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Antibiotic A10255 (Thioplabin) Enhances Fibrin Binding and Activation of Plasminogen

SHIGEKI OHYAMA, YASUKO WADA and KEIJI HASUMI*

Department of Applied Biological Science, Tokyo Noko University,
3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan

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Three thiopeptide metabolites that enhance fibrin binding of plasminogen were isolated from a culture of *Streptomyces* sp. R1401. A combination of spectroscopic analyses revealed that these compounds were identical with the antibiotic A10255B, E and G. These agents enhanced fibrin binding of plasminogen and plasminogen/urokinase-mediated fibirinolysis at concentrations of 5~20 μM. A10255B reversibily increased urokinase-catalyzed activation of plasminogen by lowering K_m, while the agent did not enhance urokinase activity when substrates other than plasminogen were used, indicating that the agent affects plasminogen to increase its affinity to urokinase. A smaller but significant increase in activation was also observed when conformationally relaxed plasminogen derivatives such as Lys-plasminogen and mini-plasminogen were used. Two related thiopeptide antibiotics with a *C*-terminal amide had no effect on plasminogen activation, suggesting a role of the terminal carboxyl of the A10255 molecule in activity.

The fibrinolytic system (plasminogen (Plg)/plasmin system) is involved not only in fibrin dissolution but also in various physiological and pathological events, including inflammation, tissue remodeling, ovulation, angiogenesis, tumor metastasis and tissue invasion of pathogens[1~5]. The fibrinolytic reaction is initiated by proteolytic activation of circulating Plg (Glu-Plg) to plasmin. This activation is physiologically catalyzed by two Plg activators (PAs), urokinase-type PA (uPA) and tissue-type PA (tPA)[6]. The Plg activation is regulated at the level of PAs as well as at the level of Plg localization[7,8]. Glu-Plg consists of an NH_2-terminal peptides (NTP), five kringle domains and a protease domain[9,10]. Intramolecular binding of NTP to aminohexyl site in kringle 5 allows the Glu-Plg molecule to adopt a tight, spiral conformation, which attenuates activation by PAs[11~16]. On the other hand, Glu-Plg bound to fibrin or cell surfaces is readily activable since the molecule adopts relaxed conformation[7,16,17].

We have searched for compounds with activity to enhance fibrin binding of Plg and found that three metabolites (tentatively designated thioplabins) from *Streptomyces* sp. R1401 as active compounds. Structural analyses revealed that thioplabins are members of antibiotic A10255 complex[18~20] (Fig. 1). Here we report that antibiotic A10255 elevates fibrin binding and activation of Plg, resulting in increased fibrinolysis.

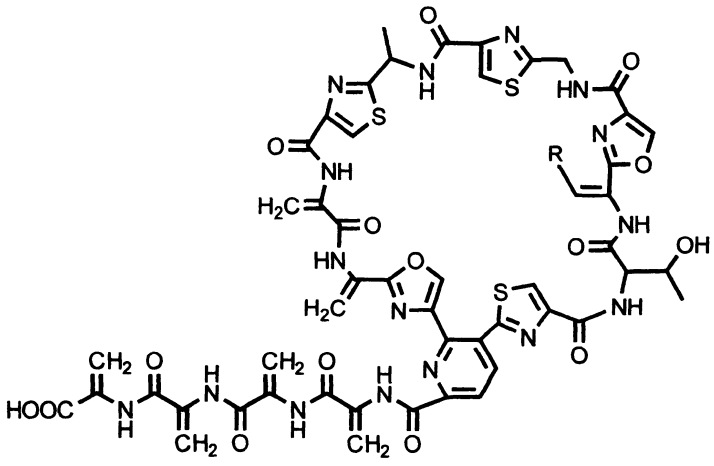
Materials and Methods

Materials

The following proteins and chemicals were from commercial sources: human Lys-Plg from Enzyme Research Laboratories, USA; bovine aprotinin from Wako, Japan; two-chain uPA from JRC Farmaceuticals, Japan; Spectrozyme UK from American Diagnostica; porcine elastase from Elastin Products, USA; human fibrinogen and bovine serum albumin from Sigma, USA; H-Val-Leu-Lys-*p*-nitroanilide (VLK-*p*NA) from Bachem, Switzerland; carrier-free $Na^{125}I$ from Amersham. Human Glu-Plg was affinity-purified from human plasma[21], and mini-Plg was obtained from Glu-Plg by bacillolysin MA digestion. CNBr fragment of fibrinogen (CNBr-Fbg) was prepared as described[22]. Siomycin A[23] and sulfomycin I[24] were kindly provided by Shionogi & Co. and Tanabe Pharmaceutical Co., respectively. The compositions of buffers were: buffer

* Corresponding author: hasumi@cc.tuat.ac.jp

Fig. 1. Structure of A10255G, B and E (thioplabin A, B and C).



Thioplabin	R
A (A10255G)	CH_3
B (A10255B)	CH_2CH_3
C (A10255E)	$CH(CH_3)_2$

A, 50 mM Tris-HCl, 100 mM NaCl and 0.01% (wt/vol) Tween 80, pH 7.4; buffer B, 20 mM sodium phosphate and 150 mM NaCl, pH 7.4; buffer C, Hanks' balanced salt solution containing 1 mg/ml bovine serum albumin and 50 mM HEPES, pH 7.4.

Isolation of Thioplabins (Antibiotic A10255s)

Streptomyces sp. R1401, which was isolated from a soil sample collected in Ichinomiya-machi, Yamanashi, Japan, was aerobically grown at 28°C for 4 days in 500-ml Erlenmeyer flasks containing 100 ml of medium consisted of 1% glucose, 2% corn starch, 1% soy bean meal, 0.5% Farmamedia (cottonseed-derived protein nutrient, Southern Cotton Oil Co., USA), 0.5% peptone, 0.5% yeast extract, 0.2% $CaCO_3$ and 0.01% CB442 (an antifoam), pH 7.0. The mycelial cake obtained from 5 liters of culture broth was extracted with acetone (three times with 800 ml), and the resulting filtrates were extracted with ethyl acetate (twice with 250 ml) at pH 3 after concentration to remove acetone. The organic layer was concentrated, giving 2.35 g of an oily residue, which was then fractionated by silica gel column chromatography developed with a mixture of ethyl acetate and MeOH (95 : 5). Active fraction (766 mg) was further fractionated by preparative HPLC on an Inertsil PREP-ODS (30×250 mm) column developed at 40°C with 0.1% formic acid in 60% acetonitrile at a rate of 25 ml/minute. The fractions containing thioplabin A, B and C (retention time of 16.3, 22.0 and 28.7 minutes, respectively) were evaporated to remove acetonitrile and lyophilized, giving 13.9, 34.9 and 46.3 mg of purified compounds, respectively.

Physico-chemical data for thioplabin B were as follows: ^{13}C-NMR (150 MHz, DMSO-d_6) δ 171.7, 169.2, 167.7, 166.7, 164.8, 162.7, 162.7, 162.6, 162.2, 162.2, 161.4, 161.2, 161.0, 160.0, 159.7, 157.7, 150.1, 149.7, 148.9, 148.6, 147.4, 142.0, 141.3, 141.2, 140.2, 138.0, 136.1, 134.6, 134.6, 134.4, 134.3, 132.8, 129.8, 128.0, 126.4, 125.4, 125.3, 121.4, 121.3, 108.5, 105.8, 105.1, 104.9, 104.5, 103.9, 67.0, 58.0, 47.3, 40.5, 22.8, 22.8, 21.6, 12.9; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.41 (1H, s), 8.29 (1H, s), 8.21 (1H, d), 8.17 (1H, s), 8.10 (1H, s), 8.09 (1H, d), 6.73 (1H, d), 6.59 (1H, d), 6.51 (1H, t), 6.46 (1H, d), 6.37 (1H, s), 6.21 (1H, s), 5.67 (1H, d), 5.66 (1H, s), 5.55 (1H, s), 5.51 (1H, dq), 5.25 (1H, d), 5.07 (1H, d), 4.79 (1H, d), 4.62 (1H, d), 4.27 (1H, d), 2.20 (2H, dq), 1.74 (3H, d), 1.10 (3H, d), 1.00 (3H, d); FAB-MS (*m/z*) 1245 (MH^+); UV (MeOH) 247 nm (ε 102000); IR (KBr) 1658, 1488 cm^{-1}; specific rotation $[\alpha]_D^{25}$ −106.4° (*c* 0.5, $CHCl_3$/MeOH (8 : 2)). Excepting the specific rotation data, which was not found in the literature, these results were very similar to those for A10255B[19,20]. Direct comparison confirmed that thioplabin B was identical with A10255B (generously provided by Eli Lilly and Company). Similarly, the two

congeners thioplabins A and C (with protonated molecular ion peaks in FAB-MS at *m/z* 1231 and 1259) were identified to be A10255G[20] and E[19], respectively.

Radioiodination of Plg and Fibrinogen

Glu-Plg was radioiodinated using the chloramine-T method as described[25] to a specific radioactivity of 1,500~3,000 cpm/ng. Over 97% of the radioactivity was precipitable upon treatment with 10% trichloroacetic acid. Fibrinogen was labeled by the iodine monochloride method[26] to a specific radioactivity of 200~400 cpm/ng. Upon treatment with thrombin, approximately 80% of the radioactivity of ^{125}I-fibrinogen was incorporated in the clot.

^{125}I-Plg Binding to Fibrin, Fibrinolysis and Plg Activation

Determination of ^{125}I-Plg binding to fibrin, fibrinolytic activity and Plg activation was performed as described previously[27]. Briefly, in the ^{125}I-Plg-fibrin binding assay, fibrin clots formed in 96-well plates were washed with buffer B and then incubated at 37°C for 60 minutes with buffer C containing 50 nM ^{125}I-Plg. After washing the wells with buffer B, ^{125}I-Plg bound was dissolved in 0.2 M NaOH and 2% (wt/vol) SDS at 37°C for 30 minutes. A portion of the lysate was removed and counted for radioactivity using a γ-counter. Fibrinolytic activity was determined by incubating ^{125}I-fibrin (20 μg/well) with 50 μl of buffer B containing 2.5 mg/ml gelatin, 100 nM Glu-Plg, and 0.3 U/ml uPA. Subsequently, a portion of the mixture was removed to determine radioactivity released from the ^{125}I-fibrin clot. For the Plg activation assay, 100 nM of Plg, 0.1 mM of VLK-*p*NA (a chromogenic substrate for plasmin) and uPA were incubated in 50 μl of buffer A at 37°C for up to 20 minutes. The uPA concentrations were 50, 4 and 3 U/ml for the activation of Glu-Plg, Lys-Plg and mini-Plg, respectively. From the slope of the plots of $A_{405\,nm}$ *versus* t^2, initial velocity of plasmin generation was calculated[28]. The conversion of ^{125}I-Glu-Plg to ^{125}I-plasmin was assayed by incubating 100 nM (670 cpm/μl) ^{125}I-Glu-Plg, 1,000 kallikrein inhibitor units/ml aprotinin and 50 U/ml uPA in 12 μl of buffer A at 37°C for 20 minutes. After incubation, the mixture received 4 μl of a solution containing 8% (wt/vol) SDS, 20% (vol/vol) 2-mercaptoethanol, 250 mM Tris-HCl (pH 6.8), 40% (wt/vol) sucrose and 0.08% (wt/vol) bromophenol blue. A portion (12 μl) of mixture was subjected to SDS-polyacrylamide gel electrophoresis[29] on a 10% gel. After fixing and drying, the gel was exposed to an X-ray film at −80°C overnight.

Amidolytic Activities of Plasmin and uPA

The amidolytic activities of plasmin and uPA were determined using VLK-*p*NA and Spectrozyme UK, respectively, as substrates in 50 μl of buffer A at 37°C. The concentrations of enzymes and substrates, respectively, were 20 nM and 100~400 μM for the plasmin assay and 50 U/ml and 16~100 μM for the uPA assay. The release of *p*NA was measured for up to 20 minutes at 405 nm.

Elastase Digestion of Plg

Plg (5 μM) and elastase (0.4 U/ml) were incubated in 7 μl of buffer A at 37°C for 60 minutes. After incubation, protein was precipitated with 10% trichloroacetic acid. The precipitate was washed with acetone and dissolved in 12 μl of a solution containing 2% (wt/vol) SDS, 5% (vol/vol) 2-mercaptoethanol, 62.5 mM Tris-HCl (pH 6.8), 10% (wt/vol) sucrose and 0.02% (wt/vol) bromophenol blue. A portion (11 μl) of mixture was subjected to SDS-polyacrylamide gel electrophoresis on a 15% gel.

Results and Discussion

Enhancement of Plg Binding to Fibrin

Antibiotic 10255B, E and G enhanced the binding of ^{125}I-Glu-Plg to fibrin 1.4- to 1.9-fold at a concentration of 4~16 μM (Fig. 2A, left panel). The binding of ^{125}I-Lys-Plg, a Plg derivative that lacks the NTP of Glu-Plg, was also elevated by A10255s, while the enhancement was 1.4-fold at 16 μM (Fig. 2A, right panel). The binding of both ^{125}I-Glu-Plg and ^{125}I-Lys-Plg was inhibited >70% by 6-aminohexanoic acid (6-AHA), a lysine analog that binds to lysine binding site in the kringle domains and, therefore, diminishes fibrin-kringle interaction[14,30].

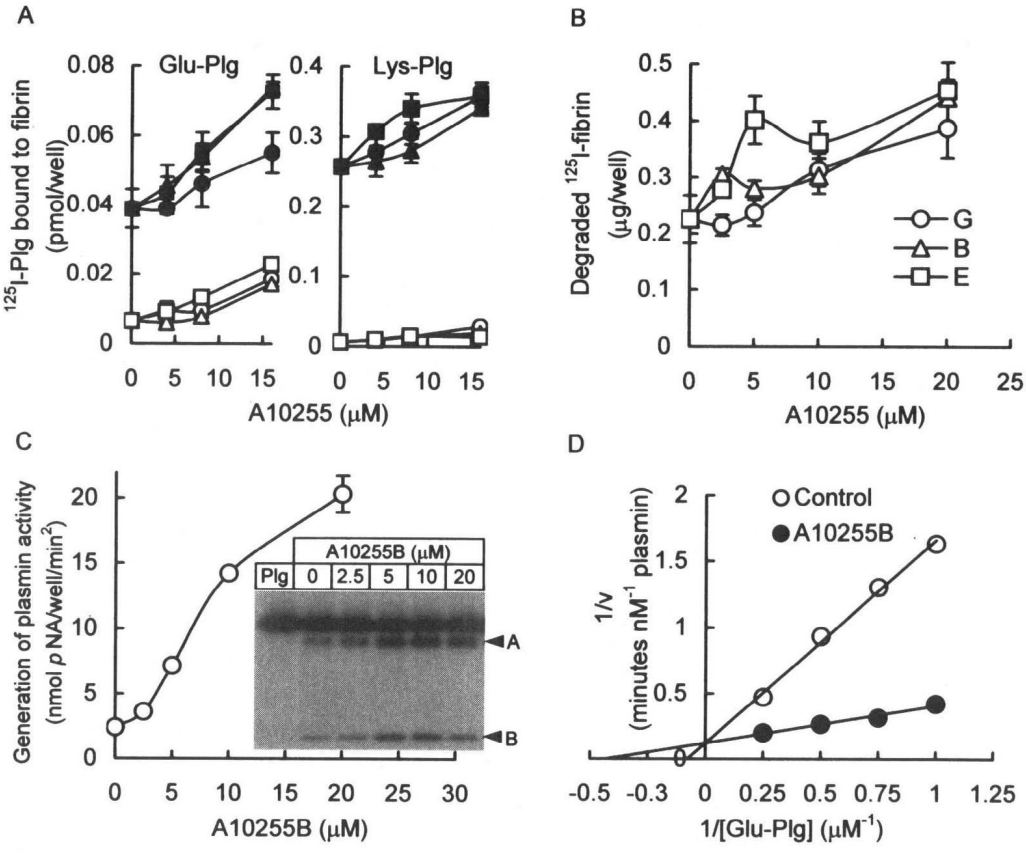
Activation of Fibrinolysis

The elevation of fibrin binding of Plg is expected to enhance fibrinolytic process. We tested for this possibility by incubating ^{125}I-fibrin with Glu-Plg and uPA in the presence of A10255s to measure ^{125}I-fibrin degradation. The results indicated that the degradation of ^{125}I-fibrin was enhanced 1.3- to 2.0-fold by A10255s at a concentration of 10~20 μM (Fig. 2B). The effect of A10255s was not observed in the absence of uPA (data not shown).

Enhancement of Glu-Plg Activation

Along with the elevation of Plg-fibrin binding, another mechanism that may account for increased fibrinolysis is

Fig. 2. Effect of A10255s on fibrin binding (A), fibrinolysis (B) and activation (C and D) of Plg.



(A) The binding of ^{125}I-Glu-Plg (left panel) and ^{125}I-Lys-Plg (right panel) to fibrin was determined in the presence of the indicated concentrations of A10255G, B or E (○, △ or □) with or without 20 mM 6-AHA (●, ▲ or ■).

(B) The degradation of ^{125}I-fibrin by Glu-Plg/uPA was measured in the presence of the indicated concentrations of A10255G, B or E (○, △ or □).

(C) The uPA-catalyzed activation of Glu-Plg was determined by measuring the generation of plasmin activity in the presence of the indicated concentrations of A10255B. Inset shows the effect of A10255B on uPA-catalyzed conversion of ^{125}I-Glu-Plg to ^{125}I-plasmin. The positions of A and B chains of plasmin are shown on the right.

(D) Lineweaver-Burk plots for uPA-catalyzed Glu-Plg activation determined in the absence (○) or presence (●) of 10 μM of A10255B at substrate (Glu-Plg) concentrations of 1~4 μM.

Each value represents the mean±S.D. from triplicate determinations.

enhancement of Plg activation. To examine effects of antibiotic A10255 on this reaction, Glu-Plg was incubated with uPA and VLK-*p*NA in the presence of A10255B to determine the rate of plasmin generation. In this assay, A10255B enhanced 1.5- to 8.4-fold the generation of plasmin activity from Glu-Plg at concentrations ranging from 2.5 to 20 μM (Fig. 2C). The enhancement of Glu-Plg activation by A10255B accompanied an increase in the generation of plasmin molecule (Fig. 2C, inset). These effects were not observed in the absence of uPA. A10255G and E also enhanced Glu-Plg activation catalyzed by uPA (data not shown).

The A10255B effect on Glu-Plg activation appeared to be reversible. Thus, when Glu-Plg was preincubated for 30 minutes with or without A10255B (10 μM) and the mixture was diluted to assay uPA-catalyzed Plg activation at A10255B concentrations of 2.5~10 μM, enhancement of Glu-Plg activation was observed only when sufficient concentrations of A10255B were present during the activation assay, regardless of the preincubation with A10255B (Table 1). The reversible nature of the A10255B effect enabled kinetic analysis of the effect on Glu-Plg activation. As shown in Fig. 2D, A10255B decreased Michaelis constant (K_m), from 12.5±0.28 to 2.3±0.04 μM,

Table 1. Reversivility of the effect of A10255B on uPA-catalyzed Glu-Plg activation.

A10255B (μM)		Generation of plasmin activity (nmol *p*NA/well/min²)
At preincubation	At activation assay	
0	0	0.74 ± 0.06
	2.5	0.88 ± 0.03
	5	2.16 ± 0.20
	10	3.16 ± 0.21
10	2.5	1.00 ± 0.05
	5	2.48 ± 0.19
	10	3.43 ± 0.11

Glu-Plg (2.5 μM) was preincubated at 37°C for 30 minutes in the absence or presence of A10255B (10 μM). Subsequently, a portion of the mixture was removed and diluted 25-fold (so that the concentration of Glu-Plg became 100 nM) and assayed for Plg activation by uPA (50 U/ml) at 37°C in the presence of the indicated concentrations of A10255B (at activation assay).

while the agent did not affect catalytic rate constant ($k_{cat}=2.19\pm0.01\times10^{-2}\,sec^{-1}$ both in the absence and presence of A10255B). The k_{cat}/K_m values in the absence and presence of the agent were 1.75×10^{-3} and $9.48\times10^{-3}\,\mu M^{-1}\,sec^{-1}$, respectively. These results suggest that A10255B enhances uPA-catalyzed Glu-Plg activation by increasing affinity of Glu-Plg to uPA.

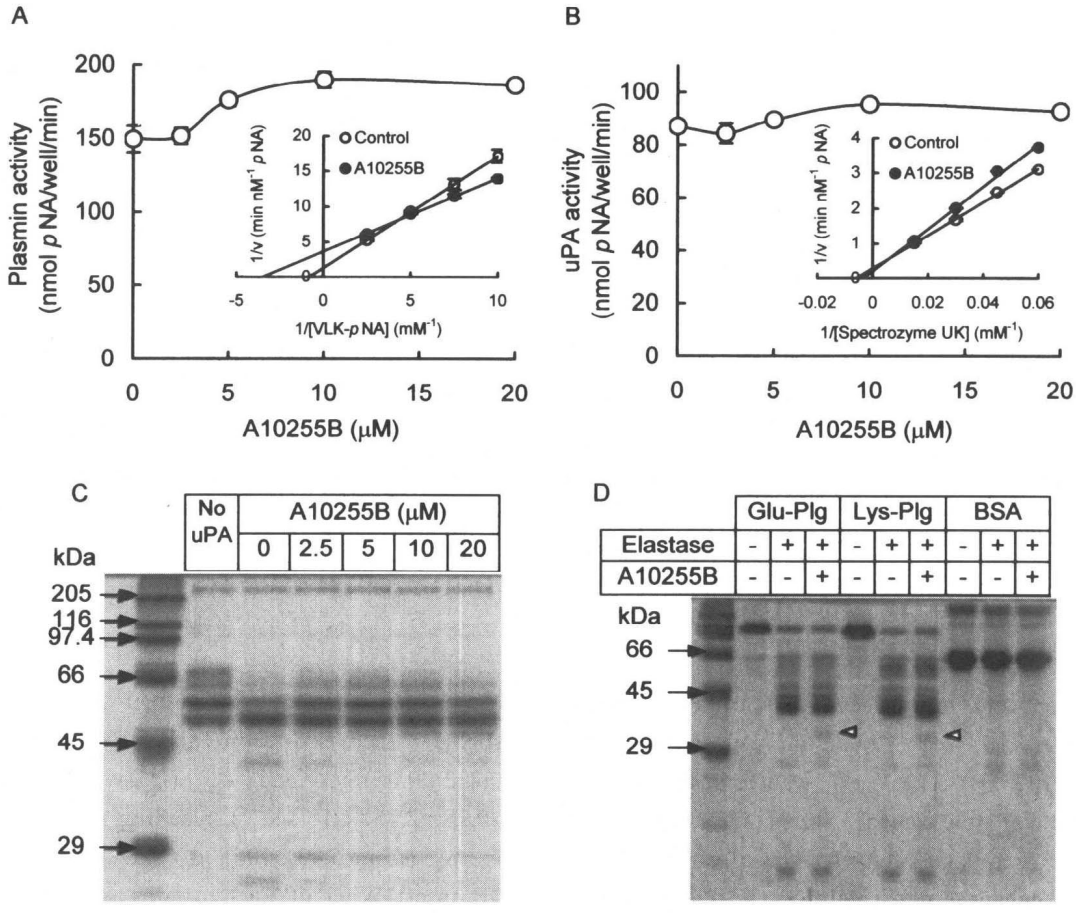
Effects on Plasmin and uPA Activities and Protease Sensitivity of Plg

The observation that A10255 enhances both Plg-fibrin binding and Plg activation suggests that the agent interacts with the Plg molecule. There remained, however, a possibility that the agent affected plasmin and/or uPA to enhance their activities. To test for this possibility, direct effects of A10255B on plasmin and uPA activities were examined. As shown in Fig. 3A, plasmin activity was only slightly increased by the agent when activity was measured at a substrate (VLK-*p*NA) concentration of 100 μM, which is the same concentration as that used in the Plg activation assays. Kinetic analysis showed that A10255B decreased both K_m (from 1.24 ± 0.38 to 0.283 ± 0.002 mM) and k_{cat} (from $6.4\pm1.5\times10^{-4}$ to $2.3\pm0.1\times10^{-4}\,sec^{-1}$), resulting in a slight increase in catalytic efficiency (k_{cat}/K_m from 5.18×10^{-4} to $8.13\times10^{-4}\,mM^{-1}\,sec^{-1}$) (Fig. 3A, inset). Fig. 3B shows the A10255B effect on uPA activity measured using a chromogenic substrate, Spectrozyme UK. The results demonstrated that the effect was minimal: A10255B slightly increased both K_m (from 0.182 ± 0.01 to 0.232 ± 0.06 mM) and k_{cat} (from $9.88\pm0.72\times10^{-3}$ to $10.28\pm2.56\times10^{-3}\,sec^{-1}$), resulting in a slight decrease in k_{cat}/K_m (from 5.43×10^{-2} to $4.43\times10^{-2}\,mM^{-1}\,sec^{-1}$) (Fig. 3B, inset). When uPA activity was assayed using fibrinogen as a substrate, A10255B did not show any enhancement of the proteolytic activity (Fig. 3C). Taken together, these results suggested that the enhancement of Plg activation by antibiotic A10255 was mainly due to its effect on Plg molecule. Another line of evidence that A10255B affects Plg molecule was that the agent caused a change in the profile of elastase cleavage of Plg. As shown in Fig. 3D, among several prominent fragments, a fragment with a molecular mass of 33~34 kDa specifically appeared in the presence of A10255B both in the digests of Glu-Plg and Lys-Plg.

Effects on Glu-Plg Activation in the Presence of Allosteric Effectors and Activation of Lys-Plg and Mini-Plg

One of possible mechanisms of the A10255B enhancement of Plg activation and Plg-fibrin binding is a conformational relaxation of Plg molecule. Glu-Plg adopts a tight, spiral conformation due to intramolecular binding of Lys^{50} or Lys^{62} in the NTP to aminohexyl site in kringle 5[31,32]. The lysine analog 6-AHA disrupts this NTP-kringle 5 interaction by binding to the aminohexyl site and relaxes

Fig. 3. Effects of A10255B on plasmin (A) and uPA (B and C) activities and Plg digestion by elastase (D).



(A and B) The activities of plasmin and uPA were determined in the presence of indicated concentration of A10255B. The substrate concentrations were 100 μM for both determinations. Insets show Lineweaver-Burk plots for plasmin (A) and uPA (B) activities determined in the absence (○) or presence (●) of 10 μM A10255B at substrate concentrations of 100~400 μM for plasmin and 16~66 μM for uPA.

Each value represents the mean±S.D. from triplicate determinations.

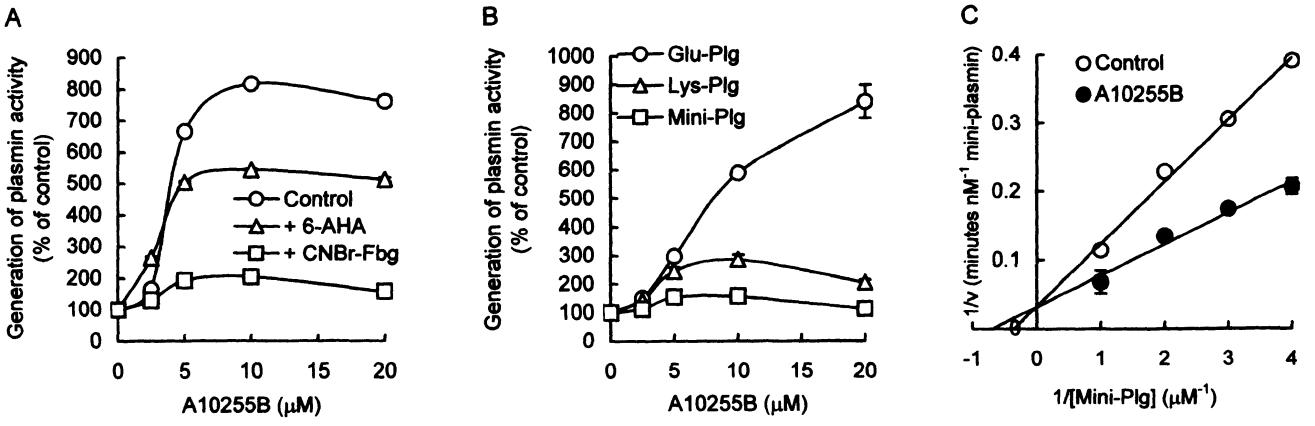
(C) uPA activity was determined using fibrinogen as a substrate in the presence of the indicated concentrations of A10255B.

(D) Elastase digestion of Glu-Plg and Lys-Plg was performed in the presence or absence of 30 μM A10255B. Bovine serum albumin (BSA) was also treated as a control.

Arrowheads denote fragment bands specifically appeared in the presence of A10255B.

Glu-Plg conformation[13,33,34]. Similarly, CNBr-Fbg also relaxes Glu-Plg conformation[14]. In the presence of these allosteric effectors, conformationally relaxed Glu-Plg was efficiently activated by uPA (see legend to Fig. 4 for actual activity). The addition of A10255B resulted in further increases in Glu-Plg activation in a dose-dependent manner, whereas the magnitude of the enhancement was markedly lowered in the presence of these effectors as compared with that in their absence (Fig. 4A). Similarly, the activation of two conformationally relaxed Plg derivatives, Lys-Plg (a derivative without NTP) and mini-Plg (a derivative lacking NTP and kringles 1 to 4), was enhanced by A10255B to a lesser extent (Fig. 4B). Although the effect on mini-Plg was small, kinetic analysis revealed that the mode of action of A10255B was similar to that observed with Glu-Plg, reducing K_m (from 2.87±0.61 to 1.46±0.15 μM) with k_{cat} unchanged (2.1×10^{-1} sec^{-1} for both in the absence and presence of A10255B) (Fig. 4C). Taken together, these results suggest that the A10255B effect is related to the conformational alteration of Plg molecule, while the effect

Fig. 4. Effects of A10255B on Glu-Plg activation in the presence of 6-AHA or CNBr-Fbg and on the activation of Lys-Plg and mini-Plg.



(A) uPA-catalyzed activation of Glu-Plg was determined at uPA and Glu-Plg concentrations of 50 U/ml and 100 nM, respectively, in the presence of the indicated concentration of A10255B without allosteric effector (○) as well as with 20 mM 6-AHA (△) or 120 μg/ml CNBr-Fbg (□). The rates of plasmin generation (nmol *p*NA/well/min^2) in the absence of A10255B were 0.515±0.009 for no effector, 2.662±0.064 for 6-AHA and 2.854±0.040 nmol for CNbr-Fbg.

(B) uPA-catalyzed activations of Glu-Plg (○), Lys-Plg (△) and mini-Plg (□) were determined in the presence of the indicated concentration of A10255B. The concentrations of uPA were 50 U/ml for Glu-Plg, 4 U/ml for Lys-Plg and 3 U/ml for mini-Plg. The rates of plasmin generation (nmol *p*NA/well/min^2) in the absence of A10255B were 2.423±0.138 for Glu-Plg, 2.760±0.237 for Lys-Plg and 1.058±0.021 for mini-Plg.

(C) Lineweaver-Burk plots for mini-Plg activation determined in the absence (○) or presence (●) of 10 μM A10255B. The concentration of uPA and mini-Plg were 20 U/ml and 0.25~1.0 μM, respectively.

Each value represents the mean±S.D. from triplicate determinations.

may be distinct from that of kringle ligands.

Activity of Other Thiopeptide Antibiotics

Antibiotic A10255 belongs to the thiopeptide antibiotics family. So we tested two structurally related thiopeptide antibiotics, sulfomycin I and siomycin A for their ability to enhance uPA-catalyzed Glu-Plg activation. These compounds, however, showed no activity at concentrations up to 60 μM. A10255s have a terminal carboxyl group, while these compounds have a terminal amide group. We also found that derivatives of A10255B lacking one or two *C*-terminal dehydroalanine moiety but with *C*-terminal amide were similarly inactive at 10 μM. Thus, it is likely that the terminal carboxyl group of A10255s plays an important role to exert activity.

Conclusion

In the present study, we have found that the thiopeptide antibiotic A10255s enhanced fibrin binding and activation of Plg. These effects appeared to be due to an interaction between the agent and Plg. The magnitudes of the A10255s' effect on Plg activation changed depending on the conformational status of Plg, suggesting that the agent affected Plg molecule to alter conformation that influences its activation. In this regard, the action A10255s is similar to that of lysine analogs such as 6-AHA. However, 6-AHA does not affect the activation of Lys-Plg and mini-Plg. Moreover, 6-AHA is inhibitory to Plg-fibrin binding[14,30]. The A10255 effects are quite different from the action of lysine analogs in these regards, and the change of Plg conformation induced by A10255s is suggested to be distinct from the change brought about by lysine analogs, which bind lysine binding site in kringles and cause Glu-Plg molecule to adopt an extended conformation[13,34].

We previously found that staplabin and its analogs (SMTPs) enhanced both activation and fibrin binding of Plg[27,35,36]. The action of these compounds is similar to the action of A10255s, including the effects on Glu-Plg activation in the presence of allosteric effectors and on the

activation of Lys-Plg and mini-Plg[36]. Although staplabin and SMTPs exert their activity at relatively higher concentrations (80~400 μM), the concentration required for the A10255s action is 5~20 μM.

Physiological activation of Plg is regulated at the level of its conformation, and this mechanism enables localized propagation of the plasminogen/plasmin system. Thus, pharmacological modulation of Plg conformation is an attractive means to enhance the plasminogen/plasmin system in diseases such as thromboembolism and atherosclerosis. Antibiotic A10255, which enhances both fibrin binding and activation of Plg, may provide useful template for the development of such drugs.

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