Regulation by \( \alpha_2 \)-Antiplasmin and Fibrin of the Activation of Plasminogen With Recombinant Staphylokinase in Plasma

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The effects of \( \alpha_2 \)-antiplasmin and fibrin on the activation of plasminogen by recombinant staphylokinase (STAR) were studied in an effort to elucidate further the molecular basis of the fibrin-specificity of this fibrinolytic agent. In purified systems consisting of 1.5 \( \mu \)mol/L intact or low-\( M \) plasminogen and 3 \( \mu \)mol/L \( \alpha_2 \)-antiplasmin, at 37°C and in the absence of fibrin, STAR did not induce plasminogen activation and plasmin-\( \alpha_2 \)-antiplasmin complex (PAP) formation. Addition of a purified fibrin clot (30% vol at a concentration of 3 mg/mL) to mixtures containing intact plasminogen caused approximately 40% plasminogen activation within 2 hours, whereas in mixtures containing low-\( M \) plasminogen, no activation was observed. In contrast, 10 nmol/L streptokinase (SK) induced 74% to 100% plasminogen activation within 2 hours in mixtures containing either intact or low-\( M \), plasminogen, in both the absence and the presence of fibrin. In citrated human plasma in the absence of fibrin, 30 nmol/L STAR did not induce measurable plasminogen activation and PAP formation (<1.5% within 2 hours), whereas addition of a plasma clot (12% vol) resulted in complete clot lysis and conversion of 19% ± 8% of the plasminogen to PAP within 2 hours. Addition of a second plasma clot produced 23% ± 2% additional plasminogen activation. Equi potent concentrations for plasma clot lysis of SK (100 nmol/L) induced 54% ± 11% plasminogen activation in the absence and 49% ± 16% in the presence of fibrin. Addition of 50 nmol/L 6-aminohexanoic acid (6-AHA) abolished the effect of fibrin on plasminogen activation with STAR, but not on activation with SK. In \( \alpha_2 \)-antiplasmin–depleted human plasma in the absence of fibrin, 30 nmol/L STAR did not induce fibrinogen breakdown (>90% residual fibrinogen after 6 hours), whereas 30 nmol/L preformed plasmin-STAR complex induced extensive fibrinogen degradation (70% within 20 minutes). Thus, in the absence of fibrin, \( \alpha_2 \)-antiplasmin inhibits the activation of plasminogen by STAR, by preventing generation of active plasmin-STAR complex. Fibrin stimulates plasminogen activation by STAR via mechanisms involving the lysine-binding sites of plasminogen, probably by facilitating the generation of plasmin-STAR complex and by delaying its inhibition at the clot surface.

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plasma and its activity determined by titration with plasmin.\(^{20}\) Plasma was pooled human plasma obtained from at least five healthy blood donors. \(\alpha\)-Antiplasmin-depleted plasma was obtained from normal plasma by immunosorption on an insolubilized monoclonal antibody (MA-34F7) directed against \(\alpha\)-antiplasmin.\(^{21}\) After depletion, this plasma contained less than 2% residual \(\alpha\)2-antiplasmin as determined by enzyme-linked immunosorbent assay (ELISA),\(^{22}\) whereas fibrinogen and plasminogen remained within the normal range. Plasminogen-depleted plasma was prepared by adsorption with lysine-sepharose,\(^{23}\) and contained \(\leq 1%\) plasminogen, as determined by ELISA.\(^{22}\)

\[^{125}\text{I}-\text{fibrinogen}\] was purchased from Amersham (Buckinghamshire, UK), the chromogenic substrate \text{S}-2225 (\(\text{o}-\text{valyl-leucyl-lysine-}\text{p-nitroanilide}\)) from Chromogenix (Antwerp, Belgium), \(\text{p-nitrophenyl-\text{p}’-guanidinobenzoate (NPGB}\) from E. Merck (Darmstadt, Germany), poly-L-lysine \((M_n \ 560,000)\) from Sigma (St Louis, MO), \(\text{6-aminohexanoic acid (6-AHA) from BDH Chemicals (Poole, UK)}\).

**Analytical techniques.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with the Phast-System (Pharmacia, Upplands, Sweden) using 10% to 15% gradient gels and Coomassie brilliant blue staining. Reduction of the samples was performed by heating at 100°C for 3 minutes in the presence of 1% SDS and 1% dithioerythritol. Densitometric scanning of the SDS-PAGE was performed with the Gel Scan Accessory of the Beckman DU 60 spectrophotometer (Fullerton, CA). Quantitation of radioactive gel bands was performed using a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA). Plasmin-\(\alpha\)-antiplasmin complex (PAP) was determined by ELISA, using urokinase-activated plasma for calibration, as described previously.\(^{24}\) Fibrinogen levels in plasma samples were monitored with a clotting rate assay,\(^{24}\) and plasminogen and \(\alpha\)-antiplasmin levels by chromogenic substrate assays.\(^{35,36}\)

**Effect of fibrin on the activation by STAR or SK of mixtures of purified plasminogen and \(\alpha\)-antiplasmin.** STAR or SK (final concentration, 10 nmol/L) was added to mixtures of intact plasminogen or low-M\(_2\) plasminogen (final concentration, 1.5 nmol/L) and \(\alpha\)-antiplasmin (final concentration, 3 nmol/L) in 0.1 mol/L phosphate buffer, pH 7.4, at 37°C. At times 0 and after 1, 2, and 4 hours, samples were removed from the incubation mixtures, heated immediately at 100°C for 1 to 3 minutes in the presence of 1% SDS, and subjected to SDS-PAGE under nonreducing conditions. The concentration of PAP in the samples was determined by densitometric scanning of the gels, and expressed as the percentage of the plasminogen concentration. The effect of fibrin on the generation of PAP was investigated by addition of a 60 \(\mu\)L purified fibrin clot (3 mg/mL fibrinogen concentration), prepared as described previously,\(^{27}\) to the 200-\(\mu\)L reaction mixture.

To characterize further the effect of fibrin quantitatively, increasing concentrations of its CNBr-digested fibrinogen substitute (final concentration, 50 to 5,000 nmol/mL) were added to mixtures of STAR (final concentration, 10 nmol/L) and intact or low-M\(_2\) plasminogen (final concentration, 1.5 nmol/L), containing 24 to 50 × 10\(^5\) \(\text{cpm/mL of } \text{125I-plasminogen}\) with \(\alpha\)-antiplasmin (final concentration, 3 nmol/L) in 0.1 mol/L phosphate buffer, pH 7.4, at 37°C. At times 0 and after 0.5, 1, 1.5, and 2 hours, samples were removed for SDS-PAGE under nonreducing conditions, as described above, followed by autoradiography. The concentration of PAP at each time point was determined by quantitation of the radioactive gel bands and expressed as the percentage of the plasminogen. For each concentration of CNBr-digested fibrinogen, the rate of PAP formation was determined from linear plots of the concentration of PAP versus time.

In addition, the effect of poly-L-lysine (final concentration, 1 to 10 \(\mu\)mol/L) or of 6-AHA (final concentration, 50 mmol/L to 1 \(\mu\)mol/L) on the rate of PAP generation was quantitated, as described above.

The effect of NPGB (final concentration, 100 \(\mu\)mol/L) on the activation of plasminogen in mixtures of STAR (final concentration, 10 nmol/L) and intact plasminogen (final concentration, 1.5 \(\mu\)mol/L, containing 50 × 10\(^5\) \(\text{cpm/mL of } \text{125I-plasminogen}\) in the absence or the presence of CNBr-digested fibrinogen (final concentration, 0 to 5 \(\mu\)mol/L) was monitored over 2 hours. Therefore, samples were subjected to SDS-PAGE under reducing conditions, followed by autoradiography; generated plasmin was quantitated from the ratio of the radioactivity in the gel bands corresponding to the plasmin A- and B-chains, and the total radioactivity recovered from the gel.

**Effect of fibrin on the activation by STAR or SK of the plasma fibrinolytic system.** The time course of the lysis of 60 \(\mu\)L \(\text{125I-fibrin-labeled plasma clots} submerged in 500 \(\mu\)L citrated human plasma at 37°C following addition of STAR (final concentration, 3 to 200 nmol/L) was monitored over 2 hours, as described previously.\(^{10}\) These data (not shown) indicated that 30 nmol/L STAR and 100 nmol/L SK yielded a comparable time course of clot lysis, producing complete lysis within 2 hours.

Activation of the fibrinolytic system in plasma (containing 5 × 10\(^7\) \(\text{cpm/mL of } \text{125I-plasminogen}\) , in the absence or presence of an unlabeled plasma clot following addition of STAR (final concentration, 30 nmol/L) or SK (final concentration, 100 nmol/L), was then monitored for 6 hours, by measurement of residual fibrinogen, plasminogen and \(\alpha\)-antiplasmin levels, and of generated PAP levels, as described above.

In additional experiments, the time course of the lysis of 60 \(\mu\)L \(\text{125I-fibrin-labeled plasma clots} (prepared from plasma containing 50 mmol/L 6-AHA) submerged in 500 \(\mu\)L citrated human plasma containing 50 mmol/L 6-AHA was monitored at 37°C following addition of STAR (final concentration, 30 nmol/L) or SK (final concentration, 100 nmol/L). Residual fibrinogen levels and generated PAP levels were measured as described above.

In separate experiments, inhibition of preformed \(^{125}\text{I-plasmin-STAR complex (final concentration, 45 nmol/L, containing 1.5 × 10^6 \text{cpm/mL of } \text{125I-plasmin}}\) by \(\text{alpha-antiplasmin in plasma (300-\(\mu\)L sample)} in the absence or presence of a 60-\(\mu\)L fibrin clot, was monitored over 2 hours by nonreduced SDS-PAGE and autoradiography, as described above.

**Effect of fibrin on the activation of the fibrinolytic system with \(\alpha\)-antiplasmin-depleted plasma.** Activation of the fibrinolytic system in normal human plasma or in \(\alpha\)-antiplasmin-depleted plasma was monitored for 6 hours at 37°C by measurement of residual fibrinogen levels, following addition of STAR or preformed \(^{125}\text{I-plasmin-STAR complex (final concentration, 30 nmol/L, both in the absence or presence of CNBr-digested fibrinogen (final concentration, 1 \(\mu\)mol/L)}\) in addition control experiments, lower concentrations of plasmin-STAR (final concentration, 8 to 30 nmol/L) were incubated with \(\alpha\)-antiplasmin–depleted plasma and residual fibrinogen levels were determined after 1 hour.

**Stability of STAR in a plasma milieu.** STAR (final concentration, 30 nmol/L) or SK (final concentration, 100 nmol/L) was preincubated for 4 hours at 37°C in 500 \(\mu\)L normal human plasma before addition of a 60-\(\mu\)L \(\text{125I-fibrin-labeled clot} prepared from whole plasma or from plasminogen-depleted plasma. Clot lysis was monitored over 2 hours and compared with the time course of clot lysis obtained with 30 nmol/L STAR or with 100 nmol/L SK, without preincubation. Residual fibrinogen, plasminogen, and \(\alpha\)-antiplasmin levels, and generated PAP levels, were determined just before addition of the fibrin clot.

In additional experiments, clot lysis, residual fibrinogen levels,
and generated PAP levels with 30 nmol/L STAR were monitored over 8 hours following addition of a [125I]-labeled plasma clot to plasma. In these experiments, a freshly prepared plasma clot was added to the same plasma sample at times 0 and again after 3 hours and 6 hours.

RESULTS

Effect of fibrin on the activation by STAR or SK of mixtures of purified plasminogen and α2-antiplasmin. Addition of STAR (final concentration, 10 nmol/L) to mixtures of intact plasminogen (final concentration, 1.5 μmol/L) and α2-antiplasmin (final concentration, 3 μmol/L) did not produce detectable plasminogen activation within 4 hours, as shown by the lack of generation of PAP on nonreduced SDS-PAGE (Fig 1A, insert, lane 1). However, addition of a 60-μL purified fibrin clot (3 mg/mL fibrinogen) to the 200-μL reaction mixture resulted in significant activation of plasminogen, as shown by generation of PAP (Fig 1A, insert, lane 2). With low-M₆ plasminogen (final concentration, 1.5 μmol/L) in the incubation mixture, STAR did not generate PAP in the absence (Fig 1A, insert, lane 3) or in the presence (Fig 1A, insert, lane 4) of the fibrin clot. In contrast, under the same experimental conditions, addition of SK (final concentration, 10 nmol/L) to mixtures of either intact plasminogen or low-M₆ plasminogen produced extensive generation of PAP within 2 hours, both in the absence (Fig 1B, insert, lanes 1 and 3) and in the presence of a purified fibrin clot (Fig 1B, insert, lanes 2 and 4).

Quantitation of PAP complexes by densitometric scanning of SDS-PAGE confirmed a progressive and extensive generation of PAP with SK with both molecular forms of plasminogen, both in the absence and in the presence of fibrin (74% to 100% plasminogen activation within 2 hours) (Fig 1B). However, with STAR, significant generation of PAP was only observed with intact plasminogen in the presence of a fibrin clot (43% plasminogen activation within 2 hours) (Fig 1A).

Following addition of CNBr-digested fibrinogen (final concentration, 50 to 1,000 nmol/L) to mixtures of plasminogen (final concentration, 1.5 μmol/L), containing a trace amount of [125I]-plasminogen and α2-antiplasmin (final concentration, 3 μmol/L), STAR (final concentration, 10 nmol/L) caused a time- and concentration-dependent generation of PAP. This is illustrated by autoradiography (Fig 2, insert A) of nonreduced SDS-PAGE of samples taken after 1 hour from incubation mixtures with different concentrations of CNBr-digested fibrinogen. The rate of PAP generation was determined from plots of the concentration of PAP versus time (not shown), and was expressed as a function of the concentration of CNBr-digested fibrinogen (Fig 2). The half-maximal rate of PAP generation was obtained at a concentration of 290 nmol/L. In contrast, with low-M₆ plasminogen, no significant PAP generation was observed under the same experimental conditions, using up to 5 μmol/L CNBr-digested fibrinogen (Fig 2, insert B). CNBr-digested fibrinogen (final concentration, 100 nmol/L) did not significantly affect the rate or extent of generation of an active site in equimolar (100 nmol/L) mixtures of intact or low-M₆ plasminogen and STAR, as monitored with S-2251 (data not shown).

In additional experiments, a similar concentration-dependent generation of PAP in mixtures of native plasminogen and α2-antiplasmin was observed in the presence of poly-L-lysine (final concentration, 1 to 10 μmol/L), with a maximal rate of PAP generation of approximately 0.5 nmol/L/s, which was reached at 5 μmol/L poly-L-lysine, and with a half-maximal rate of PAP generation at 2 μmol/L. In contrast, in the presence of 6-AHA (final concentration, 50 nmol/L to 1 μmol/L) no significant PAP generation was observed under the same experimental conditions (data not shown).

Addition of 10 nmol/L STAR to mixtures of native plasminogen (final concentration, 1.5 μmol/L) and NPGB (final concentration, 100 μmol/L) did not generate plasmin within 2 hours at 37°C, neither in the absence nor in the presence of up to 5 μmol/L CNBr-digested fibrinogen, as monitored by SDS-PAGE under reducing conditions (not shown).

Effect of fibrin on the activation by STAR or SK of the plasma fibrinolytic system. With 30 nmol/L STAR, no significant activation of the fibrinolytic system within 2 hours at 37°C was observed in plasma in the absence of a fibrin clot, as shown by unaltered levels of fibrinogen, plasminogen, and α2-antiplasmin and negligible levels of PAP (Fig 3A). Essentially the same results were obtained after incubation for 6 hours (data not shown). In the presence of a fibrin clot (Fig 3C), a significant decrease of the fibrinogen concentration to 78% ± 5% (mean ± SD, n = 3) of baseline at 2 hours, when complete clot lysis had occurred, was observed (P < .003 v value in the absence of clot), whereas the α2-antiplasmin levels had decreased to 65% ± 16% of baseline (P = .04 v value in the absence of clot). Plasminogen levels in these mixtures could not adequately be determined due to interference of the fibrin degradation fragments with the functional plasminogen assay. As judged from the PAP concentration, measured by ELISA, 20% ± 8% of the plasminogen was converted into a complex with α2-antiplasmin.

In contrast, with 100 nmol/L SK, extensive fibrinolytic activation was observed in plasma in the absence of a fibrin clot (Fig 3B), as shown by residual fibrinogen, plasminogen, or α2-antiplasmin levels after 2 hours at 37°C of 15% ± 2%, 12% ± 5%, or 5% ± 5% of baseline, respectively. The PAP concentration, as measured by ELISA, showed conversion of 53% ± 19% of the plasminogen into PAP complex. In the presence of a fibrin clot (Fig 3D), similar results were obtained, with residual fibrinogen levels of 15% ± 2% of baseline (P = NS v absence of clot), residual α2-antiplasmin levels of 5% ± 5% of baseline (P = NS v absence of clot), and generated PAP levels corresponding to conversion of 49% ± 16% of the plasminogen to PAP complex (P = NS v value in the absence of clot). Similar results for PAP levels were obtained by SDS-PAGE of plasma samples containing a trace amount of [125I]-labeled plasminogen and quantitation of radioactive gel bands (Fig 3, insert): these values were 0.5% and 31% for STAR in the absence and in the presence of a
fibrin clot, respectively, with corresponding values of 62% or 55%, respectively, for SK.

Addition of STAR or SK (final concentration, 30 nmol/L or 100 nmol/L, respectively) to 500 μL plasma containing a 60-μL 125I-fibrin–labeled plasma clot in the presence of 50 mmol/L 6-AHA, did not result in significant clot lysis (≤5% after 5 hours; data not shown). However, residual fibrinogen levels decreased to 40% of baseline (mean of two experiments) after a 1-hour incubation with SK, while with STAR, no fibrinogen degradation was observed (≥88% residual fibrinogen after 4 hours). Generated PAP levels after a 1-hour incubation were 103% ± 8% with SK (mean ± SD, n = 3), while with STAR no PAP could be detected (<1.5% after 5 hours). In separate experiments, addition of a preformed 125I-plasmin-STAR complex to normal human plasma with or without a fibrin clot, resulted in rapid and nearly quantitative complex formation with α2-antiplasmin (not shown).

**Effect of fibrin on the activation of the fibrinolytic system with STAR in α2-antiplasmin–depleted plasma.** Fibrinogen degradation was monitored for 6 hours following addition of STAR or plasmin-STAR complex (final concentration, 30 nmol/L) to normal human plasma or to α2-antiplasmin–depleted plasma, both without and with ad-
Addition of CNBr-digested fibrinogen as a fibrin substitute (final concentration, 1 μmol/L). Residual fibrinogen levels 6 hours after addition of STAR were greater than 90%, both in normal plasma and in α2-antiplasmin–depleted plasma (Fig 4). Addition of CNBr-digested fibrinogen resulted in fibrinogen degradation to 50% of baseline within 2 hours in normal plasma and to 20% of baseline within 20 minutes in α2-antiplasmin–depleted plasma.

Addition of 30 nmol/L preformed plasmin-STAR complex to normal plasma did not produce significant fibrinogen breakdown within the 6-hour observation period at 37°C. However, in α2-antiplasmin–depleted plasma, 30 nmol/L plasmin-STAR induced rapid and extensive fibrinogen breakdown (to 30% of baseline within 20 minutes) (Fig 4), whereas lower concentrations of plasmin-STAR (<20 nmol/L) did not induce fibrinogen breakdown (data not shown). This apparent discrepancy can probably be explained by the presence of approximately 2% (20 nmol/L) residual α2-antiplasmin in the depleted plasma, as confirmed by the conversion of approximately 60% of 30 nmol/L 125I-plasmin-STAR into PAP complex on SDS-PAGE (data not shown).

Stability of STAR and SK in a plasma milieu. STAR (final concentration, 30 nmol/L) or SK (final concentration, 100 nmol/L) was preincubated for 4 hours at 37°C with 500 μL normal human plasma before addition of a 60-μL 125I-fibrin–labeled clot prepared from whole plasma or from plasminogen-depleted plasma. With STAR, residual fibrinogen, plasminogen and α2-antiplasmin levels before addition of the clot were (mean ± SD, n = 3) 82% ± 10%, 81% ± 6%, and 98% ± 15%, respectively, and the level of PAP was 53% ± 11% (Fig 5B, insert). After preincubation of STAR for 4 hours in plasma, time-dependent lysis of both a whole-plasma clot and a plasminogen-depleted plasma clot was obtained, comparable to that obtained without preincubation (Fig 5A). With SK, the time course of the lysis of a whole-plasma clot was also similar with or without preincubation. However, after preincubation of SK for 4 hours, no detectable lysis of a plasminogen-depleted plasma clot was observed within 2 hours, whereas without preincubation 30% clot lysis was obtained (Fig 5B).

The stability of STAR in human plasma was further confirmed in an experiment with repeated addition of a freshly prepared plasma clot at different time points. With 30 nmol/L STAR, complete clot lysis was obtained within 2 hours, with residual fibrinogen levels of (mean ± SD, n = 3) 73% ± 8% and generated PAP levels of 29% ± 1%. After clot lysis, these levels remained unaltered for up to 8 hours (Fig 6). Addition of a second plasma clot to the incubation mixture after 3 hours again resulted in complete clot lysis within 2 hours, with residual fibrinogen levels of 48% ± 8% and generated PAP levels of 52% ± 2%. Addition of a third plasma clot after 6 hours still resulted in quantitative clot lysis within 2 hours, with corresponding fibrinogen and PAP levels of 27% ± 2% and 55% ± 3%, respectively (Fig 6).

**DISCUSSION**

Staphylokinase (STA) forms a 1:1 stoichiometric complex with plasminogen,29 which exposes an active site only after conversion of the plasminogen molecule to plasmin. The plasmin-STA complex is rapidly inhibited by α2-antiplasmin,1,3,4 but this results in dissociation of functionally active STA from the complex and recycling of STA to other plasminogen molecules. Nevertheless, STAR is a relatively fibrin-specific agent in a human plasma milieu, which causes little systemic fibrinolytic activation both in the ab-
In the present study, we investigated this apparent paradox by measuring the rate and extent of generation of plasmin-STAR complex in mixtures of plasminogen and α2-antiplasmin in buffer and in human plasma in vitro, both in the absence and in the presence of fibrin. The extent of plasmin-STAR complex generation was monitored by quantitation of the levels of PAP, a product of the reaction of plasmin-STAR with α2-antiplasmin.

Our results, obtained in mixtures of purified plasminogen

Fig 4. Fibrinogen degradation in normal (solid symbols) or α2-antiplasmin depleted (open symbols) plasma, following addition of 30 nmol/L STAR (△, ▽) or 30 nmol/L plasmin-STAR (●, ○). The effect of CNBr-digested fibrinogen (final concentration, 1 μmol/L) on fibrinogen breakdown with 30 nmol/L STAR is also shown (▲, ▼). Residual fibrinogen levels, expressed as the percentage of baseline, are plotted as a function of time. Data represent the mean ± SD of three experiments.
FIBRIN SPECIFICITY OF STAPHYLOKINASE

Fig 5. Time course of the lysis of a fibrin-labeled plasma clot prepared from whole plasma (C) or from plasminogen-depleted plasma (D, A), submerged in plasma, with 30 nmol/L STAR (A) or 100 nmol/L SK (B) without preincubation (open symbols) or after preincubation for 4 hours at 37°C (solid symbols). The inserts show the residual fibrinogen (1), plasminogen (2), and α2-antiplasmin (3) levels, expressed in percent of the baseline value, and the PAP levels (4), expressed as the percentage of the plasminogen concentration, after preincubation of plasma with 30 nmol/L STAR or 100 nmol/L SK for 4 hours at 37°C. Data represent the mean ± SD of three experiments.

and α2-antiplasmin, show that α2-antiplasmin efficiently prevents activation of plasminogen by STAR, but not by SK. Addition of a purified fibrin clot or of CNBr-digested fibrinogen, a substitute for solid-phase fibrin, triggers activation of native plasminogen, but not of low-M₆ plasminogen by STAR. In these experiments, a twofold molar excess of α2-antiplasmin over plasminogen was used to allow rapid and quantitative complexation of generated plasmin, which is required for quantitative assay of generated PAP. Furthermore, addition of 6-AHA, which impairs inhibition of the plasmin-STAR complex by α2-antiplasmin to a similar extent as CNBr-digested fibrinogen,Δ does not trigger generation of PAP, whereas poly-L-lysine as a substitute for solid-phase fibrinΔ does stimulate generation of PAP. These findings indicate that the effect of fibrin cannot be restricted to delaying the inhibition of plasmin-STAR by α2-antiplasmin, but that fibrin facilitates generation of plasmin-STAR complex. Alternatively, fibrin may enhance the activation rate of plasminogen by STAR as a result of enhanced plasmin-catalyzed conversion of Glu-plasminogen to Lys-plasminogen or of conformational alterations in the plasminogen molecule. The catalytic efficiency of the plasmin-STAR complex for activation of Lys-plasminogen indeed is twofold to threefold higher than that for activation of Glu-plasminogen.Δ However, we have previously shown that addition of saturating concentrations of fibrin-like stimulators (des AA fibrin or CNBr-digested fibrinogen) results in an enhancement of the activation rate of plasminogen by STAR of maximally fourfold, as compared with twofold for the activation by SK.Δ

The observation that no plasminogen activation by STAR occurs in the presence of excess NPGB confirms that generation of traces of plasmin-STAR complex is essential to initiate the activation process, as shown previously.Δ Addition of fibrin does not protect plasmin-STAR from inhibition by NPGB, again indicating that the protective effect of α2-antiplasmin requires additional interactions involving the lysine-binding sites.

In a human plasma milieu in vitro, in both the presence and the absence of fibrin, STAR induces little systemic
The stability of STAR in human plasma and the potentiating effect of fibrin on the activity of STAR are further illustrated by repetitive addition of plasma clots to mixtures of plasma and STAR. Indeed, after complete lysis of a first plasma clot within 2 hours, systemic fibrinolytic activation halts, but resumes after addition of a second plasma clot, which is lysed with equal efficiency as the first clot. However, no information is available on the nature of the fibrin degradation products present in the plasma after clot lysis.

Our results suggest that in the absence of fibrin, but in the presence of physiologic concentrations of plasminogen and α2-antiplasmin, STAR does not activate plasminogen in plasma at appreciable rates. In the presence of fibrin, generation of plasmin-STAR complex appears to be facilitated, whereas generated complex is protected from rapid inhibition by α2-antiplasmin via a mechanism involving the lysine-binding sites of the plasmin moiety. Plasmin-STAR complex at the fibrin surface may thus efficiently activate plasminogen. Binding of plasmin-STAR to fibrin does probably not result in dissociation of the complex (kd, 55 nmol/L),8 as this would destroy the active plasminogen activator moiety. These regulatory properties of fibrin and α2-antiplasmin provide a comprehensive explanation of the fibrinspecificity of STAR in a plasma milieu.

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FIBRIN SPECIFICITY OF STAPHYLOKINASE


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