During blood coagulation, polymorphonuclear leukocytes release elastase in amounts that can exceed 100 nmol/L. We therefore studied the interaction between human leukocyte elastase and human \( \alpha \)-thrombin. Elastase cleaved the thrombin B chain (Ala 150-Asn 151) near the \( \gamma \)-cleavage site, resulting in two fragments held together by noncovalent interactions. The NH\(_2\)-terminal fragment (FI), mol wt \( \sim 18,000 \), was disulfide-linked to the thrombin A chain. The COOH-terminal fragment (FII), mol wt \( \sim 13,000 \), contained the active-site serine and formed a covalent bond with antithrombin III. Heparin accelerated proteolysis of \( \alpha \)-thrombin by elastase. Proteolysed \( \alpha \)-thrombin (T\(_x\)) retained full amidolytic activity; however, the concentration of T\(_x\) causing 50% maximal platelet aggregation and adenosine triphosphate (ATP) release was 7.9 nmol/L (1.1 nmol/L for \( \alpha \)-thrombin and 220 nmol/L for \( \gamma \)-thrombin). Fibrinogen clotting activity of T\(_x\) and \( \gamma \)-thrombin was 32% and 1% that of \( \alpha \)-thrombin, respectively. Elastase released during the coagulation process may modulate thrombin activity. In addition, elastase-modified thrombin may be a useful probe of the structure and function of the \( \gamma \)-cleavage region.

**ELASTASE** IS the major neutral protease released by human polymorphonuclear leukocytes (PMNs) during blood coagulation or activation by soluble immune complexes, C5a, or endotoxin. Elastase is released into the circulation in acute leukemia, in septicemia, and in a variety of other inflammatory conditions. Several studies suggest that the decrease in the concentration of coagulation factors that occurs in these conditions may be the result of direct proteolysis by PMN elastase. Elastase also inactivates human antithrombin III, \( \alpha \)-plasmin inhibitor, and Cl inactivator. Furthermore, it inhibits thrombin-induced platelet stimulation in association with limited proteolysis of platelet membrane glycoprotein Ib. In circulating blood, leukocyte elastase is inactivated mainly by \( \alpha \)-antitrypsin; however, this inhibitor is readily inactivated by oxidizing agents such as those generated by phagocytosing PMNs. More recently, studies using cell contact systems have shown that \( \alpha \)-antitrypsin does not completely inhibit the proteolytic activity of PMN-derived elastase.

In this study, we investigated the effects of PMN elastase on human \( \alpha \)-thrombin. PMN elastase cleaved the thrombin B chain at Ala 150-Asn 151, within four amino acids of the \( \gamma \)-cleavage site. Cleavage resulted in formation of a larger NH\(_2\)-terminal and a smaller COOH-terminal fragment held together by noncovalent association. The elastase-modified thrombin possessed amidolytic activity similar to that of \( \alpha \)-thrombin but exhibited reduced fibrinogen clotting and platelet-stimulatory activity. Elastase degradation of \( \alpha \)-thrombin was enhanced by heparin. These findings suggest that elastase may be involved in the physiologic modulation of \( \alpha \)-thrombin function.

**MATERIALS AND METHODS**

Human polymorphonuclear leukocyte elastase, specific activity 92%, was purified from outdated WBC concentrates or from purulent sputum. Human \( \alpha \)-thrombin and \( \gamma \)-thrombin were prepared and stored at \(-70^\circ \text{C} \) in 0.75 mol/L of NaCl at concentrations of 2 to 4 mg/mL. As determined by active-site titration (p-nitrophenyl-p-guanidinobenzoate), the specific activities of the \( \alpha \)-thrombin and \( \gamma \)-thrombin preparations were 95% and 78%, respectively. Antithrombin III was obtained as a gift from the American Red Cross Fractionation Center, Bethesda, MD. As analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the inhibitor migrated as a single band; \( >96\% \) of the antithrombin III formed a higher mol-wt complex with \( \alpha \)-thrombin.

**Proteolysis of \( \alpha \)-thrombin by elastase.** \( \alpha \)-Thrombin (40 nmol/L) was incubated with elastase (thrombin/elastase molar ratios from 1:1 to 100:1) for various times (37°C) in 0.02 mol/L of Trizma base (Tris), pH 7.4, or in Tris buffer containing 0.15 mol/L of NaCl. Incubations were performed in the presence or absence of 1 U/mL of sodium heparin (140 U/mg) from porcine intestine (Elkins-Sinn, Cherry Hill, NJ). The reaction was stopped either by incubating for 10 minutes (37°C) with 0.7 mmol/L of MeO-Suc-Ala-Ala-Pro-Val-CH\(_2\)Cl (Enzyme Systems Products, Livermore, CA) to inhibit elastase activity or by boiling in SDS as detailed later. Samples were quantitated for residual elastase activity with MeO-Suc-Ala-Ala-Pro-Val-CH\(_2\)Cl (Enzyme Systems Products) as described. Only those samples demonstrating \( >95\% \) inhibition of initial activity were used in the following studies.

**SDS-PAGE.** Samples for electrophoresis (4 to 8 \( \mu \)g per lane) were mixed with an equal volume of 0.06 mol/L of Tris, 2% SDS, 5% glycerol, 0.001% bromphenol blue, and 2% dithiothreitol (SDS-DTT), solubilized by heating at 100°C for 3 minutes, and stored (\(-70^\circ \text{C}\)). SDS-PAGE was performed by the method of Laemmli using a 3.8% stacking gel and a 9% to 18% gradient separation gel. Electrophoresis was terminated when the buffer front was 1 cm from the bottom of the gel. Gels were fixed in 25% isopropanol and 10% acetic acid. The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

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acetic acid prior to being stained with Coomassie blue (0.1%). Densitometry was performed at 633 nm using an LKB Ultrascan Laser Densitometer and LKB model 2210 dual-channel recorder.

Mol wt markers included phosphorylase b (mol wt 94,000), bovine serum albumin (BSA) (mol wt 67,000), ovalbumin (mol wt 43,000), carbonic anhydrase (mol wt 30,000), soybean trypsin inhibitor (mol wt 20,100), α-lactalbumin (mol wt 14,400), and aprotonin (mol wt 6,500).

**Interation of elastase-modified thrombin with antithrombin III.** α-Thrombin (11 μmol/L) was incubated either alone or with elastase (1.7 μmol/L) for 60 minutes (37°C) in 0.02 mol/L of TRIS pH 7.4 containing 1 U/mL of porcine intestinal heparin. The mixture was incubated for 10 minutes (37°C) with 0.7 mmol/L of MeO-Suc-Ala-Ala-Pro-Val-CH2Cl prior to addition of antithrombin III (30 μmol/L). Samples were taken at various times for analysis by SDS-PAGE.

**Amino acid sequence of elastase-modified thrombin.** Samples were prepared for sequencing as described by extensive dialysis against 0.1% trifluoroacetic acid (TFA) and freeze-drying. The resultant phenylthiohydantoin (PTH) derivatives were resolved by reverse-phase HPLC on a Waters 501 pump, 440 detector (254 nm) and 740 integrating recorder. Isoelectric separation of the common PTH amino acids was achieved at 55°C with 0.01 mol/L of sodium acetate, pH 4.54 and acetonitrile (38:62, vol/vol) at a flow rate of 1 mL/min. PTH yields were automatically calculated by peak area and expressed as a percentage of the respective 200-pmol standard. Duplicate thrombin samples were subjected to sequence analysis.

**Amidolytic activity.** Samples of α-thrombin and elastase-treated thrombin (>95% conversion to the cleaved form on SDS-PAGE) were analyzed for amidolytic activity with Tos-Gly-Pro-Arg-paranitroanilide (GPA, Vega) by modification of the method of Kirchof and colleagues. GPA was dissolved in methanol (14% mol/L) was applied to a Beckman 890M spinning cup sequencer. Amino acid sequence of elastase-modified thrombin.

**Human fibrinogen (Kabi Grade L) was dissolved in 0.3 mol/L of NaCl, 0.1 mol/L of sodium citrate pH 6.4, containing 0.01 mol/L of ε-aminocaproic acid (Aldrich, Milwaukee), and stored (−70°C). For thrombin assays, fibrinogen was diluted (6.4 mg/mL) in 10 mmol/L of imidizole, 10 mmol/L of CaCl2, 0.15 mol/L of NaCl pH 7.4. Aliquots (0.09 mL) were prewarmed to 37°C prior to the addition of various concentrations of thrombin prepared in 0.2 mL of the same buffer (37°C) containing 0.1% BSA. Clotting time was determined by fiometer (Becton Dickinson No. 60415).

**Platelet stimulation studies.** Washed platelet suspensions were prepared by modification of the technique of Mustard and co-workers as described. Platelet stimulation studies were performed at 37°C with 0.45-mL aliquots of washed platelets (2 × 10⁸/mL) stirred at 1,000 rpm in a Chrono-Log model 460 Lumicycle Dual double-channel aggregometer. Aggregation and luminescence in each channel was recorded with a Chrono-Log model 703 strip chart dual-pen recorder. Aggregation was quantitated by planimetry. Results were expressed as percentage of the area under the control curves.

Secretion was measured by the luminescence reaction of secreted ATP with 4 μL of firefly luciferin and luciferase (40 mg/mL, Chrono-Lume No. 395, Chrono-Log) added 1 to 5 minutes prior to stimulation of the platelets. Secretion was measured as the increase in fluorescence and expressed as the percentage of the increase in the control.

The threshold stimulus determined for individual platelet preparations was defined as the lowest concentration that caused >90% increase in light transmission at 5 minutes when added to stirred platelets at 37°C and resulted in fluorescence at least 90% of maximum. Immediately after measurements of aggregation and secretion, 40 μmol/L of ATP (Sigma FF-ATP, 1 mg/mL, and 40 mg/mL of MgSO4 · 7 H2O) was added for instrument calibration.

**RESULTS**

**Incubation of α-thrombin with elastase.** α-Thrombin was incubated alone or with elastase for various times (37°C). After MeO-Suc-Ala-Ala-Pro-Val-CH2Cl was added to inhibit elastase activity, samples were reduced, electrophoresed, and stained with Coomassie blue. The B chain of thrombin migrated as a single band at mol wt 32,500 after incubation for 0 to 30 minutes with buffer alone (Fig 1, lanes 1 through 10). The small (mol wt) α-thrombin A chain was not visualized in this system. Incubation of thrombin with elastase (thrombin/elastase molar ratio 8:1) resulted in proteolysis (lanes 1 through 4). After incubation with elastase for 15 seconds, lower mol wt bands were observed (lane 1). Those at mol wt ~28,000 co-migrated with the elastase isoform seen in the elastase controls (lanes 11 and 12). New bands also appeared at mol wt 21,000, 16,000, and 15,000. These bands were diminished or absent if elastase was inactivated with α1-antitrypsin (experiment not shown) or with greater concentrations of MeO-Suc-Ala-Ala-Pro-Val-CH2Cl prior to the addition of SDS to the mixture. This suggests that these peptides may result from either (a)
elastase-proteolysis of SDS-denatured thrombin or (b) stimulation of elastase activity by SDS.32-34

With longer incubations (lanes 2 through 4), the concentration of thrombin B chain at mol wt 32,500 was diminished; by 30 minutes proteolysis of 50% of the thrombin B chain occurred. The three bands at mol wt 21,000, 16,000, and 15,000 were no longer apparent after 30 minutes, and two additional bands representing core fragments appeared at mol wt 18,500 and 13,500, designated fragment I (F1) and fragment II (FII), respectively. In four separate experiments, the mol wt of F1 ranged from 17,000 to 18,500, and that of FII ranged from 12,000 to 13,500.

In experiments not shown, F1 and FII could not be separated under non-denaturing conditions (0% to 1 mol/L of NaCl; 0% to 3% Triton X-100, Sigma, St Louis). This suggests that F1 and FII are held together by strong noncovalent interactions, as reported for the γ-thrombin B chain fragmentation peptides.35-38

Heparin binds to both thrombin and elastase.39-43 Therefore, the effect of heparin on elastase-mediated proteolysis of α-thrombin was examined. α-Thrombin was incubated with elastase in Tris buffer with 1 U/mL of heparin (lanes 5 through 8). Within 15 seconds after the addition of elastase to α-thrombin, F1 and FII were observed (lane 5). The breakdown products at mol wt 21,000, 16,000, and 14,000 seen in lanes 1 and 2 were not observed in the presence of heparin. Within 3 minutes (lane 6), >50% of α-thrombin was proteolysed to F1 and FII; by 30 minutes (lane 8), the conversion of α-thrombin to F1 and FII was complete.

These results indicate that heparin accelerates elastase-mediated proteolysis of α-thrombin. They also suggest that heparin protects α-thrombin from conversion into the mol wt 21,000, 16,000, and 14,000 fragments.

Elastase cleavage of factor IX is less extensive in the absence of calcium.44 Therefore, the above experiments were performed in the presence of CaCl2 (2 to 6 mmol/L). Calcium did not alter or inhibit proteolysis of thrombin by elastase.

When analyzed on SDS-PAGE without disulfide bond reduction, thrombin fragments F1 and FII were identified at mol wt 23,000 and 13,500 (gel not shown). The increase in the mol wt of F1 prior to reduction suggested that it was covalently linked to the thrombin A chain.

Interaction of elastase-modified thrombin with antithrombin III: Identification of the active-site-containing fragment. To determine if F1 or FII contained the active-site serine, antithrombin III was incubated either with α-thrombin or with the elastase-modified protease in the presence of 1 U/mL of heparin (inhibitor/enzyme molar ratio ~3:1). After various times, samples were analyzed by SDS-PAGE (Fig 2). α-Thrombin incubated in the presence of elastase (lane 1) was converted into the elastase-modified form, as indicated by the presence of F1 and FII. A faint band representing elastase was seen at mol wt 28,000. Lane 2 contained α-thrombin incubated in the absence of elastase for comparison, and lane 3 contained antithrombin III (mol wt 80,000). Incubation of antithrombin III with the modified thrombin for 15 seconds, 5 minutes, and 60 minutes (lane 4, 5, and 6, respectively) resulted in formation of a band at mol wt 72,000, the mol wt compatible with that of a complex between antithrombin III (mol wt 58,000) and FII (mol wt 13,500). In addition, after 15-second incubation (lane 4) the intensity of free FII was diminished relative to F1; within 5 minutes of incubation (lane 5), FII was no longer apparent. These findings suggest that the active-site serine is contained within FII.

For comparison, antithrombin III was incubated with α-thrombin for 15 seconds, 5 minutes, and 60 minutes, respectively (lanes 7 through 9). The α-thrombin–antithrombin III complex was visible at mol wt 88,500. A faint band was apparent at mol wt ~72,000, with electrophoretic mobility similar to the FII–antithrombin III complex. This mol wt ~72,000 complex has been shown to be the result of degradation of the α-thrombin–antithrombin complex by free thrombin.45 The relationship of this complex to the FII–antithrombin complex remains undetermined.

These results demonstrate that antithrombin III inhibits elastase-modified thrombin by combining with the active-site serine within FII. These findings suggest that elastase cleaves the α-thrombin B chain in the vicinity of the γ cleavage site.46 This results in the formation of an NH2-terminal fragment (F1), mol wt 18,000, that is disulfide linked to the thrombin A chain and a COOH-terminal fragment (FII), mol wt 13,000, containing the active-site serine.
To determine if elastase can degrade preformed α-thrombin–antithrombin complexes, thrombin was incubated with antithrombin for 5 minutes as in lane 8 prior to incubation with elastase (thrombin/antithrombin molar ratio 9:1). Fifteen seconds after addition of elastase (lane 10), a complex was observed at mol wt 72,000; by 5 minutes (lane 11), the α-thrombin–antithrombin complex at mol wt 88,500 was markedly reduced in intensity, and protein at mol wt 72,000 was increased. A faint band migrated at mol wt 16,000. Neither fragment FI nor fragment FII was observed in lanes 10 or 11, and there was an increase in stained material moving just behind the buffer front. These findings suggest that elastase cleavage of the α-thrombin–antithrombin complex may differ from cleavage of free α-thrombin.

Amino acid sequence of elastase-modified thrombin. Because the thrombin samples were analyzed directly after proteolysis, at least one new amino terminal sequence was anticipated, in addition to the usual A chain and B chain sequences. Amino acid analysis confirms the presence of a new NH2-terminal sequence corresponding to residues 151 through 156 of the human B chain of thrombin (Table 1). Residues 1 (Asn), 4 (Lys), and 6 (Gln) are unequivocal; the yields at residues 2 (Val), 3 (Gly), and 5 (Glu) are in excess of theoretical yields, assuming that only one or two peptides exist. Further efforts are under way to resolve these components so that peptide yields and the extent, if any, of protein fragmentation at the carboxy terminal regions of the three constituent polypeptides detected there can be quantitatively determined.

Amidolytic activity of elastase-modified thrombin. Increasing concentrations of elastase-modified thrombin were added to microtiter plate wells containing GPA (Fig 3). Color generation was proportional to the concentration of enzyme in the range of 13.6 to 270 nmol/L. The amidolytic activity of the modified enzyme appeared to be nearly identical to that of α-thrombin.

Fibrinogen clotting activity of elastase-modified thrombin. The fibrinogen clotting activity of elastase-modified thrombin (>98% conversion to the modified form in the presence of heparin 1 U/mL) was compared with that of α-thrombin and γ-thrombin (Fig 4). At each thrombin concentration, the clotting time of the modified thrombin was greater than that of α-thrombin but less than that of γ-thrombin. As determined by comparing the slopes of the three lines obtained by linear regression analysis (Fig 4 legend), elastase-modified thrombin was 32% as potent as α-thrombin in clotting fibrinogen; γ-thrombin was 1% as potent as α-thrombin. The relative slopes of the three curves were unaltered by the presence or absence of heparin or by the addition of elastase to α-thrombin immediately prior to mixing with fibrinogen (experiments not shown).

Platelet stimulatory activity of elastase-modified thrombin. To compare the platelet-stimulatory activity of elastase-modified thrombin with that of α-thrombin and γ-

Table 1. Sequence of Elastase-Treated Human α-Thrombin

<table>
<thead>
<tr>
<th>Cycle</th>
<th>A-chain</th>
<th>B-chain</th>
<th>Additional Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thr (0.6)</td>
<td>Ile (1.2)</td>
<td>Asn (0.8)</td>
</tr>
<tr>
<td>2</td>
<td>Phe (1.0)</td>
<td>Val (1.9)</td>
<td>Val (1.9)</td>
</tr>
<tr>
<td>3</td>
<td>Gly (1.6)</td>
<td>Glu (0.5)</td>
<td>Gly (1.6)</td>
</tr>
<tr>
<td>4</td>
<td>Ser (NQ)</td>
<td>Gly (0.7)</td>
<td>Lys (0.8)</td>
</tr>
<tr>
<td>5</td>
<td>Gly (1.4)</td>
<td>Ser (NQ)</td>
<td>Gly (1.4)</td>
</tr>
<tr>
<td>6</td>
<td>Glu (0.6)</td>
<td>Asp (0.4)</td>
<td>Gln (0.3)</td>
</tr>
</tbody>
</table>

Human α-thrombin (2.6 nmol) was subjected to sequence analysis. Yields (in parentheses) are corrected to final yield, expressed in nanomoles. The sequence, Additional Peptide, corresponds to residues 151 through 156 of the human B-chain. Thr, threonine; Ile, isoleucine; Asn, asparagine; Phe, phenylalanine; Val, valine; Gly, glycine; Glu, glutamic acid; Ser, serine; Lys, lysine; Gln, glutamine, NQ, not quantitated.

Fig 3. Amidolytic activity of elastase-modified thrombin. α-Thrombin (T) or elastase-modified thrombin (Te) was diluted (0.3 to 270 nmol/L) in buffer and incubated at 23°C with Tos-Gly-Pro-Arg-paranitroanilide (GPA) (0.5 mg/mL). Color development was analyzed as detailed in the Materials and Methods section. Each point is the average of duplicate determinations. Curves were determined by linear regression analysis. For T, y = 39.2x - 38.1 (r = 0.988); for Te, y = 39.7x - 41.4 (r = 0.988).

Fig 4. Fibrinogen clotting activity of elastase-modified thrombin. α-Thrombin (T), elastase-modified thrombin (Te), or γ-thrombin (Ty) was diluted in buffer and prewarmed to 37°C. After fibrinogen was added (2.0 mg/mL), fibrin clotting time was determined as detailed in the Materials and Methods section. Each point represents an individual clotting time measurement. Curves were determined by linear regression analysis. For T, y = 77.7x + 17.02 (r = 0.968); for Ty, y = 245.6x + 8.66 (r = 0.992); and for Tc, y = 6629x + 12.44 (r = 0.941). Study shown is representative of studies on four separate Tc preparations. Equivalent concentrations of T, Ty, and Tc had similar amidolytic activities against Tos-Gly-Pro-Arg-paranitroanilide (GPA).
thrombin, platelet aggregation and secretion were measured simultaneously with a lumigreggomeret. Examples of standard curves of α-thrombin–induced platelet aggregation and ATP-mediated luminescence are shown in Fig 5. The concentration of α-thrombin required to liberate 50% of maximally releasable ATP from platelets was 1.1 nmol/L. This was similar to the concentration required to cause 50% of maximal aggregation. In contrast, the concentration of modified thrombin required to liberate 50% of releasable ATP and to cause 50% of maximal aggregation was ~7.9 nmol/L. As a result, both curves representing aggregation and secretion were shifted to the right. Using the concentration required to produce 50% maximal stimulation for comparison, the modified thrombin was ~14% as potent as α-thrombin in stimulating both platelet aggregation and platelet release. In three additional experiments using three different preparations of the modified thrombin, the modified thrombin was 10% to 19% as potent as α-thrombin.

γ-Thrombin was a less potent platelet stimulus than elastase-modified thrombin (Fig 5 insert). The concentration required to cause 50% of maximum platelet aggregation and release was ~220 nmol/L (0.5% as potent as α-thrombin).

Elastase has been reported to inhibit thrombin-induced platelet aggregation and secretion.9 The final concentration of elastase in the cuvette (~3.5 nmol/L), however, was less than that required to inhibit platelet function in this system.9 In addition, incubation of similar concentrations of chloroacetyle methyle ketone inhibited elastase (95% inhibited by MeO-Suc-Ala-Ala-Pro-Val-CH₂Cl) with α-thrombin (0 to 60 minutes) did not inhibit thrombin-induced platelet aggregation or secretion.

**DISCUSSION**

These studies demonstrate that human α-thrombin, upon proteolysis by PMN elastase, exhibits diminished fibrinogen clotting activity and diminished platelet stimulatory activity, as measured by platelet aggregation and platelet ATP release. Loss of α-thrombin function is associated with limited cleavage of the thrombin B chain.

The concentration of prothrombin in plasma is sufficient to generate ~150 U/mL of α-thrombin.46,47 In addition to being inactivated by circulating inhibitors, α-thrombin selectively binds to fibrin, thereby becoming less susceptible to inactivation by its plasma inhibitors.48-50 These studies suggest that fibrin acts as a reservoir for enzymatically active thrombin. Human PMNs release elastase as blood clots44; in addition, PMNs penetrate preformed blood clots, accumulate within thrombin, and ingest fibrin.51-53 Recently, Weitz and co-workers documented that the fibrinogen peptide Aα1-21 is a specific in vivo indicator of elastase-mediated proteolysis of fibrinogen and higher levels of Aα1-21 were found in patients with α-antiprotease deficiency than in healthy controls.54 In a similar manner, the limited digestion of α-thrombin by elastase could be an important physiologic mechanism for regulation of the activity of both free and fibrin-bound thrombin.

PMN elastase cleaves the thrombin B chain at Ala 150-Asn 151. This site is four amino acids to the NH₂-terminal side of the autoproteolytic or tryptic γ-thrombin cleavage site; the β domain is not cleaved. Proteolysis by PMN elastase occurs in areas rich in apolar amino acids.55-57 In contrast to the positively charged thrombin β cleavage domain, the γ cleavage site (Lys 154-Gly 155) resides within a domain containing several apolar amino acids.56-58 This area should be susceptible to cleavage by elastase.55-57 These considerations are compatible with our findings that the larger NH₂-terminal fragment, Fl (mol wt ~18,000) is disulfide linked to the thrombin A chain; and the smaller COOH-terminal fragment, FII (mol wt ~13,000) contains the active-site serine, as indicated by the recovery of a covalent bond between FII and antithrombin III. These results are supported by the recent report that porcine pancreatic elastase also cleaves a single peptide linkage in prothrombin corresponding to Ala 150-Asn 151 within the α-thrombin B chain, thereby facilitating localization of the staphylocoagulase-binding region to the COOH-terminal fragment.58

PMN elastase binds to glycosaminoglycans of biologic importance, including heparin, heparin sulfate, dermatan sulfate, chondroitin sulfate, and hyaluronic acid.49 Although glycosaminoglycans inhibit the amidolytic activity of elastase against a small oligopeptide substrate,50 heparin stimulates elastase-mediated proteolysis of elastin.59 Thus, our finding that heparin accelerates elastase-mediated proteolysis of α-thrombin is not without precedent. It also suggests that the physiologic interaction of elastase with thrombin might be a surface-mediated phenomenon. Finally, because heparin binds to both thrombin and elastase,43 a template mechanism similar to that for the thrombin–antithrombin III reaction is implied, whereby the interaction of both thrombin and elastase with heparin are involved in the expression of catalytic activity.60-63

Elastase-modified thrombin might be an important probe of the thrombin γ cleavage domain. In marked contrast to...
autolytic or tryptic digestion of α-thrombin, elastase-mediated proteolysis of the γ region does not require proteolysis near the β cleavage site prior to cleavage in the γ region. Cleavage at the β site reduces fibrinogen clotting activity. Therefore, it is not surprising that the elastase-modified enzyme, with an intact β domain, retains some fibrinogen clotting activity. Likewise, we found (Fig 5) that the platelet stimulatory activity of elastase-modified thrombin (14% that of α-thrombin) is greater than that of γ-thrombin (0.5% that of α-thrombin). These findings, confirming the results of others for human γ-thrombin, suggest that thrombin-induced fibrinogen clotting and platelet stimulation are altered by cleavage near the γ cleavage site. In contrast, cleavage near the γ cleavage site does not appear to inhibit inactivation by antithrombin III. The limited digest of α-thrombin by elastase should prove useful in the investigation of the structure and function of human thrombin.

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Human neutrophil elastase alters human alpha-thrombin function: limited proteolysis near the gamma-cleavage site results in decreased fibrinogen clotting and platelet-stimulatory activity

MS Brower, DA Walz, KE Garry and JW 2d Fenton