 hour (arbitrarily defined as "fully anticoagulated"). There was no significant difference in the APTT in normal plasma and that of depleted of LACI, suggesting that endogenous plasma LACI does not play a significant role in the regulation of the intrinsic coagulation pathway in the presence or absence of heparin.

Role of endogenous plasma LACI in the regulation of extrinsic coagulation. Normal plasmas were preincubated with anti-LACI-Ig or normal rabbit Ig and their PTs measured to determine the role of endogenous plasma LACI in the regulation of extrinsic coagulation. As shown in Table 1, the PTs were shorter for plasma treated with anti-LACI-Ig than that with normal Ig. However, the difference between the antibody-treated plasma and the control were small at 1:10, 1:100, and 1:1,000 dilutions of TF. A moderate difference in the PTs was observed at a 1:10,000 dilution of TF. Similar results were obtained using untreated plasma and plasma depleted of endogenous LACI by immunoadsorption with anti-LACI-Ig Sepharose 4B (data not shown). These results suggest that the capacity and/or ability of endogenous LACI to inhibit TF-induced coagulation is rather small under these conditions.

Effect of heparin on extrinsic coagulation. The effect of heparin on the PTs of normal plasma and the same plasma depleted of endogenous LACI were measured using various concentrations of TF. Figure 1A shows the results using 1:1,000 dilution of TF (PT = 77 seconds for the control without heparin). In the LACI-depleted plasma, increasing concentrations of heparin (0 to 0.6 U/mL plasma) progressively prolong the PT in essentially a linear fashion. In plasma containing endogenous LACI, the heparin response was sigmoidal. At 0.1 to 0.2 U heparin/mL plasma, the PT was the same or marginally longer than those in the LACI-depleted plasma. At 0.3 and 0.4 U heparin/mL plasma, the PTs were 1.5- and 2.6-fold longer than those in the LACI-depleted plasma. At 0.5 U heparin/mL plasma, the plasma became fully anticoagulated.

Figure 1B shows the results using a 1:100 dilution of TF (PT = 41 seconds for the control without heparin). In LACI-depleted plasma, the PT also linearly increased with increasing heparin concentration, but it requires about a sixfold higher concentration of heparin to achieve the same PTs as those in Fig 1A. In plasma containing endogenous LACI, the heparin response was also sigmoidal, but the threshold concentration of heparin required to achieve the fully anticoagulated state occurred at a concentration greater than 1.5 U heparin/mL plasma.

Figure 1C shows a similar experiment using a 1:10 dilution of TF (PT = 24 seconds for the control without heparin). In the LACI-depleted plasma, heparin-induced prolongations of the PTs were much less than those in Fig 1A and B. In the LACI-containing plasma, the PT remained less than 500 seconds up to 4 U heparin/mL plasma.

The above results taken together suggest that several mechanisms may be involved in the regulation of extrinsic coagulation. First, heparin can prolong TF-induced clotting moderately in the absence of endogenous LACI; second, endogenous plasma LACI possesses a weak anticoagulant effect against TF-induced clotting in the absence of heparin; and third, beyond a certain threshold concentration, heparin dramatically enhances the inhibition of TF-induced clotting in the presence of plasma LACI, suggesting that LACI serves as a cofactor for heparin in the inhibition reaction; and fourth, the concentration of heparin required for the inhibition of extrinsic coagulation is dependent on the concentration of TF present.

Effect of exogenously added LACI on the PT of normal plasma. The experiments described above were restricted to plasma containing or depleted of endogenous LACI. The results suggest that endogenous plasma LACI may play an important role in the inhibition of TF-induced clotting when the amount of TF is small, but it may be inadequate when the amount of TF is large. To extend the range of control to conditions where endogenous LACI are inadequate, exogenous LACI was added to normal plasma to examine its effect on the PT. Figure 2A shows that using a constant concentrations of TF (1:10,000, 1:1,000, and 1:100 dilutions of TF), the PTs were linearly related to the
Fig 1. Effect of heparin on the PT of a normal plasma and a LACI-depleted plasma at various TF concentrations. The plasmas used were either untreated (—■—) or depleted of endogenous LACI antigen (—○—) by immunoadsorption as described in the Methods. TF reagent was diluted 1:1,000 (A), 1:100 (B), or 1:10 (C) for the determination of PT. The dashed lines in panels A and B were extrapolations based on the results that the plasmas remained unclotted for more than 1 hour at 0.5 (A) and 2 (B) Units heparin/mL plasma, respectively.

Concentration of exogenous LACI added to the plasma. The concentration of LACI required for prolongation of the PT increased with increasing concentrations of TF used and this is reflected in the slope of the PT-LACI concentration response curves. At a higher concentration of TF (1:10 dilution of TF), the PT-LACI concentration response curve is not linear as shown in Fig 2B. The reasons for the difference in concentration response under different experimental conditions are currently unclear.

Synergistic anticoagulant action of LACI and heparin. The above experiments demonstrate that addition of heparin or LACI separately to plasma produces dose-dependent inhibition of TF-induced clotting (Figs 1 and 2). In addition, heparin appears to potentiate the inhibition of TF-induced clotting by endogenous LACI (Fig 1). To quantitate the extent of potentiation, a LACI-depleted plasma was supplemented with heparin, purified LACI, or a combination of both to compare their anticoagulant effects. Figure 3A shows the relationship of the PT to the concentrations of exogenously added heparin and LACI. Prolongation of the PT with increasing concentrations of heparin or LACI alone are linear. When heparin and LACI are simultaneously present in plasma, the total effect on the clotting time varies with the concentration of the compounds used. At low concentrations (eg, <0.2 U heparin/mL plus <1 µg LACI/mL), the clotting time does not significantly deviate from that expected for the individual components. At higher concentrations (>0.3 U heparin/mL plus 1.5 µg LACI/mL), the clotting time increasingly deviates from those expected from the individual components and the potentiation effect becomes apparent. For example, 0.5 U heparin/mL plus 2.5 µg LACI/mL has a PT of approximately 1,000 seconds, while 1 U heparin/mL or 5 µg LACI/mL both have PTs of less than 200 seconds.

Pharmacologically, drug interactions can be analyzed by the isobole (isoeffective curve) method using the interaction index as a criteria to differentiate zero interaction, synergism, or antagonism. The drug interaction index is defined as DA/DA + DB/DB, where DA and DB are concentrations of A and B in the combination, respectively, and DA and DB are the concentrations of A and B separately that are isoeffective with the combination. The value of the interaction index reflects the type of interaction: a value of 1 suggests zero interaction; a value of less than 1 indicates synergism; and a value of greater than 1 shows antagonism.
combination of LAC1 and heparin on the PT of a LACI-depleted plasma. (A) Effect of LACI, heparin, and a combination of LAC1 and heparin on the PT of a LACI-depleted plasma. A LACI-depleted plasma was supplemented with LACI (—x—), heparin (—o—), or a combination of LAC1 and heparin (—•—), and their PTs were determined as described in Materials and Methods using 90 μL of 1:100 dilution of the TF reagent. (B) Isobolar analysis of the LACI/heparin interaction. Concentrations of LACI alone, heparin alone, and LAC1/heparin in combination, which give the same PTs (isoeffective clotting times of 80, 100, 120, 140, 160, 180, and 200 seconds), were determined from the curves in (A). Da and Db are the concentrations of LACI and heparin separately that are isoeffective with the LACI/heparin combination at concentrations of da and db, respectively. The values of da/Db + db/Db reflect whether the two agents interact. A value of 1 suggests zero interaction; a value greater than 1 indicates antagonism; and a value less than 1 shows synergy.

Based on the data of Fig 3A, isoeffective concentrations of the compounds (ie, LACI, heparin separately, and their combinations that give the same clotting times) can be obtained for the calculation of the interaction indexes. Figure 3B shows the interaction index as a function of clotting time. The result clearly shows an increasing synergy or potentiation (interaction index <1) with increasing clotting time due to the combined use of increasing concentrations of LACI and heparin.

Heparin enhances the inhibition of TF-induced clotting by LACI. To estimate the relative potency of LACI in the absence and the presence of heparin, a LACI-depleted plasma was supplemented with various concentrations of LACI and heparin and their PTs were determined. Figure 4 shows the PT as a function of the concentration of LACI in the absence of heparin, and in the presence of 0.5, 1.0, and 2.0 U heparin/mL plasma, respectively. If one assumes that the slope reflects the potency, then the relative potency of LACI increases 3.4-, 8.5-, and 75-fold in the presence of 0.5, 1.0, and 2.0 U heparin/mL plasma, respectively, over that in the absence of exogenously added heparin.

Effect of sulfated polysaccharides and their combination with LACI on the PT of a plasma. In view of the ability of heparin to inhibit TF-induced clotting in a LACI-dependent and independent manner, other sulfated polysaccharides were also tested for their anticoagulant effects. Figure 5 shows the effect of various sulfated polysaccharides on the PT of normal plasma. All the compounds tested exhibited the ability to prolong the clotting time, but this effect was observed at very different concentrations. By weight, the relative potencies of these compounds are in the following order: low molecular weight heparin (mean M₄ = 5100) > unfractionated heparin > low molecular weight heparin (mean M₄ = 3700) > pentosan polysulfate > dermatan sulfate > dextran sulfate > heparan sulfate. To examine whether these compounds also potentiated the LACI-dependent anticoagulant activity, experiments similar to that described in Fig 3A were performed. Figure 6A through F shows the effect of LACI, sulfated polysaccharides, and their combinations on the PT of a normal plasma. All the compounds tested potentiated the inhibition of TF-induced clotting by LACI, but at very different concentrations. The concentrations of the sulfated polysaccharides that potentiated the LACI anticoagulant activity were in a similar range

Fig 4. Effect of LACI, heparin, and the combination of LAC1 and heparin on the PT of a plasma depleted of endogenous LACI. Plasma was depleted of endogenous LACI by immunoadsorption on an anti-LACI-Ig Sepharose 4B column. PT was determined as described in the Methods using 90 μL of 1:100 dilution of the TF reagent. LACI-depleted plasma was supplemented with various amounts of LACI (—o—), heparin (—•—), and LACI in combination with 0.5 (—□—), 1.0 (—△—), and 2.0 (—□—) U heparin/mL plasma, respectively. Equations and correlation coefficients from linear regression analysis are shown.
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Fig 5. Effect of sulfated polysaccharides on the PT of normal plasma. PT was determined as described in the Methods using 90 µL of 1:100 dilution of the TF reagent, 10 µL of sulfated polysaccharides, 100 µL of a pooled plasma, and 100 µL of 25 mmol/L CaCl₂. The sulfated polysaccharides used are low molecular weight heparin (mean M₁₀, 5,100; LMWH 5100); unfractionated heparin (UFH); low molecular weight heparin (mean M₁₀, 3,700; LMWH 3700); pentosan polysulfate (PPS); dermatan sulfate (DS); dextran sulfate (mean M₁₀ = 6,000 to 8,000; DXS); and heparan sulfate (HS).

to those used in Fig 5 and the relative potencies are in the same order as above.

DISCUSSION

Based on the results of this study and other information from literature, several important conclusions can be made concerning the effect of heparin and LACI in the control of intrinsic and extrinsic coagulation:

(1) When coagulation is activated through the intrinsic pathway, plasma antithrombin III is the primary regulator of the cascade. Heparin at low concentrations (<0.6 U/mL) progressively prolongs the clotting time, and at higher concentrations (>0.8 U/mL) effectively shuts down the intrinsic cascade.

(2) When coagulation is activated through the extrinsic pathway, plasma LACI and antithrombin III are the key regulators of the cascade. Heparin enhances the formation of the inert TF/factor VIIa/factor Xa/LACI/Ca²⁺ complex and catalyzes the inhibition of the downstream proteases by antithrombin III. Inhibition by heparin/LACI causes an effective shutdown of the extrinsic coagulation, whereas inhibition by heparin/antithrombin III only causes a mild, dose-dependent prolongation of time to clotting (Fig 1). The effectiveness of heparin in controlling the extrinsic coagulation is dependent on the amount of TF present. With small amounts of TF, heparin can fully stop the extrinsic cascade primarily by enhancing the inhibition of TF/factor VIIa/factor Xa by LACI. With larger amounts of TF, heparin together with endogenous plasma LACI and antithrombin III is not adequate in controlling extrinsic coagulation (Fig 1C).

(3) In the absence of heparin, LACI inhibits the TF-induced coagulation rather inefficiently. However, in the presence of heparin (at concentrations similar to those used clinically), the inhibition of TF-induced clotting by LACI is greatly enhanced (Figs 1 through 4). LACI appears to function as a cofactor for heparin.

(4) When TF exists in relatively large amounts, exogenous supplementations of LACI or heparin to the plasma only cause limited prolongation of clotting time. Supplementation of LACI and heparin in combination, in contrast, sharply enhances the inhibition of TF-induced clotting (Figs 3 and 4). LACI and heparin appear to exert a synergistic anticoagulant action.

(5) A variety of sulfated polysaccharides are capable of enhancing the anticoagulant effect of LACI, but they require vastly different concentrations to be effective (Figs 5 and 6). The structural requirements for the enhancing effect remain to be elucidated.

Previously, it had been demonstrated that the simultaneous presence of antithrombin III and heparin abrogated the factor Xa-dependent inhibition of TF/factor VIIa by LACI in the purified system. In contrast, this study shows that heparin greatly enhances the inhibition of TF-induced coagulation by LACI in whole plasma. The discrepancy accentuates the difference between the intricate cascade and regulatory mechanism of whole plasma and the more simplistic, artificial assembly of the purified components. The exact cause of the difference is not clear, but one possible explanation is as follows: In the purified system, heparin/antithrombin III may effectively eliminate factor Xa and preclude the latter from interaction with LACI and TF/factor VIIa. In whole plasma, TF/factor VIIa converts the factor X into factor Xa, which forms the prothrombinase complex with factor Va on the procoagulant phospholipid. This physiological form of factor Xa is protected from antithrombin III and heparin action, but apparently can form the inert TF/factor VIIa/factor Xa/LACI/Ca²⁺ complex. The mechanism by which heparin enhances the formation of the inert complex has not been established. It
is possible that the enhancement of factor Xa inhibition by LACI in the presence of heparin may be partly responsible for the enhanced inhibition of TF-induced coagulation. Further detailed mechanisms involving the interaction of heparin with TF/factor VIIa and lipoproteins, and the assembly of the final inert complex remain to be elucidated.

In recent years, it has been widely believed that the extrinsic coagulation pathway plays a key role in physiological and pathological clotting, while the significance of the intrinsic pathway still remains unclear. The discovery that LACI is a plasma cofactor for heparin is important in light of the above notion, because it raises the possibility that the antithrombotic action of heparin in vivo may be to a large extent due to its ability to enhance the inhibition of the extrinsic pathway of coagulation. Currently, APTT and thrombin clotting time are used as laboratory tests for monitoring heparin treatment in clinical situations. These tests totally exclude the possible contribution of LACI-dependent inhibition of the extrinsic pathway and the results often indicate that they poorly reflect the antithrombotic effect and bleeding tendency of heparin. In view of the present findings, it seems relevant to explore the relationship of the effect of heparin on LACI/extrinsic pathway to the outcome of heparin treatment.

It has been established that the endothelial cell surface contains anticoagulantly active heparin sulfate proteoglycan. The heparin sulfate from the endothelial cell was found to contain the heparin polysaccharide sequence. In addition, binding of heparin to vascular endothelium has been described after intravenous injection into animals in vivo, and endothelial cells in culture have been shown to bind heparin in vitro. These molecules on the endothelial cell surface are thought to confer antithrombotic properties to the endothelium. Since LACI appears to possess affinity for heparin, it is likely that LACI binds to the endothelial cell surface through the glycosaminoglycans and plays an important role in the inhibition of extrinsic coagulation when TF is generated. In our previous study of
a rabbit model of TF-induced intravascular coagulation, we found that the doses of LACI required to prevent fibrinogen consumption was on the order of 100 μg LACI/kg body weight. Under those conditions, the ex vivo plasma samples show very weak prolongation of PT, which can hardly account for the complete prevention of the fibrinogen consumption in vivo. The possibility therefore exists that LACI injected in vivo may bind to the heparin-like molecule on the endothelial cell surface and the inhibition of TF-induced clotting is potentiated. Whether injected LACI binds to the endothelium in vivo remains to be rigorously established in future experiments. Recently, it has been shown that heparin induces release of endogenous LACI causing a twofold to 10-fold increase in the LACI concentration in blood. Based on this observation, it has been hypothesized that LACI is associated with endothelial glycosaminoglycans in vivo and can be displaced by binding to injected heparin. The heparin binding site(s) on LACI has not been experimentally identified. A likely site is the carboxy-terminus where a cluster of positively charged amino acids resides. Because of the unique mechanism and ability of LACI in the inhibition of TF-induced coagulation, LACI is being considered as a potential therapeutic protein for the treatment/prevention of thrombotic diseases. This study suggests that the use of heparin and LACI in combination for therapeutic applications is highly attractive for the following reasons: first, heparin is widely available and may reduce the amount of LACI required for treatment by potentiating the LACI function; second, heparin and LACI in combination inhibit both the intrinsic and extrinsic pathways of coagulation; and third, the combination may be effective in clinical conditions where heparin alone is not sufficient, eg, disseminated intravascular coagulation where TF may be generated in large amounts.

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Lipoprotein-associated coagulation inhibitor (LACI) is a cofactor for heparin: synergistic anticoagulant action between LACI and sulfated polysaccharides

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