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Effects of Human Granulocyte Elastase on Fibrinolysis

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The specific limited proteolysis of human plasminogen by human granulocyte elastase results in the cleavage of three peptides from the heavy chain of plasminogen. These three peptides bind to lysine- and fibrin-Sepharose. The remainder of the molecule possesses a molecular weight of 38000 and represents a fully activatable plasminogen lacking the lysine binding site(s) in the heavy chain.

Upon treatment of this low molecular weight form of plasminogen with urokinase, esterolytic, fibrinolytic and amidolytic activities appear. The low molecular weight form of plasmin consists of two disulfide-linked polypeptide chains with molecular weights of approximately 25000 and 13000.

The kinetic parameters of hydrolysis of synthetic substrates by the low molecular weight form of plasmin and native plasmin, respectively, are similar. Furthermore, the inhibitions of the two enzymes by several inhibitors of the hydrolysis of Blue Dextran-fixed fibrin are identical.

The initial rate of activation with urokinase is about two times faster for the low molecular weight form of plasminogen than for native plasminogen, and the activation of native plasminogen by urokinase is inhibited by plasma α_2 -antiplasmin, whereas the activation of the low molecular weight form of plasminogen is not.

Urokinase-induced clot lysis is inhibited efficiently by α_2 -antiplasmin, but the inhibitory effect of α_2 -antiplasmin is removed by heavy chain fragments containing lysine binding site(s), which are obtainable from plasminogen by limited elastase digestion. Urokinase-induced fibrinolytic activity of plasma is promoted by human granulocyte elastase in an apparently time-dependent manner. The findings further indicate that the lysine binding site(s) in the heavy chain of plasmin(ogen) are of great importance in relation to the rate of its reaction with α_2 -antiplasmin.

Keywords—granulocyte elastase; limited hydrolysis of plasminogen; low molecular weight form of plasmin(ogen); fibrinolysis; α_2 -antiplasmin; lysine binding site(s) of plasmin(ogen)

The presence of an elastase¹⁾ in human polymorphonuclear leucocyte (PMN) granules was demonstrated by Janoff and Scherer,²⁾ and confirmed by others.³⁻⁵⁾ Several lines of circumstantial evidence implicate PMN elastase as a mediator of connective tissue damage in certain vascular and lung disorders.^{4,6-11)} In addition, it was observed by Egbring *et al.*¹²⁾ that PMN elastase could be detected in the plasma of patients with acute leukemia and septicemia who exhibit reduced levels of factor XIII (including both subunits) and elevated levels of fibrinogen degradation products. In view of the probable pathologic and physiologic functions of human granulocyte elastase, it seemed worthwhile to study the PMN elastase function using the purified enzyme.

The present paper describes the specific limited proteolysis of human plasminogen by purified human granulocyte elastase, which leads to the formation of four major fragments: three derived from the heavy chain of plasminogen (HC-Fr), all of which bind to lysine- and fibrin-Sepharose, and the fourth, which is a low molecular weight form of plasminogen (min-plasminogen) with a molecular weight of 38000, and is fully activatable with urokinase. Furthermore, this paper describes the effects of HC-Fr derived from the heavy chain by elastase-catalyzed limited hydrolysis on the inhibition of plasmin by plasma α_2 -antiplasmin (α_2 -AP).

Experimental

Materials

Insolubilized derivatives for affinity chromatography were prepared by coupling of L-lysine, fibrinogen and ϵ -aminocaproyl-Ala-Ala-Ala to Sepharose 4B. Coupling was performed by the cyanogen bromide technique as described by Wiman and Wallén.¹³⁾ Fibrin-Sepharose 4B was obtained by treatment of fibrinogen-Sepharose 4B with thrombin.¹⁴⁾

Human plasminogen was prepared from human plasma by affinity chromatography on lysine-Sepharose essentially as described by Wallén and Wiman.¹⁵⁾ Human granulocyte elastase was prepared from extracts of leucocyte granules by affinity chromatography on ϵ -aminocaproyl-Ala-Ala-Ala-Sepharose as described by Janoff.¹⁶⁾ Plasma α_2 -AP was isolated from human plasma according to the method of Moroi and Aoki.¹⁷⁾ α_2 -AP depleted plasma was prepared according to the method of Edy *et al.*¹⁸⁾

Urokinase (202000 IU/mg) and bovine thrombin (500 NIH units/vial) were provided as gifts by Mochida Pharm. Co., Tokyo. Plasminogen-rich human fibrinogen was obtained from AB KABI, Stockholm, Sweden. Human plasma and human peripheral blood were from the Red Cross Blood Center, Fukuoka.

Bz-Arg-OEt HCl, Suc-Ala-Ala-Ala-pNA and elastatinal were obtained from the Protein Research Foundation, Osaka, ϵ -aminocaproic acid and D-Val-Leu-Lys-pNA from Daiichi Chem. Co., Tokyo, 4'-nitrophenyl 4-guanidinobenzoate HCl from E. Merck AG, Darmstadt, F.R.G., soybean trypsin inhibitor and ovalbumin from Boehringer Mannheim Yamanouchi Co., Tokyo, Kunitz trypsin inhibitor from Bayer AG, Leverkusen, F.R.G., DFP from Sigma Chem. Co., St Louis, Mo., U.S.A., and *trans*-4-aminomethylcyclohexane-1-carboxylic acid from Daiichi Pharm. Co., Tokyo. ϵ -Aminocaproyl-Ala-Ala-Ala was synthesized in this laboratory by us.

Methods

Specific Limited Proteolysis of Human Plasminogen by Human Granulocyte Elastase—An analytical-scale digest of plasminogen with elastase was investigated by SDS gel electrophoresis (Fig. 1). A preparative-scale digest of plasminogen with granulocyte elastase was then investigated. Human plasminogen (70 mg) dissolved in 30 ml of 0.1 M sodium phosphate buffer, pH 7.8, was treated with 200 μ g of human granulocyte elastase, and the solution was incubated at 25° overnight. The reaction was stopped by adding 1.5 μ l of DFP. After 30 min at room temperature, solid NH_4HCO_3 was added to make the solution approximately 0.05 M. After standing for 18 hr in the cold room, to permit the hydrolysis of excess DFP, the opalescent solution was subjected to centrifugation ($27000 \times g$, 30 min). The resulting supernatant was subjected to affinity chromatography on a lysine-Sepharose column.

Separation of Mini-plasminogen and HC-Fr—Affinity chromatography was performed on a column (1.0×30 cm) of lysine-Sepharose equilibrated with 0.1 M sodium phosphate buffer, pH 7.8. The above supernatant was applied to the column in the same buffer. The column was washed with the starting buffer until the absorbance at 280 nm returned to the base line, and then eluted with the same buffer containing 10 mM ϵ -aminocaproic acid. The fractions were assayed for plasminogen activity by the BAEE esterase method using high-speed liquid chromatography¹⁹⁾ after activation by urokinase. As shown in Fig. 2A, mini-plasminogen (peak I) did not bind to the column, whereas HC-Fr (peak II) was bound. Each fraction was pooled as indicated in Fig. 2A, and concentrated by ultrafiltration with an Amicon PM-10 membrane and dialyzed at 4° overnight against 0.02 M Tris-HCl buffer, pH 8.2. The dialyzed solution containing mini-plasminogen was subjected to the following step, and the other fractions containing HC-Fr were lyophilized.

Purification of Mini-plasminogen—This was performed on DEAE-Sephadex A-50. The column (1.5×20 cm) was equilibrated with 0.02 M Tris-HCl buffer, pH 8.2 and the above dialyzed solution containing mini-plasminogen was applied to the column. The column was washed with the equilibrating buffer until the absorbance of the effluent at 280 nm was equal to that of the reservoir Tris-HCl buffer in order to remove elastase, and adsorbed protein was then eluted with the same buffer containing 0.6 M NaCl. The fractions containing mini-plasminogen were collected and concentrated by ultrafiltration with an Amicon PM-10 membrane and lyophilized as purified mini-plasminogen.

Preparation of the Low Molecular Weight Form of Plasmin (Mini-plasmin)—Mini-plasmin was prepared in a reaction mixture containing of 5 IU/ml urokinase, approximately 0.38 mg/ml lyophilized mini-plasminogen, 0.05 M Tris-HCl, 0.2 M NaCl and 25% (v/v) glycerol, pH 7.8. The active site titration of aliquots removed from the reaction mixture at different times showed that the urokinase-catalyzed conversion was complete after 24 hr at 4°. After incubation, reaction mixtures were frozen at -20° and were later used as mini-plasmin stock solutions.

Assays of Plasmin Activity—All kinetic experiments were run at least three times at 25°. Most reaction mixtures were prepared in 0.05 M Tris-HCl-0.1 M NaCl, pH 7.8. Mini-plasmin and native plasmin concentrations were determined by titration with 4'-nitrophenyl 4-guanidinobenzoate as described by Chase and Shaw.²⁰⁾ The kinetic parameters of enzyme-catalyzed hydrolysis of the synthetic substrates were determined essentially as described by Christensen.²¹⁾

Assay of Arginine Esterase Activity—The arginine esterase activities of mini-plasmin and native plasmin were determined by the BAEE esterase method using the high-speed liquid chromatography pro-

cedure devised by us.¹⁹⁾

Assay of Amidolytic Activity—The activities of enzyme-catalyzed hydrolysis of D-Val-Leu-Lys-pNA were determined as described by Teger-Nilsson *et al.*²²⁾

Inhibition of Mini-plasmin by General Plasmin Inhibitors—To a suspension of 10 mg of Blue Dextran-fixed fibrin powder²³⁾ in 2 ml of 0.1 M sodium phosphate buffer, pH 7.8, 0.2 ml of inhibitor solution and 0.2 ml of plasmin solution were added. The mixture was incubated at 37° for 30 min, then 4 ml of 0.05 M acetic acid was added, and the resulting suspension was rapidly filtered through Toyo No. 2 filter paper. The absorbance of the filtrate was measured at 625 nm with distilled water as a control.

Effects of Plasma α_2 -AP and HC-Fr on Fibrinolysis—Experiments were carried out by the following two methods. (1) Normal plasma or α_2 -AP depleted plasma (0.2 ml) containing HC-Fr was mixed with 0.1 ml of urokinase solution (1000 IU/ml) and 0.2 ml of thrombin solution (20 NIH units/ml) to make a final clot volume of 0.5 ml each in test tubes (10 × 75 mm) in an ice-water bath. The tubes were then immediately placed in a 37° water bath where clotting occurred, and the clot lysis was timed. Increasing amounts of HC-Fr (0–40 μ g/ml) were previously dissolved in normal and α_2 -AP-depleted plasma. A control was run with plasma containing no HC-Fr. (2) In this case, the incubations were carried out in 0.1 M borate buffer containing 0.1 M NaCl, pH 8.0. Plasminogen-rich human fibrinogen solution (1%, 0.2 ml) was mixed with 0.1 ml of urokinase solution, 0.05 ml of α_2 -AP solution (5 nm/ml), 0.05 ml of HC-Fr solution (0–200 μ g/ml) and 0.1 ml of thrombin solution. The clot lysis time in a 37° water bath was recorded with a control in which the HC-Fr solution was replaced with borate-saline. The concentration of α_2 -AP was determined by titration against a plasmin solution of known concentration, as described by Wiman and Collen.²⁴⁾

Effect of Pre-treatment of Plasma with Elastase on Clot Lysis—Human normal plasma (0.2 ml) was mixed with 0.1 ml of 0.05 M imidazole buffer, 0.1 M NaCl, pH 7.35, and 0.5 or 2 μ g of human granulocyte elastase dissolved in 0.05 ml of imidazole buffer and incubated at 37°. At intervals, 10 μ l of elastatinal solution (1 mg/ml) was added to stop the reaction, followed by 0.05 ml of urokinase solution and 0.1 ml of thrombin solution. The clot lysis time was recorded.

Gel Electrophoresis—Polyacrylamide gel electrophoresis in the presence of SDS was carried out on 10% gels according to Weber and Osborn.²⁵⁾ One mg of plasminogen and 10 μ g of human granulocyte elastase were dissolved in 1 ml of 0.1 M Tris-HCl buffer, 0.05 M lysine, pH 8.0. The mixtures were incubated in the presence or absence of elastatinal, and after various times, a 50- μ l aliquot was placed in a plastic tube containing 5 μ l of 0.1 M DFP and 100 μ l of 1% SDS dissolved in 0.01 M sodium phosphate buffer, pH 7.0, with or without 3% 2-mercaptoethanol. One hundred μ l of this mixture was used for gel electrophoresis. For determination of molecular weight, the electrophoresis was carried out in the absence of 2-mercaptoethanol, and bovine serum albumin, RNA polymerase α -subunit, soybean trypsin inhibitor and cytochrome c were used as marker proteins. Their molecular weights were plotted against the electrophoretic mobility on a semilogarithmic scale. The disulfide bonds of native plasmin and mini-plasmin were reduced with 2-mercaptoethanol before electrophoresis.

Assay of Elastase—Suc-Ala-Ala-Ala-pNA was used for the determination of elastase activity, according to Bieth *et al.*²⁶⁾

Protein Determination—Protein concentrations were determined by the method of Lowry *et al.*²⁷⁾ with bovine serum albumin as a standard.

Results

Limited Proteolysis of Human Plasminogen by Human Granulocyte Elastase

Fig. 1 shows a reduced SDS gel electrophoretic analysis of human plasminogen digested with human granulocyte elastase. In the presence of elastatinal, plasminogen was not cleaved by elastase. In the absence of elastatinal, the native plasminogen was cleaved to a slightly smaller species in the initial period (10 min), and at longer times (180 min) it was cleaved to four main fragments. The estimated molecular weights of each fragment liberated are shown in Fig. 1.

Separation of Mini-plasminogen and HC-Fr

As shown in Fig. 2A, mini-plasminogen was eluted from a lysine-Sepharose 4B column by washing with starting buffer (peak I) and HC-Fr were recovered from the column by elution with the same buffer containing 20 mM ϵ -aminocaproic acid (peak II). Mini-plasminogen in peak I did not bind to lysine-Sepharose when peak I was rechromatographed on lysine-Sepharose, as indicated in Fig. 2B. The insert in Fig. 2A shows the electrophoretic patterns of peak I and peak II. Mini-plasminogen derived from peak I gave one major protein band with an apparent molecular weight of 38000 on gel in the absence of reducing agents, while

HC-Fr derived from peak II gave three bands, corresponding to apparent molecular weights of 35000, 27000 and 11000. Affinity chromatography on a fibrin-Sepharose column gave results very similar to those obtained with lysine-Sepharose. DEAE-Sephadex A-50 chromatography was employed to purify mini-plasminogen, as shown in Fig. 3.

Mini-plasmin

Purified mini-plasminogen was converted to mini-plasmin by urokinase as described in "Methods." Fig. 4 shows the SDS polyacrylamide gel electrophoresis patterns of reduced native plasmin and reduced mini-plasmin. The reduced native plasmin showed the typical pattern of the two plasmin subunits, and the reduced mini-plasmin showed two protein bands

Fig. 1. Effect of Human Granulocyte Elastase on Human Plasminogen as Monitored by SDS Gel Electrophoresis

(i) Native human plasminogen incubated with human granulocyte elastase in the presence of elastatinal for 180 min at 37°. Native human plasminogen was then treated with human granulocyte elastase in the absence of elastatinal at 37° for 10 min (2); 30 min (3); 60 min (4); 120 min (5); 180 min (6).

Fig. 3. DEAE-Sephadex A-50 Column Chromatography of Mini-plasminogen

The concentrated active fraction (containing 30 mg of protein) was applied to a DEAE-Sephadex column. The column was eluted as described in "Methods" and fractions of 5 ml were collected at a flow rate of 30 ml per hr.

Fig. 2. A. Chromatographic Separation of Mini-plasminogen and HC-Fr on Lysine-Sepharose 4B

Elution was carried out at a flow rate of 20 ml per hr, and fractions of 10 ml were collected.

B. Rechromatography of Peak I Containing Mini-plasminogen on Lysine-Sepharose 4B

Conditions were the same as in A.

corresponding to apparent molecular weights of 25000 and 13000. These results show that the heavy chain of human plasminogen underwent limited hydrolysis by human granulocyte elastase, whereas the light chain was not attacked. Mini-plasminogen did not bind to lysine-Sepharose as indicated in Fig. 2, while mini-plasmin did bind to lysine- and fibrin-Sepharose, and was eluted with the buffer containing ϵ -aminocaproic acid as indicated in Fig. 5A and 5B.

Catalytic Activity of Mini-plasmin

Table I summarizes the catalytic activities of mini-plasmin and native plasmin. Towards typical synthetic plasmin substrates, mini-plasmin showed activities very similar to those of native plasmin. The kinetic parameters of mini-plasmin and native plasmin were identical in the hydrolysis of Bz-Arg-OEt and D-Val-Leu-Lys-pNA at pH 7.8, 25°. The Fibrinolytic activities were also identical, as illustrated in Fig. 6.

Fig. 4. SDS Polyacrylamide Gel Electrophoresis of Reduced Native Plasmin and Reduced Mini-plasmin

(1) Human native plasmin; (2) the material in gel 1 treated with 2-mercaptoethanol; (3) mini-plasmin; (4) the material in gel 3 treated with 2-mercapto ethanol.

Fig. 6. Determination of Fibrinolytic Activity by the Blue Dextran-fixed Fibrin Method

●, Native plasmin; ○, mini-plasmin.

Fig. 5. A. Affinity Chromatography of Mini-plasmin on a Column of Lysine Sepharose 4B

The conditions of chromatography were as described for the separation of mini-plasminogen and HC-Fr in "Methods."

B. Affinity Chromatography of Mini-plasmin on a Column of Fibrin-Sepharose 4B

The conditions of chromatography were as described for Fig. 5A.

TABLE I. Kinetic Parameters of Mini-plasmin and Native Plasmin at 25°

Substrate	Mini-plasmin		Native plasmin	
	$K_m(\text{mM})$	$K_c(\text{s}^{-1})$	$K_m(\text{mM})$	$K_c(\text{s}^{-1})$
Bz-Arg-OEt	0.17	12.4	0.19	12.2
H-D-Val-Leu-Lys-pNA	0.25	11.8	0.29	11.4

Inhibition Studies

As indicated in Table II, the inhibition properties of three natural proteinase inhibitors (soybean trypsin inhibitor, ovomucoid and Kunitz trypsin inhibitor) and four synthetic inhibitors (ϵ -aminocaproic acid, L-lysine, *trans*-4-aminomethylcyclohexane-1-carboxylic acid and DFP) towards mini-plasmin and native plasmin were identical.

TABLE II. Inhibition of Mini-plasmin and Native Plasmin by Several Inhibitors

Inhibitor	Concentration (mM) of 50% inhibition	
	Native plasmin	Mini-plasmin
ϵ -Aminocaproic acid	20	25
L-Lysine	50	60
<i>trans</i> -4-Aminomethyl cyclohexane-1-carboxylic acid	0.5	0.3
DFP	0.1	0.1

	Concentration ($\mu\text{g/ml}$) of 50% inhibition	
	Native plasmin	Mini-plasmin
Soybean trypsin inhibitor	15	20
Ovomucoid	25	30
Kunitz trypsin inhibitor	5	5

The concentration inhibiting 50% of the fibrinolytic activity, determined spectrophotometrically at pH 7.8 with Blue dextran-fixed fibrin as the substrate.

Activation Properties of Mini-plasminogen

The plasmin substrate D-Val-Leu-Lys-pNA was used to investigate the plasmin activity. As shown in Fig. 7, the initial rate of activation with urokinase was about two times faster for the mini-plasminogen than for the native plasminogen. When the native plasminogen was mixed with α_2 -AP in an approximately 4:1 molar ratio, the activation rate by urokinase was reduced to about one-third. In contrast, when the mini-plasminogen was mixed with α_2 -AP at a similar molar ratio the activation was not inhibited.

Effect of HC-Fr on Urokinase-induced Clot Lysis

In the experiments using purified fibrinogen and plasminogen, lysis of a fibrin clot induced by urokinase was inhibited when α_2 -AP was also incorporated in the clot, and the clot lysis time was prolonged. However, the α_2 -AP-prolonged lysis time was shortened to that in the absence of α_2 -AP when HC-Fr was incorporated in the clot as shown in Fig. 8. Such an effect of HC-Fr was also observed when normal plasma was used instead of purified fibrinogen, plasminogen and α_2 -AP. Clot lysis in α_2 -AP-depleted plasma was also tested, and HC-Fr showed no appreciable effect, as illustrated in Fig. 9.

Fig. 7. Activation of Mini-plasminogen and Native Plasminogen by Urokinase

The plasmin activities were assayed before and after attempted activation with urokinase using D-Val-Leu-Lys-pNA as a substrate. Δ , Native plasminogen; $\blacktriangle$, native plasminogen was mixed with α_2 -AP; $\circ$, mini-plasminogen; $\bullet$, mini-plasminogen was mixed with α_2 -AP.

Fig. 9. Effect of HC-Fr on Urokinase-induced Clot Lysis in Plasma

●, Normal plasma; ○, α_2 -AP-depleted plasma.

Fig. 8. Effect of HC-Fr on Urokinase-induced Clot Lysis in the Purified System

●, In the presence of α_2 -AP; ○, in the absence of α_2 -AP.

Fig. 10. Effect of Preincubation of Plasma with Human Granulocyte Elastase on Its Clot Lysis

●, Preincubation with $2 \mu\text{g}$ of elastase and clot formed in the absence of urokinase; ○, preincubation without elastase and clot formed in the presence of urokinase; $\blacktriangle$, preincubation with $0.5 \mu\text{g}$ of elastase and clot formed in the presence of urokinase; Δ , preincubation with $2 \mu\text{g}$ of elastase and clot formed in the presence of urokinase.

Promotion of Clot Lysis by Human Granulocyte Elastase

When normal plasma was incubated with human granulocyte elastase at a concentration of 1–4 $\mu\text{g}/\text{ml}$, the urokinase-induced fibrinolytic activity of plasma was enhanced in an apparently time-dependent manner (Fig. 10).

Discussion

Human granulocyte elastase was reported to inhibit thrombosis by inactivating blood

coagulation factors²⁸⁾ and by hydrolyzing fibrin and fibrinogen.²⁹⁾ However, it is not known what effects the enzyme has on the fibrinolysis system, which is closely related to the blood coagulation system *in vivo*. Thus, we subjected human plasminogen to limited hydrolysis by human granulocyte elastase and then studied the effects of this preparation on the fibrinolysis system.

Sottrup-Jensen *et al.*³⁰⁾ showed that the limited hydrolysis of human plasminogen by porcine pancreatic elastase yielded a low molecular product from plasminogen. Our mini-plasminogen preparation that was produced by treatment of plasminogen with human granulocyte elastase seemed to be identical with the preparation of Sottrup-Jensen *et al.*, based on the molecular weight measured by SDS gel electrophoresis and the absence of affinity for lysine-Sepharose and fibrin-Sepharose.

Christensen *et al.*³¹⁾ reported that, when the low molecular weight form of plasminogen produced by porcine pancreatic elastase was subjected to activation, no kinetic difference from native plasmin was observed in the hydrolysis of synthetic substrates. In our experiments using human granulocyte elastase for the digestion of plasminogen, mini-plasmin showed no significant difference from native plasmin in affinity for synthetic substrates or in fibrinolytic activity.

The experimental results of Christensen *et al.*³¹⁾ and Wiman *et al.*³²⁾ indicated that at the initial stage of interaction the low molecular weight form of plasmin, unlike native plasmin, hardly bound with α_2 -AP. Thus, the lysine binding site(s) in the heavy chain of plasmin plays an important role in the interaction of α_2 -AP with plasmin, suggesting that the lysine binding site(s) is absent in the low molecular weight form of plasmin. Our experimental results showed that mini-plasmin was hardly inhibited by α_2 -AP. As is the case with native plasmin, the fibrinolytic activity of mini-plasmin was inhibited not only by natural proteinase inhibitors, but also by synthetic inhibitors believed to exhibit their suppressive effect by binding with the lysine binding site. In other words, an essential region for the expression of the fibrinolytic activity is not the lysine binding site(s) in the heavy chain of plasmin, but the other lysine binding site that is Kringle region 5 in the light chain of plasmin and shows affinity with lysine after plasminogen is activated to plasmin. Therefore, this lysine binding site (Kringle region 5) is important for the enzyme-substrate binding. This conclusion is supported by the observation that the activated mini-plasmin, unlike the mini-plasminogen, was adsorbed on lysine- and fibrin-Sepharose and then eluted with ϵ -aminocaproic acid. Fig. 7 shows that, when the lysine binding site(s) in the heavy chain of plasminogen was available, the conversion of plasminogen to plasmin was also inhibited by α_2 -AP.

In the absence of HC-Fr containing the lysine binding site(s) of the heavy chain, the clot lysis time of native plasmin was elongated by α_2 -AP. In the presence of HC-Fr, in contrast, the elongation of the clot lysis time by α_2 -AP was nullified, resulting in the same clot lysis time as observed in the absence of α_2 -AP. Similar observations were also made with normal plasma. Namely, the clot lysis time of normal plasma was shortened by addition of HC-Fr, whereas the clot lysis time of plasma containing no α_2 -AP remained unaffected by HC-Fr. It seems probable that the binding of HC-Fr with α_2 -AP competitively inhibits the interaction of plasmin with α_2 -AP. Furthermore, the results in Fig. 10 suggest that the fibrinolytic activity in blood vessels will rise after the limited decomposition of plasminogen is induced by granulocyte elastase.

Based on our observations, the lysine binding site(s) in the heavy chain of plasmin(ogen) which can be released by human granulocyte elastase is considered to be more important for binding with α_2 -AP in connection with control of the fibrinolysis system than for binding with fibrin. In addition, it seems very probable that human granulocyte elastase has physiological roles in the blood coagulation and fibrinolysis system, when the limited decomposition of plasminogen by granulocyte elastase occurs in blood vessels with subsequent promotion of fibrinolysis.

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References and Notes

- 1) Enzymes: elastase (EC 3.4.21.11); plasmin (EC 3.4.21.7); thrombin (EC 3.4.21.5); urokinase (EC 3.4.99.26). Abbreviations: benzoyl-L-arginine ethyl ester, BAEE; benzoyl, Bz; diisopropylphosphofluoridate, DFP; sodium dodecyl sulfate, SDS; succinyl, Suc; *p*-nitroanilide, pNA.
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