An Elastase-Dependent Pathway of Plasminogen Activation†

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ABSTRACT: In reaction mixtures containing Glu-plasminogen, α_2-antiplasmin, and tissue plasminogen activator or urokinase, either pancreatic or leukocyte elastase enhances the rate of plasminogen activation by 2 or more orders of magnitude. This effect is the consequence of several reactions. (a) In concentrations on the order of 100 nM, elastase degrades plasminogen within 10 min to yield des-kringle$_{1-4}$-plasminogen (mini-plasminogen), which is 10-fold more efficient than Glu-plasminogen as a substrate for plasminogen activators. Des-kringle$_{1-4}$-plasminogen is insensitive to cofactor activities of fibrin(ogen) fragments or an endothelial cell cofactor. (b) Des-kringle$_{1-4}$-plasmin is one-tenth as sensitive as plasmin to inhibition by α_2-antiplasmin: $k'' = 10^6$ M^{-1} s^{-1} versus 10^7 M^{-1} s^{-1}. (c) α_2-Antiplasmin is disabled efficiently by elastase, with a k'' of 20 000 M^{-1} s^{-1}. The elastase-dependent reactions are not influenced by 6-aminohexanoate. In diluted (10-fold) blood plasma, the capacity of endogenous inhibitors to block plasmin expression is suppressed by 30 μM elastase. It is proposed that elastases provide an alternative pathway for Glu-plasminogen activation and a mechanism for controlling initiation of fibrinolysis by urokinase-type plasminogen activators.

Plasminogen is activated endogenously by urokinase-type plasminogen activators or by tissue-type plasminogen activators (tPA).[1] Each reaction is characterized by hydrolysis of one bond in the plasminogen molecule to yield the two-chain enzyme plasmin. Plasmin, in turn, can influence its own formation by the conversion of Glu-plasminogen to Lys-plasminogen, by activation of single-chain pro-urokinase to fully active two-chain urokinase, and by converting single-chain tPA into two-chain tPA [for a review, see Bachmann (1987)].

The activation of plasminogen by tPA is regulated by the plasmin substrate fibrin (Astrup, 1956). As cofactors, fibrin or fibrin(ogen) fragments provide sites for condensation of plasminogen and tPA into a ternary complex in which the rate of plasminogen activation is enhanced (Wiman & Collen, 1978). Both plasminogen and tPA have kringle domains that bind lysine or 6-aminohexanoate and which serve as recognition sites for fibrin (Hoylaerts et al., 1982; Ranby, 1982). Cofactor activities also have been associated with denatured proteins (Radcliffe & Heinze, 1981), polyamines (Allen, 1982), and an endothelial cell component (Hajjar & Nachman, 1988; Machovich & Owen, 1988). Inhibition of each of these cofactor activities with alkylamines suggests a common mechanism of action. On the other hand, regulation of the activation of plasminogen by urokinase has not been recognized, aside from an effect of fibrin on activation of pro-urokinase.

Properties of the products that arise in elastase digests of plasminogen and plasmin (Clemmensen et al., 1986; Sugiyama et al., 1987; Takada et al., 1988) provide a basis for elastase to regulate plasminogen activation by urokinase as well as by tPA. During conversion of Glu-plasminogen to mini-plasminogen, kringles$_{1-4}$ are removed from the parent molecule with elastases to yield des-kringle$_{1-4}$-plasminogen (Sottrup-Jensen et al., 1978; Moroz, 1981; Schaller et al., 1987), which is more sensitive than Glu-plasminogen to activation. Moreover, elastase disables the inhibitor system of enzymes of plasminogen activation, at least the activity of α_2-antiplasmin (Brower & Harpel, 1982; Shieh & Travis, 1987) and plasminogen activator inhibitor 1 (Levin & Santell, 1987). These attributes would predict plasminogen activation to be regulated by elastase; this prediction, however, rests on the sensitivity of each individual protein of the system to hydrolysis by elastase, which has not been determined quantitatively. In the present work, we have investigated the system quantitatively and find that porcine plasminogen activation by urokinase or tPA is accelerated by catalytic concentrations of pancreatic or leukocyte elastase and, when α_2-antiplasmin is included, becomes elastase dependent.

Experimental Procedures

Elastase (porcine pancreas), Pronase, lysine–Sepharose, and DFP were obtained from Sigma Chemical Co., St. Louis, MO. Human leukocyte elastase, with a specific activity of 18–20 units/mg, was a gift of Dr. J. Travis (Baugh & Travis, 1976). Tissue plasminogen activator (human melanoma), fibrin(ogen) fragments (Verheijen et al., 1982), Spectrozyme PL (H-D-norleucyl-hexahydro-tyrosyl-lysine-*p*-nitroanilide), and Spectrozyme tPA (CH_3SO_2-D-CHT-Gly-Arg-*p*-nitroanilide) were obtained from American Diagnostica, Hartford, CT. α_2-Antiplasmin and urokinase were purchased from Calbiochem-Behring and Abbott, respectively. Porcine plasminogen was prepared by lysine–agarose affinity chromatography (Summaria et al., 1976). Des-kringle$_{1-4}$-plasminogen was obtained from limit digests of Glu-plasminogen: plasminogen (500 μg/mL) was incubated with elastase (20 μg/mL) at 37 °C for 2 h. Reactions were terminated with DFP and dialyzed. Kringle domains$_{1-4}$ were removed by chromatography on lysine–agarose. A partially purified endothelial cell tPA cofactor

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[1] Abbreviations: tPA, tissue plasminogen activator; PA, plasminogen activator; DFP, diisopropyl fluorophosphate; DIP, diisopropylphospho.

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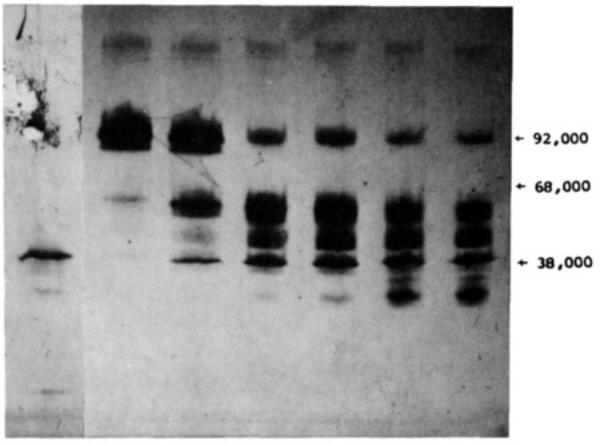

FIGURE 1: Digestion of porcine Glu-plasminogen with elastase. Porcine Glu-plasminogen (152 μg/mL) was incubated with elastase (20 μg/mL) in 0.1 M Tris-HCl–0.15 M NaCl, pH 7.4, at 22 °C. At intervals, samples were removed, added to one-tenth volume of 10% $NaDodSO_4$, heated at 90 °C for 3 min, and analyzed by gel electrophoresis. Lane 1, isolated des-kringle$_{1-4}$-plasminogen; lane 2, Glu-plasminogen; lanes 3–7, Glu-plasminogen incubated with elastase for 2, 10, 20, 40, and 80 min, respectively.

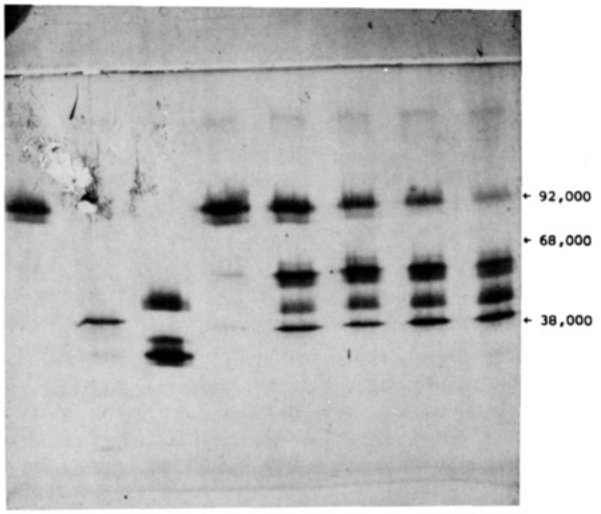

FIGURE 2: Digestion of porcine Glu-plasminogen with Pronase. Porcine Glu-plasminogen (152 μg/mL) was incubated with Pronase (2 μg/mL) in 0.1 M Tris-HCl–0.15 M NaCl, pH 7.4, at 22 °C. At intervals, samples were treated with 10 mM DFP, added to one-tenth volume of 10% $NaDodSO_4$, heated at 90 °C for 3 min, and then analyzed by gel electrophoresis. Lane 1, Glu-plasminogen; lane 2, isolated des-kringle$_{1-4}$-plasminogen; lane 3, elastase digest of Glu-plasminogen bound to lysine–Sepharose (digested kringle structures); lanes 4–8, Glu-plasminogen incubated with Pronase for 2, 10, 20, 40, and 80 min, respectively.

was prepared as described previously (Machovich & Owen, 1988).

Polyacrylamide gel electrophoresis in $NaDodSO_4$ was carried out with a Pharmacia Phast system. Amino acid sequence analysis was performed with an Applied Biosystems gas-phase sequencer.

Plasminogen activation reactions with tPA or urokinase were carried out at 22 °C in 0.1 M Tris-HCl 0.15 M NaCl, pH 7.4, or in 50 mM sodium phosphate, pH 7.4, respectively. When elastase was present in the activation reaction, plasminogen was incubated with this enzyme for 10 min before the addition of the activator. For assay of plasmin activity, samples (5–10 μL) of activation mixtures were diluted into 300 μL of 0.2 mM peptide substrate for plasmin (Spectrozyme PL) in 0.1 M Tris-HCl–0.15 M NaCl, pH 7.4, at 30 °C; the change in A_{405} was measured with an Abbott ABA-100 bichromatic analyzer.

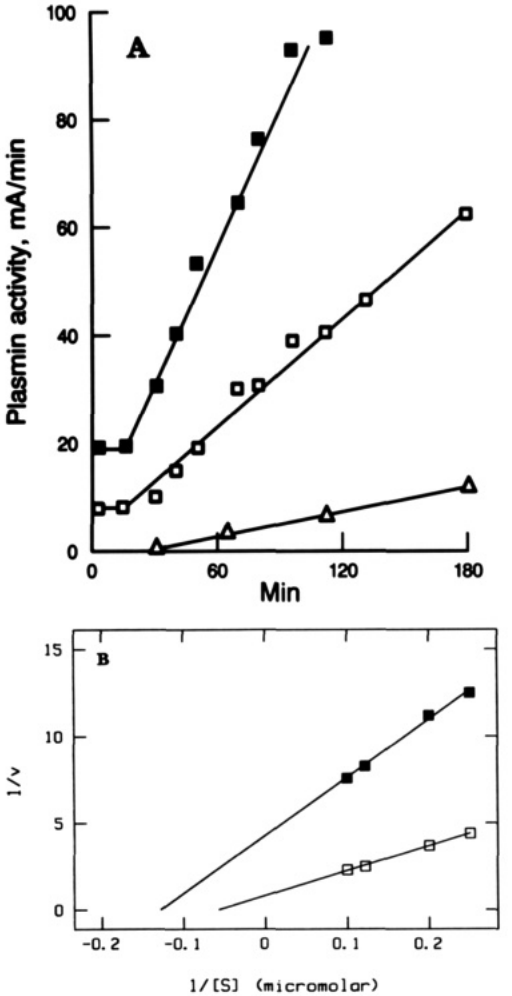

FIGURE 3: Plasminogen activator activity of Pronase. Porcine Glu-plasminogen (160 μg/mL) was incubated with Pronase [2.4 μg/mL (■); 1.4 μg/mL (□)] or tPA [2.8 μg/mL (△)] in 0.1 M Tris-HCl–0.15 M NaCl, pH 7.5 at 22 °C. At intervals, samples were assayed for plasmin activity. (B) Lineweaver–Burk plot of plasminogen activation by Pronase [1.25 μg/mL (■); 2.5 μg/mL (□)]; data were obtained from 40-min incubations and concentrations of porcine Glu-plasminogen between 2.5 and 10 μM.

RESULTS

Degradation. Proteolysis of plasminogen with elastase is illustrated by Figure 1. Virtually complete proteolysis was attained within 10 min with 20 μg/mL pancreatic elastase, yielding three primary stable products having M_r 30 000–50 000. The observed second-order rate constant of plasminogen hydrolysis by human leukocyte elastase was calculated from densitometry to be 6000 M^{-1} s^{-1}. Our sequence analysis confirmed the finding of Schaller et al. (1987) that des-kringle$_{1-4}$-plasminogen purified from the digest (Figure 1, land 2) arose from hydrolysis of the A449–Q450 peptide bond.

Des-K$_{1-4}$-plasminogen resists additional proteolytic degradation. While the kringle domains of plasminogen are digested rapidly, des-kringle$_{1-4}$-plasminogen undergoes no additional degradation either with elastase or with Pronase as well (Figures 1 and 2). When isolated des-kringle$_{1-4}$-plasminogen was further incubated with 2 μg/mL Pronase for 4 h, additional degradation was not seen on SDS–PAGE (not shown), although Pronase was able to activate plasminogen (Figure 3); with Pronase as activator, a K_m of 9 μM was determined for porcine Glu-plasminogen. The kinetic data, as well as sequence analysis of isolated des-kringle$_{1-4}$-plasmin(ogen), formed by Pronase action (Figure 4) indicate that Pronase acts near the elastase-sensitive region of porcine plasminogen as

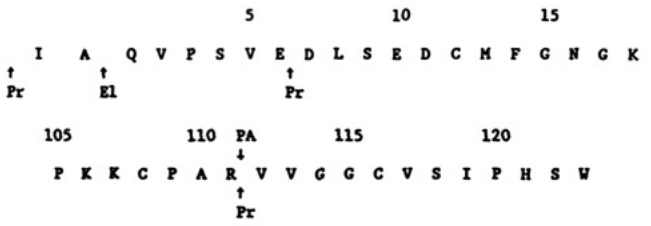

FIGURE 4: Sequence analysis of des-kringle$_{1-4}$-plasminogen. Porcine Glu-plasminogen (4 mg/mL) was treated with Pronase (5 μg/mL) at 22 °C for 10 min. Enzyme activities were arrested with 1 mM DFP, and the reaction mixture was applied to a column of lysine–agarose (30 × 1.5 cm column) developed with 0.1 M Tris-HCl–0.15 M NaCl, pH 7.4. The unbound fraction (294 μg/mL protein) was further treated with Pronase at 22 °C for 180 min, stopped with 10% acetic acid, and subjected to N-terminal analysis. Arrows indicate the bonds cleaved by porcine pancreatic elastase (El), Pronase (Pr), and urokinase type- or tissue-type plasminogen activator (PA). Numbers correspond to des-K$_{1-4}$-plasminogen obtained with elastase.

Table I: Action of Some Proteases on Porcine Glu-Plasminogen

protease[a]	product: des-kringle$_{1-4}$-plasminogen	product: plasmin
plasmin (porcine)	–	–
tPA (porcine, human)	–	+
urokinase (human)	–	+
trypsin (bovine)	–	+
chymotrypsin (bovine)	–	–
thrombin/thrombomodulin (porcine)	–	–
factor X$_a$ (porcine)	–	–
activated protein C (porcine)	–	–
cathepsin C, type X (bovine spleen)	–	–
collagenase, type VII (*Clostridium histolyticum*)	–	–
elastase (porcine pancreas)	+	–
elastase (human neutrophil)	+	–
Pronase (*S. griseus*)	+	+
streptokinase (*Streptococcus*)[b]	–	–

[a] Proteases were assayed in concentrations between 0.1 and 1 μM, and the longest incubation periods were 3 h at 22 °C. Des-kringle$_{1-4}$-plasminogen formation was determined by SDS gel electrophoresis; plasmin activity was assayed on peptide substrate. [b] Not a protease.

well as on the tPA–urokinase-sensitive bond of the des-kringle$_{1-4}$-plasminogen and plasminogen. The capacities of other proteases to catalyze des-kringle$_{1-4}$-plasminogen formation or activate plasminogen are summarized in Table I.

Neither the activity nor the molecular weight of tPA was altered by treatment with elastase, whereas Pronase degraded tPA (Figure 5).

Activation. Added to reaction mixtures containing plasminogen and tPA, elastase enhances the rate of porcine plasminogen activation in a manner mimetic of a cofactor for tPA (Figure 6). Two features that distinguish the kinetics with elastase are pronounced lag phase and persistence of the enhanced activation rate (Figure 6A). That the pseudocofactor activity of elastase is accounted for by an enhanced sensitivity of des-kringle$_{1-4}$-plasminogen to activation with tPA is illustrated in Figure 6B. Limit elastase digests of plasminogen are activated by tPA at a rate approaching the maximum rate shown in Figure 6A but do not exhibit sensitivity to either fibrin(ogen) fragments or endothelial cofactor. Adsorption of the preparation with lysine–agarose to remove the kringle domains had no significant effect on the rate of plasminogen activation with or without cofactors (Figure 6C).

The kinetic constants determined in this work for hydrolysis of the peptide nitroanilide substrate by porcine plasmin and des-kringle$_{1-4}$-plasmin are similar to each other (K_m, 50 μM; turnover number (k_3), 100 s^{-1}), as are their human counterparts (Christensen et al., 1979). While streptokinase did activate human plasminogen, it failed to convert either porcine Glu-plasminogen or des-kringle$_{1-4}$-plasminogen to active complexes either in the absence or in the presence of 10 mM 6-aminohexanoate (data not shown).

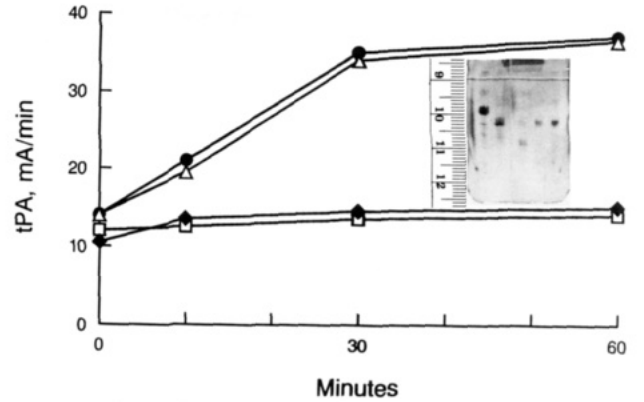

FIGURE 5: Conversion by plasmin of single-chain tPA to two-chain tPA in the presence of elastase. Tissue plasminogen activator (5.4 μg/mL) was incubated with plasmin (4.5 μg/mL) and elastase (9 μg/mL) in 0.1 M Tris-HCl–0.15 M NaCl, pH 7.4 at 22 °C, for 60 min. At intervals, samples were assayed for tPA activity with 0.2 mM Spectrozyme tPA substrate. tPA alone (♦); tPA plus elastase (□); tPA plus plasmin (△); tPA plus plasmin plus elastase (●). Inset: tPA (225 μg/mL) was incubated with elastase (2.5 μg/mL) or Pronase (2.5 μg/mL) in 0.1 M Tris-HCl–0.15 M NaCl, pH 7.4 at 22 °C, for 120 min, thereafter added to one-tenth volume of 10% NaDodSO$_4$, heated at 90 °C for 3 min, and analyzed by gel electrophoresis. Lane 1, porcine Glu-plasminogen; lane 2, bovine serum albumin; lane 3, tPA plus Pronase; lane 4, tPA plus elastase; lane 5, tPA.

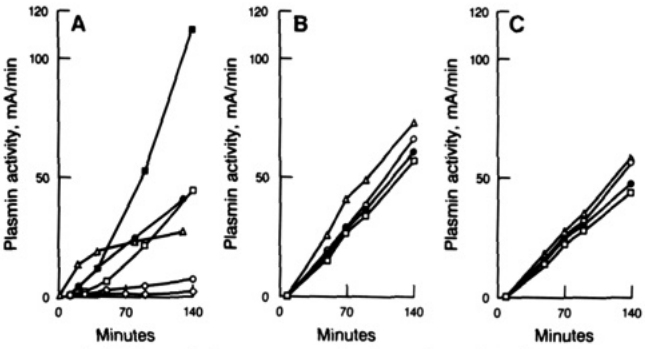

FIGURE 6: Effect of elastase on activation of porcine plasminogens by tPA. Porcine plasminogen (A), a limit elastase digest of plasminogen (B), and a limit elastase digest of plasminogen separated from kringle structures (C) were incubated with tPA (2.8 μg/mL) at 22 °C in 100 mM Tris-HCl–150 mM NaCl, pH 7.4, with the following additives: none (○); 20 μg/mL fibrin(ogen) fragments (△); 20 μg/mL endothelial cofactor (●); 2 μg/mL elastase (□); or 20 μg/mL elastase (■). As a control, plasminogen was incubated with 20 μg/mL elastase without tPA (◇). Starting plasminogen concentration in all cases was 250 μg/mL.

Inhibition. The second-order rate constant observed for inhibition of porcine des-kringle$_{1-4}$-plasmin by α_2-antiplasmin (10^6 M^{-1} s^{-1}) was one-tenth of that observed for inhibition of plasmin (Figure 7). Elastase digests of plasminogen activation mixtures and isolated des-kringle$_{1-4}$-plasmin yielded the same rate constants. Moreover, the antiplasmin activity of the α_2-antiplasmin preparation was inactivated rapidly, with a second-order rate constant of approximately 20 000 M^{-1} s^{-1} (Figure 8).

Reconstitution. As illustrated above, each effect of elastase on isolated components of the plasminogen activation system serves individually to enhance the net rate of expression of plasmin activity. The effect of elastase in a reconstituted system comprising tPA, plasminogen, and α_2-antiplasmin is

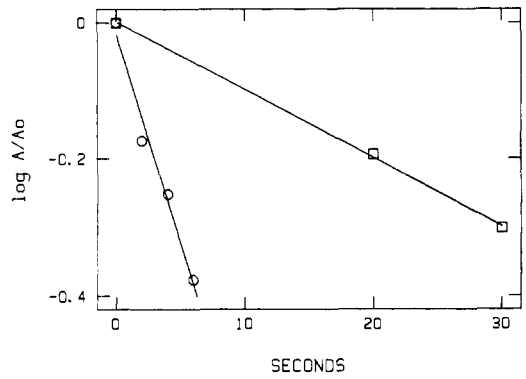

FIGURE 7: Inhibition of plasmin and des-kringle$_{1-4}$-plasmin by α_2-antiplasmin. Plasmin (○) or des-kringle$_{1-4}$-plasmin (□) was reacted with a 5-fold excess (50 nM) of α_2-antiplasmin (dissolved in 0.1 M NaCl, 1 mg/mL bovine albumin, and 0.02 M Tris-HCl, pH 8.1) for the times indicated. Then, one-seventh volume of a solution containing substrate (Spectrozyme PL, 700 μM) and 6-aminohexanoate (70 mM) was added to assay residual plasmin activity, which is expressed as the fraction of initial activity.

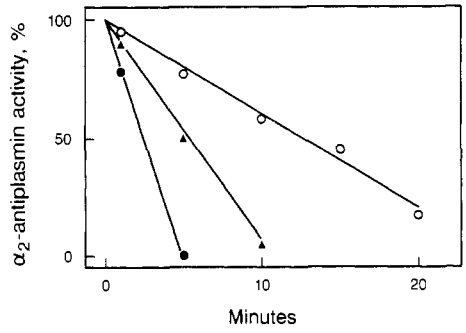

FIGURE 8: Rate of inactivation of α_2-antiplasmin by pancreatic elastase. α_2-Antiplasmin (30 μg/mL) was incubated with porcine pancreatic elastase in 50 mM sodium phosphate, pH 7.4, at 22 °C for the times indicated. Then, one-fourth volume of plasmin solution (180 μg/mL) was added and further incubated for 1 min. Thereafter, remaining plasmin activity was determined with 0.2 mM Spectrozyme PL. Elastase added: 1.25 μg/mL (○); 2.5 μg/mL (▲); 5 μg/mL (●).

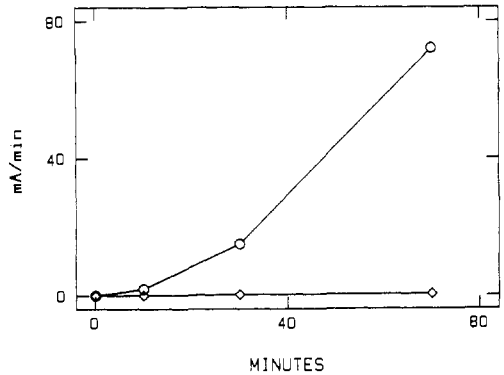

FIGURE 9: Activation of plasminogen by tPA in the presence of α_2-antiplasmin: Effect of elastase. Glu-plasminogen (200 μg/mL) was incubated (22 °C, 20 mM sodium phosphate–60 mM Tris-HCl–100 mM NaCl, pH 7.4) with tPA (2.7 μg/mL) and α_2-antiplasmin (90 μg/mL) in the absence (◇) or presence (○) of elastase (18 μg/mL).

shown in Figure 9. With the rapid-acting antiplasmin present at physiological concentrations, even the very high tPA concentration used here yields no detectable plasmin in 70 min. Elastase, however, abolishes the capacity of the antiplasmin to moderate plasminogen activation; the net rate is approximately that shown in Figure 6A with elastase.

Similar results were obtained when plasminogens were activated by urokinase (Figure 10). The rate of activation was dose dependent for pancreatic elastase in concentrations from

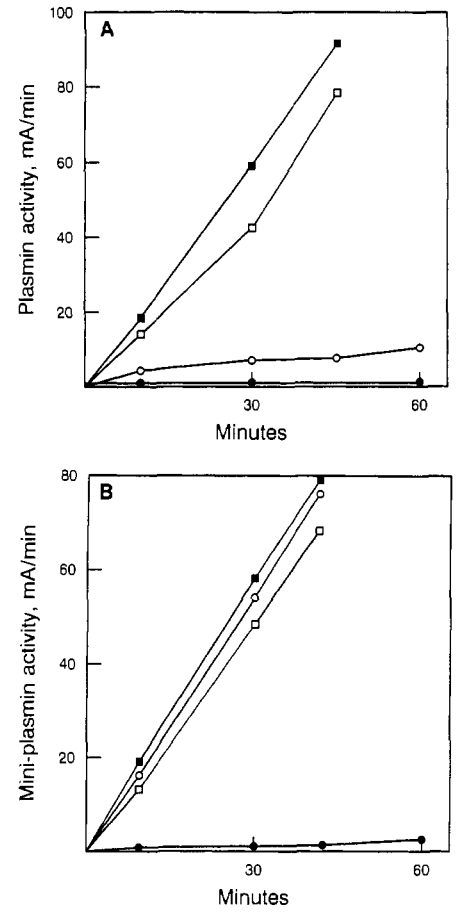

FIGURE 10: Effect of pancreatic elastase on activation of Glu-plasminogen (A) and des-kringle$_{1-4}$-plasminogen (B) by urokinase. Glu-plasminogen (95 μg/mL) or des-kringle$_{1-4}$-plasminogen (45 μg/mL) was incubated with urokinase (1.25 μg/mL) at 22 °C in 50 mM sodium phosphate buffer, pH 7.4, with the following additives: none (○); 222 μg/mL α_2-antiplasmin (●); 12.5 μg/mL elastase (□); 12.5 μg/mL elastase plus 222 μg/mL α_2-antiplasmin (■).

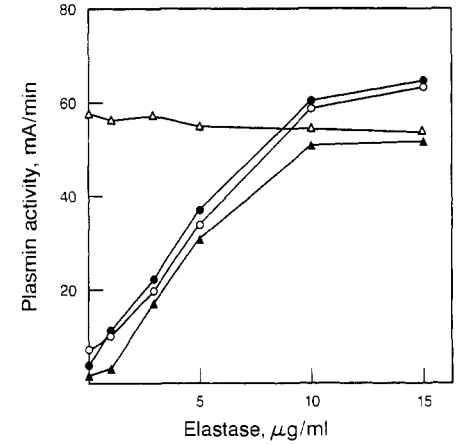

FIGURE 11: Activation of plasminogens by urokinase: Elastase concentration dependence. Porcine Glu-plasminogen (90 μg/mL) or des-kringle$_{1-4}$-plasminogen (35 μg/mL) was incubated with urokinase (1.25 μg/mL) in the presence of α_2-antiplasmin (111 μg/mL) in 50 mM sodium phosphate buffer, pH 7.4. After 30 min, at 22 °C, samples were assayed for plasmin activity. Glu-plasminogen with urokinase (○) and with urokinase plus α_2-antiplasmin (●); des-kringle$_{1-4}$-plasminogen with urokinase (△) and with urokinase plus α_2-antiplasmin (▲).

2 μg/mL to a maximum at 20 μg/mL (Figure 11). The effect of human leukocyte elastase in the reconstituted activation system was essentially the same as that of pancreatic elastase (Figure 12).

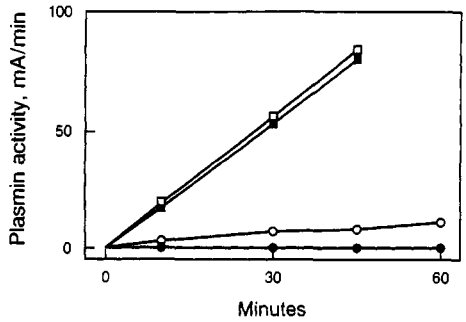

FIGURE 12: Effect of human leukocyte elastase on porcine Glu-plasminogen activation by urokinase in the presence of α_2-antiplasmin. Glu-plasminogen (70 μg/mL) was incubated with urokinase (1.25 μg/mL) at 22 °C in 50 mM sodium phosphate, pH 7.4, with the following additives: none (○); 70 μg/mL α_2-antiplasmin (●); 6.25 μg/mL elastase (□); 70 μg/mL α_2-antiplasmin plus 6.25 μg/mL elastase (■). Before addition of urokinase, plasminogen and additives were preincubated for 15 min.

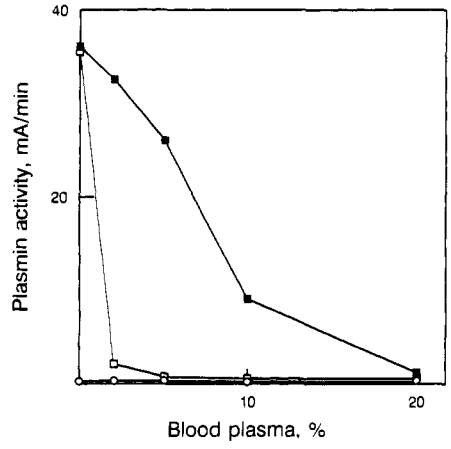

FIGURE 13: Activation of porcine Glu-plasminogen in the presence of citrated porcine blood plasma: Effect of elastase. Porcine Glu-plasminogen (300 μg/mL) was incubated with urokinase (0.5 μg/mL) and porcine pancreatic elastase (22 or 222 μg/mL) in 25 mM sodium phosphate–50 mM Tris-HCl–75 mM NaCl, pH 7.4, in the presence of increasing dilutions of citrated porcine blood plasma. Undiluted plasma concentration is taken as 100%. After 45 min at 22 °C, samples were determined for plasmin activity on 0.2 mM Spectrozyme PL substrate. Urokinase (○); urokinase plus 22 μg/mL elastase (□); urokinase plus 222 μg/mL elastase (■).

None of the elastase-dependent reactions was influenced by 6-aminohexanoate (data not shown).

Effect of Elastase in Blood Plasma. Plasma contains approximately 20 μM α_1-protease inhibitor (α_1-antitrypsin), the primary natural inhibitor of elastase (Matheson et al., 1981). Nevertheless, when elastase concentration exceeds that of its inhibitor, activation of Glu-plasminogen by urokinase in plasma is facilitated by elastase (Figure 13).

Discussion

In addition to humoral fibrinolysis, assorted cell systems have been implicated in clot catabolism. Among these, macrophages and neutrophils and several tumor cell types in particular have been observed to express fibrinolytic activities via synthesis of elastase and plasminogen activators (Plow, 1986; Markus, 1988). The only known effect of elastases on the plasmin system has been that of proteolysis of fibrin (Plow, 1986). However, a regulatory role of elastases, a protease family having aliphatic specificities and secreted by many cell types, in plasminogen activation is suggested by some features of elastase enzymology. First, elastases enhance the rate of activation of Glu-plasminogen by tPA or urokinase by as much as 10-fold (Figures 6 and 10). Second, des-kringle$_{1-4}$-plasmin is one-tenth as sensitive as plasmin to the fast-acting inhibitor α_2-antiplasmin (Figure 7). Third, α_2-antiplasmin itself is a sensitive substrate for destructive proteolysis by elastase (Figure 8). When these attributes are combined in a reconstituted system comprising Glu-plasminogen, α_2-antiplasmin, and tPA or urokinase, plasminogen activation not only is enhanced but also appears virtually dependent on a catalytic concentration of elastase (Figures 9–11). [It is interesting that plasminogen activator inhibitor 1 is also inactivated by elastase (Levin & Santell, 1987).] Such elastase dependency may reflect an inherent sensitivity of key regions of each of these proteins to proteases (Sottrup-Jensen et al., 1978; Moroz, 1981; Schaller et al., 1987). Thus, the exceptional sensitivity of plasminogen and α_2-antiplasmin to both pancreatic and leukocyte elastases and the relative insensitivity to further degradation of des-kringle$_{1-4}$-plasmin(ogen)[2] and tPA (Figures 1, 2, 5, and 8) afford argument that enzymes of elastase specificity are integral to cellular regulation of fibrinolysis. That is, for each substrate of the system, hydrolysis by elastases serves to promote, never moderate, plasminogen activation. Elastases thus provide a fully equivalent alternative to fibrin-dependent plasminogen activation, not only for tPA but also for urokinase, which is not known to be regulated by cofactors. Neutrophils, macrophages, tumors, or other cells could promote this type of reaction under circumstances where plasminogen is activated outside of thrombi, or when plasmin is being recruited to degrade proteins other than fibrin.

Elastase-dependent plasminogen activation must be controlled by α_1-protease inhibitor, the potent and abundant elastase inhibitor. Neutrophils, however, secrete large amounts of elastase along with myeloperoxidase, a poison of the α_1-protease inhibitor (Matheson et al., 1981). Furthermore, any cell engaged in secretion of sufficient quantities of an elastase would have a corresponding capacity to promote plasminogen activation (Figure 13). Some tumors, already recognized as secreting urokinase-type plasminogen activators (Markus, 1988), may warrant analysis for proteases that yield des-kringle$_{1-4}$-plasminogen.

Acknowledgments

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[2] Owing to the sequence homology between *S. griseus* proteinases A and B and streptokinase (Jackson & Tang, 1982), Pronase treated with diisopropyl fluorophosphate (DIP–Pronase) was assayed for a capacity to activate Glu-plasminogen or to facilitate Glu-plasminogen activation by tPA. In neither catalytic nor stoichiometric concentrations was DIP–Pronase able to activate either porcine or human plasminogen. These results were not altered by the addition of 6-aminohexanoate. However, the DIP–Pronase preparation enhanced the rate of porcine Glu-plasminogen activation by tPA in a dose-dependent manner. Although DFP inhibited the trypsin-like (plasminogen activator) activities of the Pronase preparation, which contains at least four proteolytic enzyme activities (Trop & Birk, 1970), some elastase-like activities were relatively insensitive to this inhibitor, as minute amounts of DIP–Pronase converted Glu-plasminogen to des-kringle$_{1-4}$-plasminogen (as shown by SDS gel electrophoresis).

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CORRECTION

Site-Directed Mutagenesis of *Escherichia coli* Ornithine Transcarbamoylase: Role of Arginine-57 in Substrate Binding and Catalysis, by Lawrence C. Kuo,* Arthur W. Miller, Sunjoo Lee, and Cheryl Kozuma, Volume 27, Number 24, November 29, 1988, pages 8823–8832.

Page 8827. In Table III, the dissociation constant for the reaction E·cp + nor = E·cp·nor of the wild-type enzyme should be 0.054 mM.