

# GRANULOCYTE ELASTASE CLEAVES HUMAN HIGH MOLECULAR WEIGHT KININOGEN AND DESTROYS ITS CLOT-PROMOTING ACTIVITY

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When blood clots, elastase is released from granulocytes, which has fibrinolytic properties and which can probably digest fibrin deposits in areas of inflammation where coagulation may occur and granulocytes accumulate (1-4). In addition, this elastase can inactivate coagulation factor IX (5, 6), fibronectin (7)  $\alpha_2$ -antiplasmin and C1-inhibitor (8) by proteolytic cleavage. It is therefore probably capable of modifying hemostatic responses particularly in the presence of inflammation.

High molecular weight kininogen (HMW kininogen)<sup>1</sup> promotes blood coagulation (9-12) and can serve as a source of vasoactive polypeptides that can produce some of the components of the inflammatory response. Plasma from persons with an hereditary deficiency of HMW kininogen is severely deficient in coagulant properties attributable to this protein, and contact-initiated generation of fibrinolytic activity in this plasma is markedly impaired (9-12). Nonetheless, individuals with this hereditary deficiency do not have a hemorrhagic or an identifiable thrombotic disorder. Since bradykinin, a vasoactive peptide that can induce vasodilation, enhance vascular permeability, and cause pain, is part of the HMW kininogen molecule and can be released from the kininogen either by plasma kallikrein or plasmin (13-16), it was important to determine if granulocytes, which accumulate in areas of inflammation, could also release kinin as a result of cleavage of HMW kininogen by granulocyte elastase. The following experiments show that a preparation of granulocyte elastase cleaved purified human HMW kininogen into multiple low molecular weight fragments and destroyed its clot-promoting activity, but did not release kinin from the molecule.

## Materials and Methods

Benzamidine and polyacrylamide were obtained from Eastman Kodak Company, Rochester, NY; Polybrene (hexadimethrine bromide) and trisodium EDTA were obtained from Aldrich Chemical Co., Milwaukee, WI. QAE Sephadex A-50, SP Sephadex C-50, DEAE-Sephadex A-50, and cyanogen bromide-activated Sepharose were all obtained from Pharmacia Fine Chemicals, Piscataway, NJ. DE-23 (diethylaminoethyl cellulose) was obtained

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<sup>1</sup> *Abbreviations used in this paper:* HMW, high molecular weight; NHP, normal human plasma; TBS, Tris buffered saline.

from Whatman, Ltd., Springfield Hill, Kent, UK.  $\alpha_1$ -antitrypsin was obtained from Calbiochem-Boehringer Corp., LaJolla, CA (lots No. L. 101296 and 701049). These preparations contained MIMI  $\alpha_1$ -antitrypsin when examined in an isoelectric focussing technique in PAGE. Soybean trypsin inhibitor, SDS, and molecular weight markers were from Sigma Chemical Co., St. Louis, MO; diethylstilbestrol was from Eli Lilly, Indianapolis, IN; Centrox O phospholipids was a gift from Central Soya, Chicago, Illinois; kaolin came from Fisher Scientific Co., Norwood, Ohio. Bradykinin came from Schwarz-Mann, Orangeburg, NY, and crystalline bovine serum albumin came from Pentex, Kalamazoo, Michigan. All chemical reagents were of analytical grade. Elastase purified from human sputum was from Elastin Products Company, Pacific, MO (lot SES63).

Buffers used in test mixtures are noted in the legends; barbital-saline buffer, pH 7.4, was used in coagulation assays and contained 2.76 gm of barbital, 2.06 gm of sodium barbital, and 7.3 gm of sodium chloride/liter.

HMW kininogen was isolated from human plasma by modifications (16) of previously published procedures (17). This preparation gave a single homogeneous band when analyzed in SDS-PAGE (see Fig. 1), except where noted (see Fig. 4). Human *plasma kallikrein* was isolated from acetone-treated plasma according to the procedure described by Nagase and Barrett (18) and modified in repeating the affinity chromatography of the kallikrein on Sepharose-4B-soybean trypsin inhibitor column after removing IgG by immunoaffinity chromatography. It had a specific activity of 57 coagulation U/mg of protein, measured in an assay testing its ability to shorten the activated partial thromboplastin time (19, 20) of plasma from an individual with a severe inherited deficiency of plasma prekallikrein (21). To measure kallikrein coagulant activity, a 1 min incubation period, instead of 8 min, was carried out before recalcification of test mixtures. Clot-promoting activity of HMW kininogen or its derivatives was measured with a modified activated partial thromboplastin time (19, 20) using a kininogen deficient plasma (12) as substrate. The coagulant property attributable to HMW kininogen or plasma kallikrein was quantified by comparing the shortening of the clotting time of kininogen deficient, or prekallikrein-deficient plasma by the test samples with the shortening achieved by identical dilutions of pooled normal human plasma (NHP), arbitrarily defined to have 1 U of activity per ml of plasma, or of purified untreated HMW kininogen. The comparison was made using a linear double logarithmic plot of the clotting time vs. the amount of normal plasma or kininogen used.

PAGE was performed in a 0.75-mm thin slab vertical Hoeffler apparatus containing 7.5% gel and 0.1% SDS. Samples were incubated at 100°C for 90 s after addition of SDS. Electrophoresis was carried out at pH 8.6 in a Tris (0.025 M) glycine (0.192 M) buffer, according to the method of Laemmli (22) and using gels with a continuous concentration of 7.5% polyacrylamide. Reduced mixtures were prepared by adding 2-ME in an amount to give a final concentration of 2.5% before adding SDS to give a final concentration of 1%.

Kinin activity was measured by applying test samples to an estrus rat uterus suspended in deJalon's solution at pH 7.4 (23) in an 8-ml organ bath maintained at 30°C. Varied amounts of synthetic bradykinin were used as standards for comparison of the amount of kinin activity in test fractions. The comparison was made by measuring the time required for the onset of contraction of the isometric muscle after the injection of the standard or test samples into the organ bath. The times were then plotted against the amount of synthetic bradykinin used on a semilogarithmic plot, and test samples were compared with this linear plot according to the times of onset of contractions induced by each sample.

Protein concentrations were estimated by reading the OD of solutions at 280 nm with a Gilford 240 spectrophotometer (model 240, Gilford Instrument Laboratories, Inc., Oberlin, OH) and comparing the readings to those of a solution of crystalline BSA. All protein preparations were dissolved in 0.05 M Tris, pH 7.4, and 0.15 M NaCl. The same buffer was used in test mixtures during incubation.

## Results

To assess the effect of elastase upon HMW kininogen, 0.02, 0.06, or 0.18  $\mu\text{g}$  of elastase was incubated with 21  $\mu\text{g}$  of purified human HMW kininogen at 37°C. After

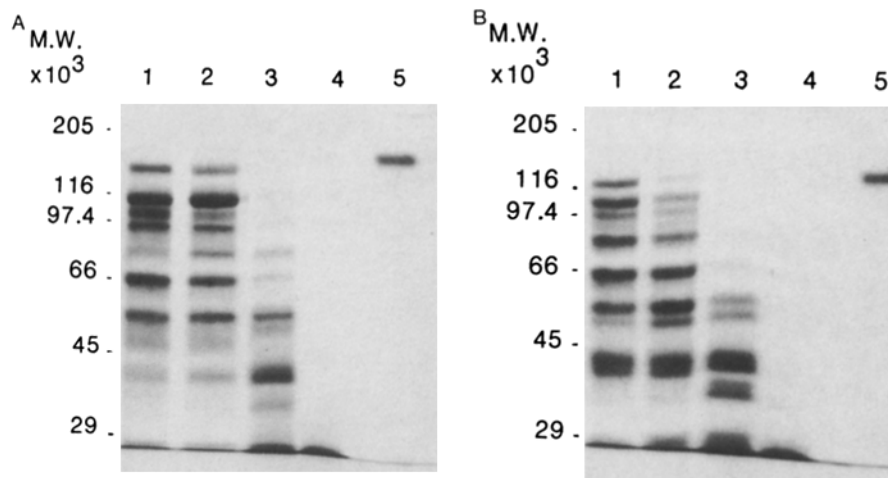


FIGURE 1. (A) SDS-PAGE of HMW kininogen digested by elastase under nonreducing conditions. HMW kininogen, 0.05 ml (420  $\mu\text{g}/\text{ml}$ ), was incubated for 15 min at  $37^\circ\text{C}$  with 0.01 ml of elastase at a concentration of 2  $\mu\text{g}/\text{ml}$  (lane 1), 6  $\mu\text{g}/\text{ml}$  (lane 2), and 18  $\mu\text{g}/\text{ml}$  (lane 3). These mixtures, as well as control samples containing 5  $\mu\text{g}$  of elastase (lane 4) and 0.05 ml of HMW kininogen containing 5  $\mu\text{g}$  of protein (lane 5), were made to 10% in SDS, placed in a  $100^\circ\text{C}$  boiling water bath for 90 s, and then run at 7.5% SDS-PAGE. The positions of the bands of molecular weight standards (not included in this photo) are noted at the left. (B) The same experiment was performed under reducing conditions using 10% in SDS and 2.5%  $\beta$ -ME.

15 min at  $37^\circ\text{C}$ , samples of each incubation mixture were mixed with SDS, heated to  $100^\circ\text{C}$  for 90 s, and then subjected to PAGE. Multiple low molecular weight cleavage fragments evolved during digestion with as little as 0.02  $\mu\text{g}$  of elastase (Fig. 1, lane 1), having molecular weights of from 40,000 to 110,000. In other experiments, after only 2 min of incubation, multiple low molecular weight components of the HMW kininogen were visible after electrophoresis under nonreducing conditions. When examined under reducing conditions, six fragments of HMW kininogen, all having molecular weights of 60,000 and less, were apparent after 40 min of incubation (Fig. 1, and Fig. 2, *top*). Therefore, HMW kininogen was extensively digested by granulocyte elastase.

*Effect of Digestion on Kinin Release.* When HMW kininogen was incubated with granulocyte elastase and samples applied to an isolated estrus rat uterus during the digestion period, there was no contraction of the isolated muscle (Table I, lines 3 and 4). When the digests were then exposed to a preparation of human plasma kallikrein, kinin activity evolved rapidly in an amount equal to that released by the kallikrein from HMW kininogen not previously digested by elastase (Table I, line 5). The absence of kinin activity from these incubation mixtures was not due to the destruction of the kinin by elastase, for the elastase failed to inactivate synthetic bradykinin (Table I, lines 6 and 7).

To determine if elastase digestion might have enhanced the rate of later release of kinin from HMW kininogen by plasma kallikrein, 42  $\mu\text{g}$  of HMW kininogen was incubated with elastase (3  $\mu\text{g}$ ) for 30 min at  $37^\circ\text{C}$ , or with buffer for the same period, and the rates at which kinin activity was then released in each mixture by

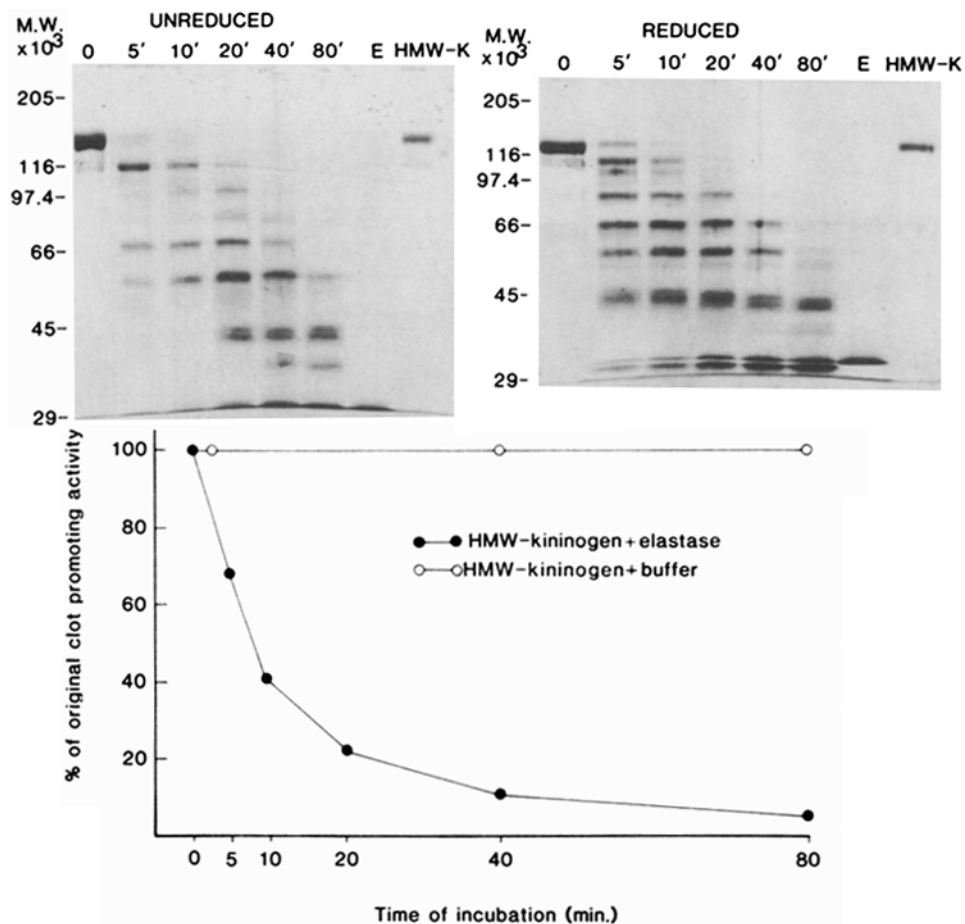


FIGURE 2. The digestion of HMW kininogen by elastase and decay of its clot-promoting activity. HMW kininogen, 0.45 ml (210  $\mu\text{g}/\text{ml}$ ), was mixed with 0.05 ml of elastase (10  $\mu\text{g}/\text{ml}$ ) in TBS. After incubation at 37°C for the times noted, 0.04 ml samples were made to 10% in SDS or 10% in SDS and 2.5% in  $\beta$ -ME and were analyzed on 7.5% SDS-PAGE after 90 s at 100°C (lanes 1-6, respectively). Lane 7 contained a control sample of 5  $\mu\text{g}$  of elastase, and lane 8, 3  $\mu\text{g}$  of control HMW kininogen. For coagulation studies (*lower panel*), 0.01 samples of the same kininogen mixtures were tested at times noted on the abscissa for their ability to correct the activated partial thromboplastin time of kininogen deficient plasma after being diluted 1:20 in barbital saline buffer, pH 7.4. The graph shows the percent of initial HMW kininogen coagulation activity found in the same incubation mixtures in the presence (●) or absence (○) of elastase. The upper panels illustrate SDS-gel electrophoresis of the samples containing elastase carried out at the times of incubation indicated (*top*), under nonreducing (*left panel*) and reducing (*right*) conditions.

a preparation of plasma kallikrein were compared. In this experiment, there was no enhancement, but a minor reduction in the amount of kinin activity released by the kallikrein in the mixture containing elastase-treated HMW kininogen (Fig. 3).

*Effect of Granulocyte Elastase on Clot-promoting Activity of HMW Kininogen.* The domain of the HMW kininogen molecule responsible for its clot-promoting activity is in the light chain and is distinct from the kinin-containing region (24-27). To

TABLE I  
*Elastase Does not Release Kinins from HMW Kininogen and  
 Does not Destroy Bradykinin*

Test mixtures	Kinins released from 1.0 ml of HMW kininogen
	<i>ng</i>
HMW kininogen (60 µg/ml) + buffer	0
HMW kininogen + kallikrein (5 µg/ml)	440
HMW kininogen + elastase (5 µg/ml)	0
HMW kininogen + elastase (50 µg/ml)	0
HMW kininogen + elastase (50 µg/ml) + 25°C × 30 min with kallikrein (5 µg/ml)	440
	Kinins
	<i>ng/ml</i>
Bradykinin (100 ng/ml) + Buffer	100
Bradykinin (100 ng/ml) + Elastase (20 µg/ml)	100

After incubation of the mixtures, shown in the left column, containing 22 µg of HMW kininogen, samples of each mixture were tested for kinin activity on an isolated estrus rat uterus. The enzyme was neutralized with soybean trypsin inhibitor before this assay. In the last experiment of the upper panel, HMW kininogen was first incubated with elastase, and then with kallikrein, to determine if elastase destroyed the bradykinin sequence. The experiment shown in the lower panel tested the effect of elastase on synthetic bradykinin alone. Incubations were carried out in 0.05 M TBS (0.15 M), pH 7.4.

\* Incubated at 25°C for 30 min.

test the effect of granulocyte elastase upon the clot-promoting property of HMW kininogen, elastase in amounts of 0.02–0.64 µg was incubated with each ml of either normal plasma or of purified HMW kininogen (95 µg). Then, the specific clot-promoting activity attributable to HMW kininogen was quantified by measuring the effects of dilutions of these incubation mixtures upon the clotting time of plasma from a person with a severe hereditary deficiency of plasma kininogens (12). The specific HMW kininogen clot-promoting activity of normal plasma was decreased when it had been incubated with 0.08–0.64 µg/ml of elastase (Table II, lines 1–6). When purified HMW kininogen was incubated with as little as 0.03 µg/ml of elastase, its specific clot-promoting activity was destroyed (Table II, lines 7–10). The difference in the sensitivity of the kininogen to elastase digestion in the purified mixture as contrasted to that of plasma probably mainly reflects the inhibition of the elastase by  $\alpha_1$ -antitrypsin in plasma (28); there was no  $\alpha_1$ -antitrypsin in the mixture containing purified HMW kininogen. The rate of destruction of the clot-promoting activity of the HMW kininogen is illustrated in Fig. 2 (*bottom panel*), and the fragmentation of the molecule during this incubation is shown in the SDS gel electrophoretic patterns in the upper panel. It is clear that coagulant activity was readily lost as fragmentation of the molecule occurred.

*Effect of Purified  $\alpha_1$ -Antitrypsin upon Digestion of HMW Kininogen by Elastase.* Since  $\alpha_1$ -antitrypsin is an elastase inhibitor in NHP (28), its effect on the destruction of HMW kininogen clot-promoting activity by elastase was measured. The loss of clot-promoting activity was prevented only when equimolar or greater amounts of  $\alpha_1$ -

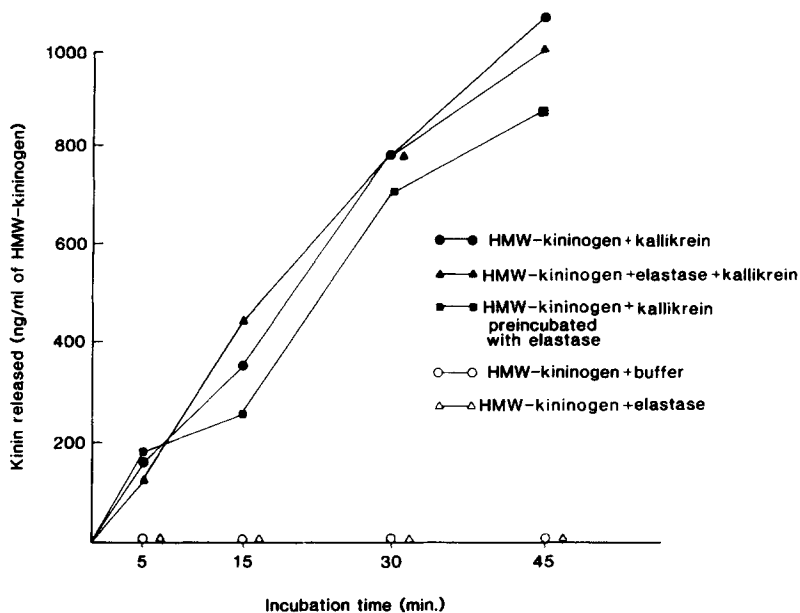


FIGURE 3. The rate of kinin release by kallikrein from HMW kininogen during digestion by elastase. HMW kininogen, 0.2 ml (210  $\mu\text{g}/\text{ml}$ ), was incubated for 10 min at 37°C alone or with 0.05 ml of elastase (60  $\mu\text{g}/\text{ml}$ ). Then, at 0 min, 0.05 ml of kallikrein (1.2  $\mu\text{g}/\text{ml}$ ) (●) or 0.05 ml elastase ( $\Delta$ ) (60  $\mu\text{g}/\text{ml}$ ) or both kallikrein and elastase ( $\blacktriangle$ ) was added to the kininogen which had first been incubated alone, as indicated, and the volumes adjusted to 0.5 ml with buffer. Kallikrein was added to the kininogen already incubated with elastase ( $\blacksquare$ ). During the second incubation at 22°C, samples were tested for the amount of kinin released at the times noted on the abscissa. All reagents were in TBS, pH 7.4. Kinin activity was quantified as described in the Materials and Methods section.

antitrypsin had been incubated with elastase before HMW kininogen was added to the mixture (Table III).

When  $\alpha_1$ -antitrypsin was added to mixtures of purified HMW kininogen and elastase that had already been incubated together for 45 min, the kininogen was extensively digested (Fig. 4 A), indicating that digestion occurred before electrophoresis in SDS. In less than equimolar amounts, the  $\alpha_1$ -antitrypsin failed to prevent digestion of HMW kininogen (Fig. 4 B), or loss of its clot-promoting activity (Table III). Suboptimal amounts of  $\alpha_1$ -antitrypsin partially impaired HMW kininogen digestion by elastase (Fig. 4 B, lanes 4 and 5), and equimolar amounts prevented its cleavage (Fig. 4 B, lanes 6 and 7). The bands of  $\sim 84,000$  molecular weight in lanes 6-9 probably represent complexes between elastase (32,000) and  $\alpha_1$ -antitrypsin (52,000) (Fig. 4 B).

Since we had noted that the clotting times of unincubated test mixtures containing HMW kininogen and  $\alpha_1$ -antitrypsin were often longer than those of mixtures containing buffer in place of inhibitor, the effect of  $\alpha_1$ -antitrypsin upon the clotting of normal plasma was assessed. When  $\alpha_1$ -antitrypsin was added to normal human plasma the clotting time of the plasma was delayed (Table IV). In this experiment, the amount of  $\alpha_1$ -antitrypsin added represented an increase of 30-60% in the levels

TABLE II  
*Effect of Elastase Incubation on HMW Kininogen Clot-promoting Activity*

Test mixtures	Elastase final concentration	Clotting time
	$\mu\text{g/ml}$	<i>s</i>
1. Buffer + NHP	0	85
2. Elastase + NHP	0.64	110
3. Elastase + NHP	0.32	102
4. Elastase + NHP	0.08	88
5. Elastase + NHP	0.04	86
6. Elastase + NHP	0.02	83
7. Buffer + kininogen-deficient plasma	0	> 300
8. Purified HMW kininogen + kininogen-deficient plasma	0	85
9. Purified HMW kininogen + elastase added		
10. after incubation at 37°C for 15 min	0.03	86
11. Purified HMW kininogen + elastase added		
12. before incubation for 15 min at 37°C	0.03	>300

NHP or purified HMW kininogen, 0.03 ml (420  $\mu\text{g/ml}$ ), was mixed either with 0.01 ml of Tris buffered saline, pH 7.4, or 0.01 ml of elastase at a concentration of 16  $\mu\text{g/ml}$ . After a 15-min incubation at 37°C, mixtures were mixed with 1.2 ml of the barbital saline at 0°C and immediately tested for specific clot-promoting activity attributable to HMW kininogen (lines 1-8) by measuring the effect of each mixture on the clotting time of plasma from an individual with an inherited deficiency of kininogens (see Materials and Methods). In the experiments shown in lines 9-10, elastase was added to purified HMW kininogen at 0°C after incubation of the kininogen, and the mixture was immediately tested for coagulant activity. In the experiment, shown in lines 11 and 12, the HMW kininogen and elastase were incubated together for 15 min at 37°C before the mixture was tested for coagulant activity.

of plasma  $\alpha_1$ -antitrypsin, assuming that the concentration in normal plasma is between 1.5 and 3.5 mg/ml (29).

### Discussion

Although purified HMW kininogen was cleaved into multiple low molecular weight fragments by purified human granulocyte elastase (Fig. 1, *A* and *B*), kinin activity was not released in the process, nor was domain of the kininogen molecule containing the kinin sequence destroyed (Table I). Moreover, the cleavage products, which were mainly of molecular weights of 60,000 and less, still contained the kinin sequence that could be released when these fragments were incubated with plasma kallikrein. These experiments do not determine if the kinin activity was due to the nonapeptide, bradykinin, or to one of the larger peptides that may be released by proteases and have kinin activity. While one cannot directly extrapolate from these results to an *in vivo* situation, these events could conceivably provide low molecular weight forms of kininogen as a source of kinin in areas of inflammation.

It is possible that elastase might regulate the generation of clot-promoting activity in part through its destruction of the clot-promoting properties of HMW kininogen (Table II, Fig. 2). Since the structures required for clot-promoting activity of HMW kininogen reside in the light chain of the molecule (24) and factors XI or prekallikrein or kallikrein can form complexes with the light chain (25), these critical regions may be digested by elastase. The histidine-rich region of the light chain, which is apparently responsible for binding of HMW kininogen to kaolin

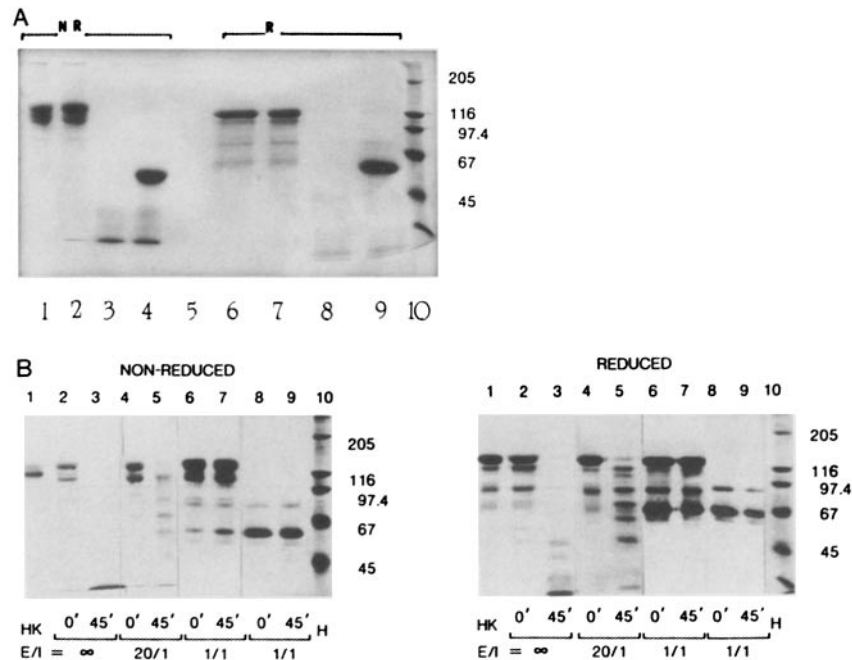


FIGURE 4. (A) Elastase (0.5  $\mu\text{g}$ ) was incubated with HMW kininogen for 0 min (lanes 2 and 7) and 45 min (lanes 3 and 8) and an equimolar amount (1.0  $\mu\text{g}$ ) of  $\alpha_1$ -antitrypsin was added at the conclusion of the incubation period, before SDS was added (lanes 4 and 9). HMW kininogen incubated with buffer is in lanes 1 and 6; lane 5 is blank. Electrophoretic analysis was done under nonreducing (NR) (lanes 1-4) and reducing (R) (lanes 6-9) conditions. A high molecular weight standard protein mixture is in lane 10. The elastase was added to 0-min mixtures after SDS and 2-ME (lanes 2 and 7). (B) High molecular weight kininogen (HK) was incubated in buffer for 45 min (lane 1), with 0.5  $\mu\text{g}$  of elastase (E/I =  $\infty$ , lanes 2 and 3), or with elastase that had already been incubated with  $\alpha_1$ -antitrypsin at 37°C for 10 min (lanes 4-7). The molar ratios of the enzyme to inhibitor in the incubation mixtures was 20:1 (lanes 4 and 5) or 1:1 (lanes 6 and 7), and no inhibitor was present in the mixture shown in lanes 2 and 3. The mixtures in lanes 8 and 9 contained equimolar amounts of enzyme and inhibitor, but no high molecular weight kininogen. H represents high molecular weight standard marker proteins that had been reduced in each instance. PAGE was run under nonreducing (left) and reducing (right) conditions.  $M_r \times 10^{-3}$  are shown on the right.

(26, 27), may have been digested, for kaolin was used in the assay to measure HMW kininogen coagulant activity. These studies do not define the points of elastase cleavage. It is possible that all of these regions have been cleaved.

When blood clots, the elastase concentration in the fluid phase is increased >10-fold (1), presumably reflecting the release of this enzyme from granulocytes either because of a secretory or a lytic event (30). Once this has occurred, the elastase in plasma is readily inhibited by  $\alpha_1$ -antitrypsin (28, 31, 33) and can also interact with  $\alpha_2$ -macroglobulin (33). In the present studies, the blockade of digestion of the kininogen with purified  $\alpha_1$ -antitrypsin supports the likelihood that the effective enzymatic activity is elastase. In subjects deficient in  $\alpha_1$ -antitrypsin, the pulmonary and hepatic pathologic changes that occur as a result of the deficiency may be largely due to elastase activity (31, 32). The ability of elastase to inactivate coagulation factor



TABLE III  
*Effect of  $\alpha_1$ -Antitrypsin ( $\alpha_1$ AT) upon Elastase-induced Destruction  
of Clot-promoting Activity of HMW Kininogen*

Incubation mixtures*	Specific HMW kininogen clotting times
	s
Buffer + HMW kininogen	85
Elastase, (0.64 $\mu$ g) + HMW kininogen	>300
$\alpha_1$ AT (1.6 $\mu$ g) + Elastase (0.64 $\mu$ g) + HMW kininogen	85
$\alpha_1$ AT (0.8 $\mu$ g) + Elastase (0.64 $\mu$ g) + HMW kininogen	120
$\alpha_1$ AT (0.4 $\mu$ g) + Elastase (0.64 $\mu$ g) + HMW kininogen	250
$\alpha_1$ AT (0.2 $\mu$ g) + Elastase (0.64 $\mu$ g) + HMW kininogen	>300
$\alpha_1$ AT (1.6 $\mu$ g) + Buffer	86

HMW kininogen, 12.6  $\mu$ g in 0.03 ml, was added to the mixtures containing 0.01 ml of elastase and/or  $\alpha_1$ AT (*left column*) that had been incubated together at 37° for 5 min and incubation continued for another 15 min. Samples from each mixture were then diluted four-fold in cold barbital saline buffer and tested for clot-promoting activity attributable to HMW kininogen by the procedure described in Materials and Methods.

\* 37°C for 5 min.

IX (5, 6) and HMW kininogen clot-promoting activity raises the possibility that it could regulate the generation of clot-promoting activity *in vivo* that might be initiated by the activation of Hageman factor, by altering interactions promoting coagulation directly through the "intrinsic" coagulation pathway and possibly by affecting mechanisms by which factor VII activity is markedly enhanced by factors IXa and Xa *in vitro* (34-37). "Elastase-like" proteases from granulocytes may inactivate coagulation factors VIII, XII, V, and XIII (38), but it is not clear that all of these factors were inactivated by elastase in these preparations.

The prolongation of the clotting time of normal plasma, which was observed when this  $\alpha_1$ -antitrypsin was added to the plasma (Table IV), may reflect its inhibition of factor XIa, which was reported earlier (39). It is important to note that the  $\alpha_1$ -antitrypsin used in these experiments was in type M1M1 upon analysis by an isoelectric-focussing technique because Z type  $\alpha_1$ -antitrypsin has a lower association constant for leukocyte elastase than the M1M1 forms of the inhibitor (40).

TABLE IV  
*Effect of  $\alpha_1$ -Antitrypsin upon the Clotting Time of Normal Plasma*

Test mixture	Clotting time
	s
Normal plasma + buffer	96
Normal plasma + $\alpha_1$ -AT, 25 $\mu$ g	111
Normal plasma + $\alpha_1$ -AT, 50 $\mu$ g	119

Normal plasma was incubated with an equal volume of either barbital saline buffer or of a solution of  $\alpha_1$ -antitrypsin containing either 25 or 50  $\mu$ g of this inhibitor, plus phospholipid and kaolin at 37°C for 8 min. Calcium chloride was then added and the clotting times (21, 22) of the mixtures were recorded, each of which represents the mean of duplicate mixtures.

Granulocyte elastase is a "neutral" serine protease and a glycoprotein having a molecular weight of  $\sim 30,000$  (41). Although it has a pH optimum of 8.5 against most of its substrates (41), in the present studies, proteolysis was readily achieved at pH 7.4. Therefore, granulocyte elastase could function under physiologic conditions of pH *in vivo*.

The fibrinolytic potential of granulocytes has been known since the beginning of this century (42, 43). The fibrinolytic property of granulocyte elastase may be one of its most significant contributions to reactions that may occur in the body during injury or inflammation. Elastase can inactivate  $\alpha_2$ -antiplasmin (8). The release of leukocyte elastase leading to fibrinolysis occurs in a calcium dependent reaction (1), and the enzyme can then release a fibrinogen fragment D cleavage product, which has been shown to be immunologically distinct from plasmin-generated fragment D (30). Therefore, elastase proteolytic activity is distinct from that of plasmin. Moreover, elastase can inactivate C1-inhibitor (8), which can regulate the first component of complement (44) and is one of the plasma inhibitors that can impair the actions of plasmin (45), coagulation factors XIIa, XIa, and kallikrein (45-47). Elastase can also alter the function of  $\alpha$ -thrombin (48), and may therefore add a critical dimension to altered hemostasis and effective fibrinolytic activity which may evolve in areas of inflammation involving both elastase and plasmin. It has been reported that the release of the granulocyte elastase can occur when granulocytes are aggregated by plasma kallikrein (49), since the release of elastase from granulocytes in prekallikrein-deficient plasma was impaired (49). When prekallikrein-deficient plasma was diluted, however, release of elastase from granulocytes in this plasma was similar to its release in granulocytes suspended in normal plasma (30). Therefore, substances other than prekallikrein must be important in this process. Thrombin does not directly release elastase from granulocytes (1), but events involving the Hageman factor dependent generation of clot-promoting activity are probably important in its release. The physiologic mechanisms of elastase generated fibrinolysis are not yet clearly defined.

### Summary

Purified human granulocyte elastase cleaved purified human high molecular weight (HMW) kininogen into multiple low molecular weight fragments, and destroyed the clot-promoting activity of the HMW kininogen. Elastase digestion did not release kinin or destroy the bradykinin portion of the HMW kininogen molecule; kallikrein could release kinin from the elastase-induced low molecular weight digestion products of HMW kininogen. Purified  $\alpha_1$ -antitrypsin prevented the destruction of the clot-promoting activity of HMW kininogen by elastase; it also delayed the clotting of normal plasma. Elastase may play a significant role in altered hemostasis as well as fibrinolysis, in areas of inflammation to which polymorphonuclear leukocytes have been attracted.

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