Effect of Heparin on the Inactivation Rate of Human Factor Xla by Antithrombin-III

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Factor Xla catalyzes an important reaction in the early phase of blood coagulation by converting factor IXa to an active enzyme (factor Xaa). Although antithrombin-III, an inhibitor of factor Xaa, normally accounts for only one-sixth of the plasma inhibitory activity against factor Xaa, its effectiveness has been reported to be enhanced by heparin. We have reinvestigated the ability of heparin to potentiate factor Xaa inhibition by both purified antithrombin-III and plasma using synthetic tripeptide amide substrates as well as a coagulant assay. No increase in the inactivation rate of factor Xaa amidolytic activity by purified antithrombin-III was observed in the presence of therapeutic heparin concentrations (1 U/ml), although inhibition of the amidolytic activity of thrombin by purified antithrombin-III was enhanced at least 20-fold by the same concentration of heparin. Furthermore, despite the ability of heparin (1 U/ml) to increase the inactivation rate of thrombin by plasma, no acceleration of the rate of inhibition of factor Xaa by plasma was observed. Similar results were found when the inhibition of factor Xaa was monitored with a coagulant assay after first removing the heparin. Only at heparin concentrations of 5 and 10 U/ml, was a 2- and 4-fold increase in the inactivation rate of factor Xaa by purified antithrombin III observed. Therefore, in both purified systems as well as plasma, heparin, at concentrations observed in clinical practice, does not accelerate the inactivation rate of human factor Xaa by antithrombin-III.

ANTITHROMBIN-III is considered to be the major inhibitor of many of the enzymes in the coagulation pathway, including thrombin, factor IXa, factor Xa, and factor XIIa. In these reactions, the addition of heparin to either plasma or purified systems promotes a remarkable increase in the rates of inactivation. However, not all coagulation proteases behave in this fashion. Factor VIIa is very slowly inhibited by antithrombin-III even in the presence of heparin. Kallikrein is inhibited slowly and incompletely by antithrombin-III, and the rate of inactivation is only increased 2-3-fold by high concentrations of heparin. Although inactivation of plasmin by antithrombin-III is enhanced by heparin at a concentration of 10 U/ml, Smith and Sundbloom have recently demonstrated that heparin at a concentration of 1.4 U/ml has no effect on this reaction. We have recently reexamined the quantitative contribution of various plasma protease inhibitors toward the inactivation rate of human factor Xaa in the absence of heparin. Factor Xaa is known to be inhibited by α₂-protease inhibitor, antithrombin-III, CI inhibitor, and α₂-plasmin inhibitor. We have quantified the relative contribution of each inhibitor toward the inactivation rate of factor Xaa by plasma to be 68%, 16%, 8%, and 8%, respectively. However, human factor Xaa and bovine factor Xaa have been reported to be inactivated much more rapidly by antithrombin-III in the presence of heparin. Therefore, it is conceivable that in the presence of therapeutic concentrations of heparin, antithrombin-III might become the predominant inhibitor of factor Xaa.

In this study, we assessed the contribution of heparin toward the inhibition of factor Xaa by antithrombin III in purified systems and in plasma using amidolytic as well as coagulant assays. At therapeutic heparin concentrations, no potentiating effect on this reaction was found. Only at very high heparin concentrations was a small degree of acceleration of the inactivation rate of factor Xaa by antithrombin-III observed.

MATERIALS AND METHODS

Plasma

Plasma, drawn into 0.38 g/dl sodium citrate, as a pool from normal donors, was obtained from George King Biomedicals, Overland Park, Kans. Kininogen-deficient plasma was donated by M. Williams. Factor-XI-deficient plasma, with less than 0.01 U/ml, was donated by F. Volinski.

Substrates

Chromogenic substrates H-Leu-Thr-Arg-p-nitroanilide (S-2511) and Bz-Leu-Thr-Arg-p-nitroanilide (S-2417) were generously donated by Kabi, Stockholm, Sweden. The former, prepared as a 4.2 mM stock solution in distilled H₂O, and the latter, prepared as a 3.8 mM stock solution in distilled H₂O, were each used to assay factor Xaa. The substrate H-d-Phe-Pip-Arg-p-nitroanilide (S-2238), purchased from Kabi Group Inc., Greenwich, Conn., was employed to assay thrombin.
Heparin

Heparin was purchased from either Riker Laboratories, Inc., Northridge, Calif. (1000 U/ml) or Wyeth Laboratories, Philadelphia, Pa. (110,000 U/ml), and each had a specific activity (SA) approximately equal to 150 U/mg with an average mol wt of 11,000.13 High affinity heparin (a gift from Dr. U. Lindahl, Swedish University of Agricultural Jueus, Upsala and Dr. S. Niewiarowski, of this institution) was purified from pig intestinal mucosal heparin (SA 190 U/mg) on antithrombin-III-Sepharose. Following gel filtration on Sephadex G-100, the fractions eluting with a mol wt of 19,000–20,000 were pooled. The specific activity was 285 U/mg13 according to the British Pharmacopoeia whole blood assay.

Antithrombin-III

Human antithrombin-III was purchased from Kabi Group Inc., Greenwich, Conn., and prepared as a stock of 25 U/ml (117 μM) in 20 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl. One unit is the amount in 1 ml of normal pooled plasma or 4.7 n mole.18 The preparation contained neither measurable factor XI or Xla activity, nor α1-protease inhibitor, α1-macroglobulin, or CI-inhibitor antigens and appeared as a single band of mol wt 62,000 on SDS gel electrophoresis in the presence of β-mercaptoethanol.10

Factor XI

Factor XI was prepared according to a modification19 of a method for isolating prekallikrein17 from human plasma. The resultant factor XI had a specific activity of 203 U/mg and appeared, after activation to Xla (see below), as a single peak of 160,000 daltons on a Bio-Rad A5M column in the presence of 0.1% SDS. Factor XI also migrated as a single band of mol wt 180,000 on SDS polyacrylamide gel electrophoresis in the absence of a reducing agent, and as a single band of mol wt 80,000 in the presence of dithiothreitol. No prekallikrein or factor XII was detected by coagulation assay. One unit is defined as the amount in 1 ml of normal pooled plasma and is approximately equal to 0.025 n mole.

Factor Xla

Factor Xla was activated with trypsin as described by Mannhalter et al.18 The activation was monitored by the appearance of factor Xla coagulant activity in the absence of kaolin. After activation, the trypsin was neutralized by a twofold molar excess of soybean trypsin inhibitor. The uncleaved soybean trypsin inhibitor was removed by chromatography on a 1-ml column of QAE-Sephadex equilibrated with 20 mM Tris-HCl, pH 8.0, containing 0.05 M NaCl.

Thrombin

Human thrombin was a gift from Dr. John Fenton II (Albany, N.Y.). The specific activity was 2900 NIH U/mg.

Other Reagents

Bovine serum albumin (crystalline) and e-amino-n-caproic acid were purchased from Sigma Chemical Company, St. Louis, Mo.; polypropylene microcentrifuge tubes (lot 72.699) from Walter Starstedt, Inc., Princeton, N.J.; DEAE-Sephadex A50 from Pharmacia Fine Chemicals, Piscataway, N.J.; Biogel A5M, Bio-Rad, Rockville Center, N.Y. All other reagents were reagent grade.

Amidolytic assay of Factor Xla

The κₜ for factor Xla (1 U/ml) was determined for H-Leu-Thr-Arg-p-nitroanilide and Bz-Leu-Thr-Arg-p-nitroanilide in 0.1 M potassium phosphate, pH 7.6, containing 0.15 M NaCl and 1 mM EDTA. Assays were performed by adding 10 μl of enzyme to 330 μl substrate and the absorbance change at 405 nm was continuously recorded using a Gilford 240 recording spectrophotometer. For activity measurements of factor Xla, H-Leu-Thr-Arg-p-nitroanilide was used at 0.68 mU, while Bz-Leu-Thr-Arg-p-nitroanilide was used at 0.61 mU.

Coagulant assay of Factor Xla

This was performed by means of a partial thromboplastin time18 with factor-XI-deficient plasma as the substrate,19 in the absence of an activating surface, and calculated from a standard curve constructed from assaying several dilutions of normal pooled plasma in the presence of kaolin (1 mg/ml).

Inhibition of factor Xla

All incubations were performed in 0.5 ml polypropylene microcentrifuge tubes at 23°C. To minimize the adsorption of factor Xla onto the test tube surface, bovine serum albumin (5 mg/ml) was incubated in the tube for 5 min prior to addition of factor Xla. Heparin or buffer, and then antithrombin-III, were added. In certain experiments, antithrombin-III and heparin were incubated prior to the addition of enzyme in order to form the antithrombin-III–heparin complex. The results were unchanged by this experimental modification. At various times, 10 μl was removed and assayed for factor Xla activity by either amidolytic or coagulant assay (after removal of heparin). The data are expressed as percent of initial factor Xla activity.

Amidolytic assay of thrombin

Thrombin was assayed using H-b-Phe-Pip-Arg-p-nitroanilide at a concentration of 0.645 mM under the conditions for factor Xla kinetic determinations.

Inhibition of thrombin

This was performed using the conditions described for the inhibition of factor Xla.

Removal of heparin from incubation mixtures

Solutions containing heparin (1–30 U/ml) were added to a suspension of DEAE-Sephadex (10 mg/ml) in 10 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl. The volumes were adjusted so that no more than 0.6 U heparin/mg anion exchanger was present. The samples were mixed and centrifuged at 12,000 g in an Eppendorf microcentrifuge for 1 min at 23°C. The supernatants were then tested for the ability to inhibit thrombin or factor Xla amidolytic or factor Xla coagulant activity by the assays, as indicated.

RESULTS

Effect of heparin on the inactivation rate of thrombin amidolytic activity and factor Xla coagulant activity by antithrombin-III

Since heparin interferes with the coagulant assay, it is necessary to remove it after incubation with the protease and antithrombin-III (AT-III), but prior to assay. Thrombin (0.008 μM) was incubated with antithrombin-III (1.67 μM) in the presence and absence of heparin (1 U/ml) (Fig. 1A) and the amido-
antithrombin-III coagulant of 20 contrast, transferred BSA. buffer. H-D-Phe-Pip-Arg-p-nitroanilide. solution. in 942 tions. to presence thrombin reaction lytic. Thirty microliters of the supernatant from the DEAE-Sephadex was transferred to a tube containing 20 µl of 30 mg/ml BSA. Twenty microliters of the solution, diluted equally with buffer instead of DEAE-Sephadex, was transferred to another tube that contained 20 µl of 30 mg/ml BSA. Thirty microliters of thrombin (3 U/ml) was added to each tube and assayed at various times for amidolytic activity using the substrate H-D-Phe-Pip-Arg-p-nitroanilide. A third tube, containing all reactants except heparin, was also assayed. The data are expressed as percent of original activity. (C) Antithrombin-III + buffer, (D) antithrombin-III + heparin, after DEAE-Sephadex. (E) antithrombin-III + heparin + buffer. (B) Five microliters of factor Xla (41 U/ml), 10 µl of antithrombin-III (117 µM), 5 µl of heparin (10 U/ml) or buffer, 5 µl of 30 mg/ml BSA, and 25 µl of 10 mM Tris-HCl, pH 7.4 containing 0.15 M NaCl were incubated at 23°C. At various times, 10 µl from each tube was transferred to a tube containing 60 µl of DEAE-Sephadex (10 mg/ml). Each sample was mixed, centrifuged, and assayed for factor Xla coagulant activity in the absence of kaolin. The data are expressed as percent original activity. (F) Antithrombin-III (G) antithrombin-III + heparin.

lytic activity was measured. The pseudo-first-order reaction rate constant (k') for the inactivation of thrombin by antithrombin-III was 0.266 min⁻¹. In contrast, the k' was at least 20-fold increased in the presence of 1 U/ml heparin, but the rate was too rapid to quantify accurately, similar to previous observations. When the heparin–antithrombin-III mixture was incubated with DEAE-Sephadex to remove heparin prior to incubation with thrombin, the k' for the inactivation of thrombin was decreased to 0.266 min⁻¹, identical to the rate constant observed with the antithrombin-III in the absence of heparin. Therefore, the heparin used in this experiment was able to potentiate the inhibitory action of antithrombin-III on thrombin.
AT-III-HEPARIN EFFECT ON FACTOR Xla

Figure 2. Effect of heparin on the inhibition of factor Xla amidolytic activity by antithrombin-III. Three microliters factor Xla (41 U/ml), 10 μl 30 mg/ml BSA, 5 μl heparin (10 U/ml) or buffer, 5 μl antithrombin III (177 μM), and 27 μl 10 mM Tris-HCl, pH 7.4 containing 0.15 M NaCl were incubated at 23°C. At various times, 10 μl from each tube was assayed for amidolytic activity using H-Leu-Thr-Arg-p-nitroanilide. (■) Antithrombin III + heparin, (□) antithrombin III + buffer.

Moreover, treatment with DEAE-Sephadex was able to completely remove that quantity of heparin from the preincubation mixture without removing the antithrombin-III.

Factor Xla (1 U/ml or 0.025 μM) was preincubated with purified antithrombin III (26 μM) in the presence and absence of heparin (1 U/ml) at 23°C (Fig. 1B). The heparin was removed by DEAE-Sephadex (see Fig. 1A) prior to assay of residual coagulant activity. No difference in inactivation rate constant (k' = 0.133 min⁻¹) was observed in the presence or absence of heparin when the heparin concentration in the incubation mixture was 1 U/ml. Similar results were observed when the incubations were performed at 37°C (data not illustrated).

Effect of Heparin on the Inhibition of Xla Amidolytic Activity by Antithrombin-III

The Kₐ for factor Xla determined for H-Leu-Thr-Arg-p-nitroanilide and Bz-Leu-Thr-Arg-p-nitroanilide was found to be 0.42 mM and 0.22 mM, respectively. The kₐ/Kₐ for the former was found to be 3.50 × 10⁴ M⁻¹ s⁻¹ and for the latter was 4.73 × 10³ M⁻¹ s⁻¹. Therefore, both substrates appear suitable for the measurement of purified human factor Xla activity.

Factor Xla (1 U/ml or 0.025 μM) was preincubated with antithrombin-III (13 μM) in the presence and absence of heparin (1 U/ml) (Fig. 2). Factor Xla was also incubated with heparin (10 U/ml). Even at this concentration, heparin had no effect on the amidolytic activity of factor Xla. Furthermore, no difference in the inactivation rate of factor Xla by antithrombin III (0.074 min⁻¹) was observed in the presence or absence of heparin, as measured by amidolytic activity. The inactivation rate observed with the amidolytic assay (Fig. 2) is about half of that by coagulant assay (Fig. 1B), as expected, since the concentration of antithrombin-III in Fig. 2 is half of that in Fig. 1B. When the heparin was removed by DEAE-Sephadex prior to the amidolytic assay (data not illustrated), the results did not differ from those in Fig. 2 where heparin was not removed.

Effect of Heparin on the Inhibition of Factor Xla Amidolytic Activity by Kininogen-Deficient Plasma

Prior to assessing the inactivation rate of factor Xla by plasma, it was important to ascertain whether heparin would accelerate the inactivation rate of thrombin amidolytic activity by plasma, under the same conditions. Kininogen-deficient plasma was utilized in both experiments in order to obviate the protective effect of that cofactor against inhibition of factor Xla by antithrombin III. Thrombin (0.023 μM) was incubated with kininogen-deficient plasma for 2.5 min, heparin (final concentration 1 U/ml) was added to the incubation mixture, and the amidolytic activity of thrombin was measured (Fig. 3A). A dramatic increase in the inactivation rate was observed after the addition of heparin, as compared to similar experiments performed in the absence of heparin. This observation established the ability of the heparin used in these experiments to enhance thrombin inactivation by plasma.

To ascertain whether heparin might affect the inactivation rate of factor Xla by the antithrombin-III in plasma, factor Xla (1 U/ml or 0.025 μM) was incubated with kininogen-deficient plasma. The decrease in factor Xla amidolytic activity was measured using Bz-Leu-Thr-Arg-p-nitroanilide (Fig. 3B). No differences were found whether or not heparin was present. In another experiment, the inactivation rate of factor Xla by kininogen-deficient plasma was measured, and after 14 min, concentrated heparin was added to the incubation mixture so that no appreciable dilution resulted. No further decrease in inactivation rate was observed after the addition of heparin (final concentration 1 U/ml). This observation agreed with the results obtained with purified antithrombin-III and supported
Fig. 3. Inhibition of factor Xla and thrombin by kininogen-deficient plasma with or without heparin. (A) Five microliters thrombin (30 U/ml), 10 μl L-amino caproic acid (0.5 M), 10 μl BSA (30 mg/ml), 5 μl heparin (10 U/ml) (final concentration 0.7 U/ml) or buffer, 10 μl kininogen-deficient plasma, and 30 μl buffer were incubated at 23°C. At various times, 10 μl from each tube was assayed for amidolytic activity using the substrate H-o-Phe-Pip-Arg-p-nitroaniline. A similar experiment was performed where 2 μl heparin was added 2.5 min after monitoring the activity. The data are expressed as percent original activity. (C) Plasma + buffer, (○) plasma + heparin, and (*) plasma, heparin added after 2.5 min. (B) Three microliters factor Xla (41 U/ml), 10 μl L-amino caproic acid (0.5 M), 10 μl 30 mg/ml BSA, 20 μl kininogen-deficient plasma, 5 μl heparin (10 U/ml) (final concentration 0.67 U/ml) or buffer, and 27 μl buffer were incubated at 23°C. At various times, 10 μl from each tube was assayed for amidolytic activity using Bz-Leu-Thr-Arg-p-nitroaniline. A similar experiment was performed where 2 μl heparin (10 U/ml) (final concentration 1 U/ml) was added after monitoring the activity for 14 min. The data are expressed as percent original activity. (O) Plasma + buffer, (●) plasma + heparin, (*) plasma, heparin added after 14 min.
the conclusion that heparin at 1 U/ml does not potentiate the factor Xla inactivation rate by antithrombin-
III.

**Effect of Heparin Concentration on the Inactivation Rate of Factor Xla Amidolytic Activity by Antithrombin-III**

To elucidate whether the failure of heparin to enhance the inactivation rate of factor Xla by antithrombin-III was a function of the concentration of heparin, factor Xla, at its plasma concentration (1 U/ml or 0.025 μM), was incubated with antithrombin-III at its plasma concentration (4.7 μM) in the presence and absence of increasing concentrations of heparin (Fig. 4). Heparin was removed, prior to assay, using DEAE-Sephadex, since at high concentrations, heparin precipitates the chromogenic substrate. The k' for the inactivation of factor Xla by antithrombin-III (4.7 μM) was identical in the absence or the presence of heparin at 1 U/ml. When the heparin concentration was 5 U/ml, the k' increased twofold, and at 10 U/ml, the k' increased fourfold. No further increase in the inactivation rate of factor Xla by antithrombin-III was observed when the heparin concentration was greater than 10 U/ml. Even at a heparin concentration of 30 U/ml, there was only a fourfold increase in the rate of factor Xla inactivation by antithrombin-III, as compared to at least an 80-fold increase in the rate of thrombin inactivation at 1 U/ml heparin.

**Effect of High-Affinity Heparin on the Inhibition of the Amidolytic Activity of Factor Xla and Thrombin by Antithrombin-III**

In order to determine whether purified, high-affinity heparin was more effective in accelerating the inactivation rate of factor Xla by antithrombin-III, factor Xla was incubated with antithrombin-III in the presence or absence of 2 U/ml high-affinity heparin (Fig. 5). No difference in inactivation rate was observed in the presence or absence of high-affinity heparin.

Thrombin was then incubated with antithrombin-III in the presence and absence of 2 U/ml high-affinity heparin. A marked increase in the inactivation rate of thrombin was noted in the presence of high-affinity
heparin as compared to what was measured in its absence. Therefore, high affinity heparin is ineffective as a potentiator of the inhibition of factor Xla by antithrombin-III at a concentration of 2 U/ml.

DISCUSSION

We have reinvestigated the effect of heparin on the inactivation rate of factor Xla by antithrombin-III in purified systems as well as in plasma using both an amidolytic assay and coagulant activity. Although heparin at 1 U/ml greatly accelerated the inactivation rate of thrombin by antithrombin-III in both purified systems (Fig. 1A) and plasma (Fig. 3A)

under the same conditions, heparin did not influence the inactivation rate of factor Xla by antithrombin-III using amidolytic (Fig. 2) or coagulant (Fig. 1B) assays for factor Xla in purified systems, or an amidolytic assay for factor Xla in a plasma system (Fig. 3B). Furthermore, a high-affinity heparin, at a concentration of 2 U/ml (Fig. 5) failed to affect the inactivation rate of factor Xla by antithrombin-III. Only when heparin was present at high concentrations (5–30 U/ml) was a modest increase in the inactivation rate of factor Xla by antithrombin-III observed (Fig. 4). This observation differs from a previous report that stated that heparin (0.7 U/ml) produced a great increase in the inactivation rate of factor Xla by antithrombin-III.

In that study, only a coagulant assay was used, and it was necessary to remove heparin using DEAE-cellulose prior to the factor Xla assay. It is possible that the DEAE-cellulose, under the experimental conditions used by the previous workers, not only removed heparin, but also a heparin–factor-Xla–antithrombin III complex, leading to a physical loss of factor Xla due to adsorption to the discarded ion-exchange resin, rather than inactivation of factor Xla by the antithrombin-III–heparin complex. Alternatively, since only indirect evidence (differences in the rate of factor Xla inactivation) was provided to assure that no residual heparin persisted that could have affected the coagulant assay, it is possible that a small amount of heparin persisted, since DEAE-cellulose has a lower capacity for heparin than does DEAE-Sephadex. The use of an amidolytic assay makes the removal of low heparin concentrations prior to factor Xla assay unnecessary. Incubation of the supernatant from the DEAE-Sephadex adsorption of the factor Xla–heparin–antithrombin-III complex with thrombin provides direct evidence that all heparin was removed from our assay system.

Kurachi and Davie have shown that bovine factor Xla is more rapidly inactivated by antithrombin III in the presence of heparin (50–100 U/ml) than in its absence. The findings of that investigation are consistent with the present study, since we find that 10–30 U/ml of heparin did increase the rate of inactivation of factor Xla by antithrombin-III, although 1 U/ml of heparin did not. The order in which heparin was added to the incubation mixture does not appear to be a major factor in determining whether or not heparin accelerates the inactivation rate of factor Xla by antithrombin-III. The inactivation rate was greatly enhanced when heparin was either added prior to the thrombin (Fig. 1A) or after the thrombin–antithrombin-III reaction was allowed to proceed (Fig. 3A). Heparin did affect the inactivation rate of thrombin by antithrombin-III, indicating that both the heparin and antithrombin-III used in these experiments were functionally intact.

The pioneering study of Rosenberg and Damus demonstrated that heparin dramatically accelerated the interaction between antithrombin-III and thrombin. The mechanism proposed by the investigators involved the binding of negatively charged heparin to positively charged lysine groups in antithrombin-III, inducing a conformational change in the inhibitor protein. Such an allosteric modification in the antithrombin-III renders an arginine in its reactive site more accessible to the serine in the active site center of thrombin. Since heparin alters the rate of formation, but not the final amount, of the stoichiometric convergent antithrombin-III–thrombin complex, the action of heparin appeared to be catalytic in type. Indeed, the ability of as little as 0.005 U/ml of heparin (0.3 × 10⁻⁴M) to accelerate the inhibition of thrombin (4.6 × 10⁻⁴M) by antithrombin-III (108 × 10⁻⁴M) supports the hypothesis of a catalytic role for heparin. In addition, factor Xa is inactivated in the presence of catalytic amounts of heparin (0.03 U/ml). The requirements of 10 U/ml heparin (600 × 10⁻⁴M) to accelerate the inhibition of plasmin (21 × 10⁻⁴M) by antithrombin-III (108 × 10⁻⁴M) suggest that heparin may play more than a catalytic role with certain enzymes. The observation of the concentration dependence of heparin for the acceleration of antithrombin-III-induced inactivation of factor Xla in this study is consistent with a mechanism similar to that observed for plasmin. The enhancement of the inactivation rate of factor Xla, at plasma concentration (0.025 μM), by antithrombin-III, at plasma concentration (4.7 μM), requires heparin at a concentration of 6.0 μM (10 U/ml) and is not observed at 0.6 μM (1.0 U/ml). These observations suggest that a stoichiometric combination might be required, paralleling the mechanism postulated for plasmin–heparin–antithrombin-III interactions and that the conformational change induced in antithrombin-III by heparin does not contribute directly to increasing the rate of factor Xla inactivation.
Kallikrein, plasmin, and factor Xla are structurally similar. After reduction, each is composed of a large noncatalytic chain of approximate mol wt 50,000 as well as a smaller chain, containing the active site serine, of approximate mol wt 30,000. In contrast, reduced thrombin, factor Xa, and factor IXa each are composed of noncatalytic chains of approximate mol wt 12,000–25,000 in addition to the catalytic polypeptide of approximate mol wt 30,000. The presence of a large noncatalytic chain might sterically impair the interaction of the enzyme with antithrombin-III and/or heparin, leading to the differences observed.

It appears unlikely that the commercial heparin used in this study failed to bind to antithrombin-III, thereby influencing our results. First, the commercial heparin used was capable of markedly potentiating antithrombin-III inhibition of thrombin. Second, high-affinity heparin, fractionated by its affinity for antithrombin-III, also failed to potentiate inhibition of factor Xla by antithrombin III.

Although heparin, at 1 U/ml, profoundly affects the inactivation rate of factor Xa and thrombin by antithrombin-III, it has no effect of factor Xla inactivation. The concentrations of heparin (5–30 U/ml) needed to modestly affect factor Xla inactivation by antithrombin-III are not in the therapeutic range. This finding is consistent with a recent report using a chromogenic substrate in which up to 12 U/ml of heparin was required for potentiation of bovine factor Xla inhibition by antithrombin-III. Thus, even in individuals receiving heparin, antithrombin-III plays a relatively minor role in the inhibition of factor Xla in plasma. Therefore, α1-antitrypsin serves as the predominant plasma inhibitor of human factor Xla in vivo, regardless of the presence or absence of heparin.6,10

REFERENCES