A Kinetic Analysis of the Tissue Plasminogen Activator and DSPAα1 Cofactor Activities of Untreated and TAFIα-treated Soluble Fibrin Degradation Products of Varying Size*

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The kinetics of tissue plasminogen activator (t-PA) and DSPAα1-catalyzed plasminogen activation using untreated and TAFIα-treated fibrin degradation products (FDPs), ranging in weight average molecular weight (Mw) from 0.48 × 10^⁶ to 4.94 × 10^⁸ g/mol, were modeled according to the steady-state template model. The FDPs served as effective cofactors for both activators. The intrinsic catalytic efficiencies of both t-PA (17.4 × 10⁶ m⁻¹ s⁻¹) and DSPAα1 (6.0 × 10⁵ m⁻¹ s⁻¹) were independent of FDP Mw. The intrinsic catalytic efficiency of t-PA was 12-fold higher than that measured under identical conditions with intact fibrin as the cofactor. At sub-saturating levels of cofactor and substrate, rates were strongly dependent on FDP Mw with DSPAα1 but not t-PA. Loss of activity with decreasing FDP Mw correlated with loss of finger-dependent binding of the activators to the FDPs. TAFIα treatment of the FDPs resulted in 90- and 215-fold decreases in the catalytic efficiencies of t-PA (0.20 × 10⁶ m⁻¹ s⁻¹) and DSPAα1 (0.028 × 10⁶ m⁻¹ s⁻¹), yielding cofactors that were still 30- and 50-fold better than fibrinogen with t-PA and DSPAα1, respectively. Our results show that for both activators the products released during fibrinolysis are very effective cofactors for plasminogen activation, and both t-PA and DSPAα1 cofactor activity are strongly down-regulated by TAFIα.

Hemeostasis requires a proper balance between the coagulation and fibrinolytic systems. In response to vascular injury, a hemostatic plug is generated by converting fibrinogen to an insoluble fibrin clot through the action of thrombin, the terminal enzyme of the coagulation cascade. Fibrinolysis, the breakdown of the fibrin clot, is achieved primarily by the activation of plasminogen to the serine protease plasmin, which catalyzes degradation of the insoluble fibrin clot to soluble fibrin degradative products (FDPs). The activation of plasminogen can be catalyzed by both endogenous activators such as tissue-type plasminogen activator (t-PA) and urokinase or exogenous activators such as streptokinase, staphylokinase, and Desmodus rotundus salivary plasminogen activators (DSPAs). These enzymes have all been used as thrombolytic agents for the dissolution of pathological thrombi, which can cause both myocardial infarction and stroke.

The fibrin clot is not only the substrate for plasmin but also a cofactor for plasmin generation by the various plasminogen activators. Both t-PA and DSPAα1 are known as fibrin-selective plasminogen activators, because the rate of plasminogen activation with both activators is increased several orders of magnitude in the presence of fibrin, as compared with fibrinogen (1). Extensive plasminogen activation in the plasma, mediated via the cofactor effect of fibrinogen, results in systemic, plasmin-mediated fibrinogenolysis and consumption of α2-antiplasmin, severely compromising the coagulation potential of the plasma (1, 2). Fibrin selectivity is thus highly desirable for systemically administered thrombolytic agents. DSPAα1 is considerably more fibrin-selective than t-PA, as the catalytic efficiency of DSPAα1 is stimulated 13,000-fold, compared with only 820-fold for t-PA, when fibrin is the cofactor instead of fibrinogen (1). Furthermore, DSPAα1 is intrinsically less fibrinogenolytic than t-PA because the catalytic efficiency of DSPAα1 is 13-fold lower than t-PA when fibrinogen is the cofactor (50 versus 640 m⁻¹ s⁻¹) (1).

The stimulation of plasminogen activation by fibrin is mediated by interactions of the two activator and plasminogen with fibrin (3). Structures within t-PA by which it interacts with fibrin are its fibronectin finger-like domain and its kringle-2 domain. The interaction of DSPAα1 with fibrin is presumably mediated solely by its finger domain, since it lacks a kringle-2 domain, although at least one other low affinity interaction is likely, because DSPAβ and DSPAγ, highly homologous relatives of DSPAα1 (89 and 91% identity, respectively) lacking the finger domain, also stimulated by fibrin, albeit to a much lesser extent (1). The interaction of plasminogen with fibrin occurs by its lysine-binding kringle domains. Two forms of plasminogen exist. Glu-plasminogen, the full-length form found circulating in plasma, interacts only weakly with intact fibrin but strongly with partially degraded fibrin possessing carboxyl-terminal lysine and/or arginine residues (4). Lys-plasminogen, a truncated version of plasminogen produced by the plasmin-catalyzed removal of a 77-residue peptide from the amino terminus, binds to both native and partially degraded fibrin tightly (4). Lys-plasminogen is a considerably better substrate for t-PA, and its formation during t-PA-mediated fibrinolysis confers positive feedback on the process (3, 4). Since the cleavage of fibrin by plasmin exposes carboxyl-terminal lysine and arginine residues, producing a fibrin surface containing high affinity plasminogen-binding sites, partial degradation of fibrin by plasmin results in the recruitment of plasminogen to the partially degraded fibrin surface (5–7). The partially de-
graded fibrin is a superior cofactor than intact fibrin for plasminogen activation (8). This effect can be eliminated by the basic plasma carboxypeptidase, TAFIa, which removes the carboxyl-terminal lysine and arginine residues from the fibrin surface (8). Plasmin production, therefore, results in a positive feedback loop that can be down-regulated through the generation of TAFIa from its precursor TAFI.

Plasmin-catalyzed digestion of fibrin produces soluble FDPs which, owing to their structural similarity to fibrin and/or fibrinogen, likely act as cofactors for plasminogen activation. We have recently demonstrated that FDPs released from a perfused clot are composed of noncovalently associated products whose masses range from 250 kDa (the mass of DD/E) to ~10,000 kDa (9). Furthermore, our work showed that the majority of the FDPs compose structures much larger than DD/E. Since the relationship between the sizes of FDPs and their cofactor effects has not been studied, we isolated FDPs with different masses to study the relationship between FDP mass and cofactor activity in reactions with t-PA and DSPA1. Furthermore, since the FDPs contain carboxyl-terminal lysine and arginine residues as a result of plasmin degradation, we also investigated the effect of the TAFIa-catalyzed removal of the carboxyl-terminal lysine and arginine residues on the cofactor activity of FDPs. The work described in this paper represents the first extensive study of the cofactor effect of FDPs on plasminogen activation catalyzed by the fibrin-specific plasminogen activators, t-PA and DSPA1.

**EXPERIMENTAL PROCEDURES**

**Materials—**Human fibrinogen, plasminogen (Pgn), prothrombin, and factor XIII (FXIII), were prepared as described previously (9). Plasmin (Pn), thrombin (Ila), and FXIIIa were prepared from the purified zymogens as described (9). Recombinant human thrombin-activatable fibrinolysis inhibitor (TAFI) was produced as described by Rofa et al. (10). The recombinant, active site serine to cysteine mutant of human plasminogen, plasminogen(ST741C), was produced as described by Horrevoets et al. (11). The florescein derivative, plasminogen(ST741C-fluorescein), hereafter referred to as 5AF-Pgn, was produced as described (11). A soluble form of human thrombomodulin, Solulin, was a kind gift of Dr. John Morser of Berlex Biosciences (Richmond, CA). The recombinant plasminogen activator from the vampire bat Desmodus rotundus, DSPA1, was a kind gift of Dr. Peter Bringmann at Schering, AG (Berlin, Germany). The recombinant tissue-type plasminogen activator, activase, was a kind gift from Dr. Gordon Vehar at Genentech (South San Francisco, CA).

**Preparation of FXIIIa Cross-linked Soluble Fibrin Deposition Products—**Soluble FDPs were prepared as described previously (9), except that 1) clots were formed in columns with 9 ml of available volume, 2) the clots were perfused with 0.2 mM plasmin instead of 0.05 mM plasmin, and 3) the FDPs from four perfusions were pooled prior to gel filtration. The pooled FDPs (~25 mg) were subjected to gel filtration on Sephacryl S-1000 and the weight average molecular weight (\(M_w\)) of the FDPs in the eluate was determined on-line using multangle laser light scattering (9). Samples from the gel filtration column having 

\[
E = \frac{E_{0} - E}{E_{0}}
\]

were concentrated to about 10 mM by centrifugal concentration as described (9). The concentration of the FDPs refers to the concentration of fragment X equivalents present in the sample. Samples were stored at 0.2 mM Hepes, 0.5 M NaCl, 0.001% Tween 80, pH 7.4 at 4 °C.

**Activation of 5AF-Pgn by t-PA and DSPA1 in the Presence of Soluble FDP Cofactors—**The activation of 5AF-Pgn (to 5AF-Pn) by t-PA and DSPA1 in the presence of soluble FDP cofactors, both native and TAFIa-treated (see below), was monitored by fluorescence spectroscopy as described previously (3). Aliquots (90 μl) of 5AF-Pgn, in 0.2 mM Hepes, 0.053 mM NaCl, 0.0256% Tween 80, 2.56 mM CaCl2, pH 7.4, were added to 2.56 mM CaCl2, 500 μl of 5AF-Pgn and the fluorescence of the samples was measured at 535 nm (3 nm slit) using a 515 nm cut-off filter. FDPs (25 μl) were then added to the wells, and the fluorescence of the 5AF-Pgn/FDP samples was determined over 10–30 min. The combination of 5AF-Pgn and FDPs gave a solution containing 0.02 μM Hepes, 0.15 μM NaCl, 2 mM CaCl2, 0.02% Tween 80, pH 7.4. Plasminogen activation reactions containing FDPs (33.3–500 nM final) and 5AF-Pgn (33.3–500 nM final) were then initiated by the addition of 15 μl of either t-PA (1–40 nM final) or DSPA1 (4–10 nM final), and the fluorescence of the reactions was monitored every 80 s for 80 min. Both t-PA and DSPA1 were in 0.02 mM Hepes, 0.15 mM NaCl, 2 mM CaCl2, 0.02% Tween 80, pH 7.4. The fluorescence of 5AF-Pgn in reactions without FDPs was determined identically to the reactions described above, except that 0.02 mM Hepes, 0.5 mM NaCl, 0.001% Tween 80, pH 7.4, was used in place of the FDPs. All experiments were performed at ambient temperature (~20 °C).

**Binding of 5AF-Pgn to Soluble FDP Cofactors of Varying Sizes—**The binding of 5AF-Pgn to the FDPs was measured based on the decrease in fluorescence of the 5AF-Pgn in the presence of FDPs. Binding was determined by measuring the fluorescence of the reactions containing 5AF-Pgn and FDPs prior to the addition of the activator (I) and subtracting it from the measured fluorescence of the 5AF-Pgn in each reaction prior to the addition of FDP, corrected for dilution (I). The dilution factors accounting for the fluorescence change upon addition of FDPs and activator were determined from the experiments described above in which no FDPs were used. The difference in fluorescence \(\Delta I = I - I_0\) was measured for all concentrations of Pgn and FDP, and the binding was analyzed by nonlinear regression of the data according to Equation 1,

\[
\Delta I = (\frac{[5AF-Pgn]}{[5AF-Pgn] + \Delta_{bound}}) \times \Delta_{bound} \quad \text{(Eq. 1)}
\]

where \([5AF-Pgn]\) is the concentration of bound 5AF-Pgn, and \(\Delta_{bound}\) is the difference in fluorescence coefficients (fluorescence units/5AF-Pgn) between free and bound 5AF-Pgn. These coefficients are referred to as IC_{free} and IC_{bound}, respectively. The concentration of bound 5AF-Pgn is found from the quadratic binding Equation 2

\[
[5AF-Pgn] = \frac{[5AF-Pgn] + [FDP] + K_I - \sqrt{(5AF-Pgn) + [FDP] + K_I}^2 - 4[5AF-Pgn][FDP]}{2} \quad \text{(Eq. 2)}
\]

where \([5AF-Pgn]\) and \([FDP]\) are the total concentrations of 5AF-Pgn and FDP, respectively, and \(K_I\) is the binding constant for the Pgn-FDP interaction. The data were fit globally by nonlinear regression to Equation 2. The independent variables were \([5AF-Pgn]\) and \([FDP]\); the dependent variable was \(I_0\), and the best fit parameters were \(\Delta_{bound}\) and \(K_I\). IC_{free} is known as the fluorescence intensity of free 5AF-Pgn divided by its concentration.

\[
I_0 = 1 - \frac{[5AF-Pgn] + [FDP]}{IC_{free}} \times \Delta_{bound} \quad \text{(Eq. 3)}
\]

**Treatment of FDPs with TAFIa—**TAFIa was activated to TAFIa by thrombin in the presence of Solulin essentially as described previously (10). Briefly, 1.0 μM TAFIa was reacted with 20 nM thrombin, 80 nM Solulin in 0.02 mM Hepes, 0.15 mM NaCl, 5 mM CaCl2, 0.001% Tween 80, pH 7.4, for 10 min at ~22 °C. The TAFIa was then stored on wet ice until used. A TAFIa titration was performed to determine the amount of TAFIa required to achieve maximal “deactivation” of the FDPs. The titration was based on the activation of 5AF-Pgn in the presence of FDPs treated with or without varying amounts of TAFIa. The 1.93 × 10^6 g/mol FDP was used as the cofactor and 5 μM DSPA1 as the plasminogen activator. FDPs (2.6 μM) were treated with varying concentrations of TAFIa (0.1–30 nM) for 60 min at room temperature. The TAFIa/FDP solutions were diluted in half with 0.02 mM Hepes, 0.5 mM NaCl, 0.001% Tween 80, pH 7.4, and a 25-μl aliquot was added to 90 μl of 180 nM 5AF-Pgn in the wells of a fluorescence microtiter plate. DSPA1 (15 μl of 43.3 nM) was added to initiate cofactor-dependent activation, and the fluorescence of the reaction was monitored over time (see below). Based on the results of the TAFIa titration, the FDP samples were treated in place of the FDPs. The FDPs (2.6 μM) were in 0.02 mM Hepes, 0.5 mM NaCl, 0.001% Tween 80, pH 7.4, and were treated with 10 nM TAFIa for 60 min at room temperature followed by a 2-h incubation at 37 °C which served to both maximally inactivate the FDPs and inactivate the TAFIa. The TAFIa-treated FDPs were stored at 4 °C.

**Data Analysis—**The rates of 5AF-Pgn activation were determined from the initial slopes of the activation reactions, using the fact that conversion of 5AF-Pgn to 5AF-Pn results in a 50% decrease in the...
Equation 4 is given by

\[ [5AF-Pgn] \text{ concentration; varying the } [Pgn] \text{ and concentrated to obtain the different } \]

show the fractions that were pooled boxes the volume of the eluate. The state template model for plasminogen activation as described by Hor- 

Since the end point fluorescence of the reactions was independent of the fluorescence, the difference between the fluorescence of the 5AF-Pgn/FDP solutions and the end point fluorescence for the 5AF-Pgn concentration defined the full-scale fluorescence change upon complete conversion of 5AF-Pgn to 5AF-Pn.

The data from the reactions were modeled according to the steady-state template model for plasminogen activation as described by Hor-revoets et al. (3), with the terms for fibrin substituted by FDPs. The rate Equation 4 is given by

\[ \text{rate} = \frac{k_{\text{cat}}[\text{Pgn}]_{\text{free}} [\text{FDP}]_{\text{app}}}{K_m(\text{cat} + [\text{FDP}] + [\text{Pgn}]_{\text{free}}(K + [\text{FDP}]_{\text{app}}))} \]  

where rate is the velocity of the reaction per nominal activator concentration; \( k_{\text{cat}} \) is the turnover number for the reaction; \([\text{Pgn}]_{\text{free}} \) is the concentration of free plasminogen (calculated from the above binding Equation 4); \([\text{FDP}]_{\text{app}} \) is the total FDP concentration; \( K_m \) is the Michaelis constant for the reaction; \( K_a \) is the dissociation constant of the activator for the FDPs, and \( K \) is a constant whose value is equal to the concentration of FDPs required to give a rate equal to half \( k_{\text{cat}} \) at saturating Pgn.

The data from the experiments were initially analyzed using direct plots. The reaction rates at each \([\text{FDP}]_{\text{app}} \) were plotted against the \([\text{Pgn}]_{\text{free}} \) and the resulting curves were analyzed by nonlinear regression according to the Michaelis-Menten equation for each set of reaction conditions (see Equation 5),

\[ \text{rate} = \frac{k_{\text{cat}}[\text{Pgn}]_{\text{free}} [\text{FDP}]_{\text{app}}}{K_m(\text{cat} + [\text{FDP}]_{\text{app}} + [\text{Pgn}]_{\text{free}}(K + [\text{FDP}]_{\text{app}}))} \]  

where \( k_{\text{cat}}(\text{app}) \) and \( K_m(\text{app}) \) are the apparent \( k_{\text{cat}} \) and \( K_m \) measured by varying the \([\text{Pgn}]_{\text{free}} \) at a given FDP concentration. The ratio \( k_{\text{cat}}(\text{app})/K_m(\text{app}) \) was plotted against \([\text{FDP}]_{\text{app}} \) and analyzed by nonlinear regression according to Equation 6,

\[ \frac{k_{\text{cat}}(\text{app})}{K_m(\text{app})} = \frac{[\text{FDP}]_{\text{app}}}{K_a + [\text{FDP}]_{\text{app}}} \]  

by using the relationships (Equations 7 and 8)

\[ k_{\text{cat}}(\text{app}) = \frac{k_{\text{cat}}[\text{FDP}]_{\text{app}}}{K_a + [\text{FDP}]_{\text{app}}} \]  

\[ K_m(\text{app}) = \frac{K_m(K_a + [\text{FDP}]_{\text{app}})}{K_a + [\text{FDP}]_{\text{app}}} \]  

to obtain values for the true \( k_{\text{cat}}/K_m \) ratio and \( K_a \). Finally, the data from all reactions for each activator were analyzed globally, according to Equation 4.

The rates of 5AF-Pn formation from the experiments using the TAFIa-treated FDPs did not show saturation with respect to \([\text{Pgn}]_{\text{free}} \), at any FDP concentration for all TAFIa-treated FDPs. We may assume, therefore, that for all TAFIa-treated FDPs, \( K_m(\text{app}) \) is much greater than \([\text{Pgn}]_{\text{free}} \) in all reactions (from Equation 5). Furthermore, since binding of 5AF-Pgn to TAFIa-treated FDPs was not detected in these experiments, \([\text{Pgn}]_{\text{free}} \) can be set equal to \([\text{Pgn}]_{\text{free}} \). By using these assumptions, the rate obtained at each TAFIa-treated FDP concentration was plotted against \([\text{Pgn}]_{\text{free}} \), using Equation 9, a modified form of Equation 5, to obtain values for the \( k_{\text{cat}}(\text{app})/K_m(\text{app}) \) ratio at each FDP concentration.

\[ k_{\text{cat}}(\text{app}) \approx \frac{[\text{FDP}]_{\text{app}}}{K_m(\text{app})} \]  

The Cofactor Activity of FDPs

Isolation of FDPs with different \( M_w \) Ranging from \( 0.48 \times 10^6 \) to \( 4.94 \times 10^6 \) g/mol—FDPs were made using a perfused clot system and subjected to gel filtration on Sephacryl S-1000. The \( M_w \) of the FDPs in the eluate was determined on-line using multiangle laser light scattering (9). Fig. 1 shows a plot of the protein concentration according to Equation 6, a modified form of Equation 5.

\[ k_{\text{cat}}(\text{app}) = \frac{k_{\text{cat}}[\text{FDP}]_{\text{app}}}{K_a + [\text{FDP}]_{\text{app}}} \]  

The Effect of TAFIa on t-PA- and DSPAa1-mediated Fibrinolysis in Plasma—Clots (200 µl), made from 66.6 µl of TAFIa-deficient human plasma, 56 µl of 0.02 M Hapes, 0.15 M NaCl, 0.02% Tween 80, pH 7.4, 4 µl of 500 nM Solulin, 20 µl of 6 nT-PA or DSPAa1, 20 µl of 0–200 nT TAFI, and 33.3 µl of 30 nM thrombin, 60 nM CuCl2 were formed in the wells of a microtiter plate. The clotting and subsequent lysis of the clots were monitored by turbidity at 405 nm at 37 °C. The lysis time, the time at which the turbidity has decreased to one-half the maximal plateau value, was determined for each sample, and the results are presented as relative lysis times, which are the lysis times for each reaction divided by the lysis time for the reaction in the absence of TAFI.

RESULTS

Isolation of FDPs with \( M_w \) Ranging from \( 0.48 \times 10^6 \) to \( 4.94 \times 10^6 \) g/mol—FDPs were made using a perfused clot system and subjected to gel filtration on Sephacryl S-1000. The \( M_w \) of the FDPs in the eluate was determined on-line using multiangle laser light scattering (9). Fig. 1 shows a plot of the protein concentration and corresponding \( M_w \) of the FDPs versus the volume of the eluate. The fractions that were pooled to give FDP samples having \( M_w \) ranging from \( 0.48 \times 10^6 \), \( 1.08 \times 10^6 \), \( 1.93 \times 10^6 \), \( 3.08 \times 10^6 \), \( 3.97 \times 10^6 \) and \( 4.94 \times 10^6 \) g/mol are indicated by shading.

Binding of 5AF-Pgn to FDPs—During the course of the t-PA/ DSPAa1 cofactor activity experiments (see below), it was found that the fluorescent plasminogen derivative, 5AF-Pgn, displayed a reduced fluorescence in the presence of the FDPs relative to 5AF-Pgn alone. We used this property to investigate the binding of the 5AF-Pgn to the FDPs. The fluorescence changes versus the concentration of FDP were collected for all FDP sizes at all 5AF-Pgn concentrations. The data from each
Cofactor Activity of FDPs

with t-PA exhibit Michaelis-Menten kinetics at any fixed FDP concentration for each of the different FDP samples when the substrate concentration is expressed as free 5AF-Pgn (Equation 5). The kcat(app) was found to increase with increasing FDP concentration, as expected in a cofactor-mediated, template-dependent reaction. No significant differences in kcat(app) between the different FDP samples, at any particular FDP concentration, were found. With the exception of the smallest FDP (Mw = 0.48 × 10^6 g/mol), the Km(app) value for the reactions was fairly insensitive to the FDP concentration. This is consistent with reactions where K0 g = K (Equation 8). For the FDP of Mw = 0.48 × 10^6 g/mol, the Km(app) was found to decrease with increasing FDP concentration, consistent with K0 g > K for this FDP. The data indicate that the true Km(app) value for reactions with t-PA (obtained at saturating FDP concentration, Equation 8) is the same for all FDPs, regardless of Mw. The kcat(app)/Km(app) ratios for all FDPs increase with increasing FDP concentration, indicative of a template mechanism.

The kcat(app), Km(app), and kcat(app)/Km(app) values for the reactions with DSPAα1, derived from the direct Michaelis-Menten plots, are shown in Table II. The values for kcat(app) and Km(app) for the small FDPs of Mw = 0.48 × 10^6 and 1.08 × 10^6 g/mol could not be determined individually, since the plots did not exhibit saturable kinetics over the concentrations of 5AF-Pgn and FDPs used (Fig. 4). For the FDPs of higher Mw the plots exhibited an approach to saturation with respect to substrate concentration and the kcat(app) increased with increasing FDP concentration. The data show that for FDPs with Mw ≥ 1.93 × 10^6 g/mol, the kcat(app) with DSPAα1 was insensitive to the Mw of the FDPs. In contrast to that seen with t-PA, the Km(app) for the larger FDPs was found to increase with increasing FDP concentration, indicating that the Km(app) < K for these reactions (Equation 8). Although the lack of saturation in the Michaelis-Menten plots of the reactions with the smaller FDPs (0.48 × 10^6 and 1.08 × 10^6 g/mol) precluded separate determinations of the kcat(app) and Km(app), and therefore any conclusions regarding the relationship between K0 g and K, the behavior of the kcat(app)/Km(app) with respect to FDP concentration was found from the slopes of the direct plots. Since the apparent catalytic efficiency was found to be a function of the FDP concentration, these data indicate that the smaller FDPs act as cofactors. The larger FDPs were found to influence plasminogen activation in a manner fairly independent of FDP size, as indicated by the behavior of the kcat(app)/Km(app) ratios with respect to FDP concentration (Table II).

By using the results from the direct plots as a guide, we fit the experimental data from each of the FDP samples with t-PA as the activator to the steady-state template model (Equation 4). When the data from all 5AF-Pgn and FDP concentrations were regressed together, we were unable to assign values simultaneously for all four parameters (kcat, Km, K0 g, and K) for each FDP size. We could, however, determine values for kcat, KA, and K, using a single value of Km, or for kcat, Km, and K, using a single value for KA, for all FDP sizes. From these two fits of the data, we found that the values for kcat and K were essentially invariant, with respect to FDP Mw, when either the Km was fixed for all FDPs (kcat = 0.065–0.081 s⁻¹, K = 122–198 nM) or when the KA was fixed for all FDPs (kcat = 0.076–0.091 s⁻¹, K = 140–300 nM). Since the results from the direct Michaelis-Menten plots (Table I) suggested that the true Km value (obtained at saturating FDP concentration) is likely the same for all FDP samples when t-PA was used as the activator, and since kcat and K values were essentially unaffected by fixing either Km or KA, we modeled the t-PA cofactor activities for each FDP size according to differences in KA, using a single Km for all FDP sizes. The results of the regression analysis are

**Fig. 2. Binding of 5AF-Pgn to FDPs of different Mw.** The binding of 5AF-Pgn to the FDPs was determined as described under "Experimental Procedures." The figure shows the fluorescence intensity of the 5AF-Pgn/FDP mixtures, relative to free 5AF-Pgn (I0), versus the FDP concentration at 500 nM 5AF-Pgn. The FDPs of different Mw (g/mol) are shown by open circles (0.48 × 10^6), open squares (1.08 × 10^6), open triangles (1.93 × 10^6), closed circles (3.08 × 10^6), closed squares (3.97 × 10^6), and closed triangles (4.94 × 10^6). The 5AF-Pgn bound to the FDPs with a global K0 g of 225 ± 60 nM. The fluorescence decrement of FDP-bound 5AF-Pgn relative to free 5AF-Pgn (ΔIbound/I0) was dependent on the Mw of the FDP as follows: ΔIbound/I0 = 5.5 ± 0.8, 11.6 ± 1.2, 19.4 ± 1.8, 20.6 ± 1.9, 20.5 ± 1.9, and 21.7 ± 2.0% for the FDPs having Mw (g/mol) of 0.48 × 10^6, 1.08 × 10^6, 1.93 × 10^6, 3.08 × 10^6, 3.97 × 10^6, and 4.94 × 10^6, respectively. The solid lines were obtained by nonlinear regression of the data to Equation 3. The lines for FDPs of Mw (g/mol) 3.08 × 10^6 and 3.97 × 10^6 overlap at this scale.
indicated by the solid lines in Fig. 3. The kinetic parameters obtained from the regression analysis for t-PA with each of the different FDP samples are shown in Table III. Consistent with the observations from the individual Michaelis-Menten plots, the value of $K_A$ is greater than the value of $K$ for t-PA with 0.48 × 10⁶ g/mol FDPs, whereas $K_A \sim K$ with FDPs $\geq 1.08 \times 10^6$ g/mol. The data show that the FDP cofactors yield a low $K_m$ (45 nM) reaction with t-PA, and the $k_{cat}$ (0.065–0.081 s⁻¹) and $K$ (122–198 nM) values of the reactions are essentially independent of FDP $M_w$. The difference in cofactor activity between
were determined by regression to the Michaelis-Menten equation. The data show that all the reactions with FDPs exhibited Michaelis-Menten sizes, using single supported by the fact that a global fit of the data for all FDPs of \( M_w \geq 1.08 \times 10^6 \) g/mol was essentially independent of FDP concentration, indicating that \( K_a > K \). The \( K_a \) values for the reactions with the FDPs of \( M_w \geq 1.08 \times 10^6 \) g/mol were essentially invariant. For FDPs of \( M_w \) 0.15 s. The rate of 5AF-Pn formation at each FDP concentration was plotted against the 5AF-Pgn concentration, and the kinetic parameters of t-PA-catalyzed 5AF-Pgn activation in the presence of FDPs of different \( M_w \) decreased with FDP concentration, indicating that \( K_a \) for these reactions. The \( k_{cat} \) versus \( K_m \) ratio for each reaction was essentially invariant (\( K_m \geq 3000 \) nm), the smallest possible FDP (\( M_w = 0.25 \times 10^6 \) g/mol).

The rate of 5AF-Pn formation at each FDP concentration was plotted against the 5AF-Pgn concentration, and the \( k_{cat}(app) \) and \( K_m(app) \) values were determined by regression to the Michaelis-Menten equation. The data show that all the reactions with FDPs exhibited Michaelis-Menten sizes, using single supported by the fact that a global fit of the data for all FDPs of \( M_w \geq 1.08 \times 10^6 \) g/mol was essentially independent of FDP concentration, indicating that \( K_a > K \). The \( K_a \) values for the reactions with the FDPs of \( M_w \geq 1.08 \times 10^6 \) g/mol were essentially invariant. For FDPs of \( M_w \) 0.15 s. The rate of 5AF-Pn formation at each FDP concentration was plotted against the 5AF-Pgn concentration, and the kinetic parameters of t-PA-catalyzed 5AF-Pgn activation in the presence of FDPs of different \( M_w \) decreased with FDP concentration, indicating that \( K_a \) for these reactions. The \( k_{cat} \) versus \( K_m \) ratio for each reaction was essentially invariant (\( K_m \geq 3000 \) nm), the smallest possible FDP (\( M_w = 0.25 \times 10^6 \) g/mol).

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The reaction rates were strictly linear with respect to the 5AF-Pgn concentrations for all TAFIa-treated FDP samples. Thus, we were unable to obtain individual \( k_{cat}(app) \) and \( K_m(app) \) values. The \( k_{cat}(app)/K_m(app) \) ratio for each reaction.
The rate of 5AF-Pn formation at each FDP concentration was plotted against the 5AF-Pgn concentration, and the $k_{cat}(\text{app})$ and $K_a(\text{app})$ values were determined by regression to the Michaelis-Menten equation. The data show that the reactions with FDPs of $M_w = 1.93 \times 10^6$ g/mol exhibit Michaelis-Menten kinetics with $k_{cat}(\text{app})$ values increasing with FDP concentration, as expected in a cofactor-mediated reaction. The $K_a(\text{app})$ values for these reactions increased with FDP concentration, indicating that $K_a < K$. The reactions using FDPs of $M_w = 1.08 \times 10^6$ g/mol did not show saturation over the 5AF-Pgn concentration range used. Thus, the $k_{cat}(\text{app})$ and $K_a(\text{app})$ values for these reactions could not be determined; however, the $k_{cat}(\text{app})/K_a(\text{app})$ ratio was found from the slope of the rate versus 5AF-Pgn plot. The $k_{cat}(\text{app})/K_a(\text{app})$ ratio for all FDP samples increased with increasing FDP concentration, indicative of a template mechanism. All data are shown $\pm$ S.E.

### Table II

**Kinetic parameters of DSPαA1-catalyzed 5AF-Pgn activation in the presence of FDPs of different $M_w$**

The parameters from the regression of the data to the steady-state template model are presented $\pm$ S.E. The reactions were modeled with $k_{cat}$, $K_a$, and $K$ being dependent on FDP $M_w$ and a single $K_m$ for all FDPs. FDP cofactors yielded lower $K_m$ values with t-PA than with DSPαA1.

### Table III

**Summary of the kinetic parameters of t-PA- and DSPαA1-catalyzed 5AF-Pgn activation in the presence of FDPs of different $M_w$**

The parameters from the regression of the data to the steady-state template model are presented $\pm$ S.E. The reactions were modeled with $k_{cat}$, $K_a$, and $K$ being dependent on FDP $M_w$ and a single $K_m$ for all FDPs. FDP cofactors yielded lower $K_m$ values with t-PA than with DSPαA1.
The cofactor and either t-PA (1,3) or DSPA obtained by others, using intact fibrin (1,3) or fibrinogen (1) as substrates.

The Effect of TAFIa on t-PA- and DSPAα1-mediated Fibrinolysis in Plasma—The fibrinolysis of clots formed in human plasma, induced by t-PA or DSPAα1, was prolonged by TAFIa in a concentration-dependent, saturable manner. As shown in Fig. 6, fibrinolysis induced with t-PA and DSPAα1 was equally prolonged at low (1-2 nM) concentrations of TAFIa, and the extent of prolongation with saturating TAFIa was marginally higher with t-PA (3-fold) than with DSPA (2.5-fold). When t-PA was the activator, TAFIa can prolong fibrinolysis by three separate mechanisms (8). As a basic carboxypeptidase, TAFIa removes exposed carboxyl-terminal lysine and arginine residues, thus preventing the plasmin-mediated up-regulation of the fibrin cofactor activity. In addition, TAFIa suppresses the plasmin-catalyzed conversion of Glu-plasminogen to Lys-plasminogen, a much better substrate for t-PA, thereby eliminating negative effects of plasmin on TAFIa.

A summary of the intrinsic $k_{\text{cat}}$, $K_m$, and $k_{\text{cat}}/K_m$ (catalytic efficiency) values for t-PA and DSPAα1-stimulated 5AF-Pgn activation using FDPs as cofactors, before and after treatment of the FDPs with TAFIa, is presented in Table V. The $k_{\text{cat}}$ and $K_m$ values presented for t-PA and DSPAα1 with native FDPs were determined for each activator by fitting the data for all FDPs to a global steady-state template model using single values for $k_{\text{cat}}$, $K_m$, and $K$ and values that were dependent on the FDP $M_w$ for $K_A$. For comparison, Table V also shows values obtained by others, using intact fibrin (1,3) or fibrinogen (1) as the cofactor and either t-PA (1,3) or DSPAα1 (1) as the activator with Glu-plasminogen (1,3) and Lys-plasminogen (3) as substrates.

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TABLE V  
Comparison of the intrinsic kinetic parameters of t-PA- and DSPA1-catalyzed plasminogen activation with FDPs, TAFIa-treated FDPs, fibrin, and fibrinogen as cofactors

The data from the reactions with t-PA and DSPA1 were globally regressed to the steady-state template model using single values for \( k_{cat}, K_m \), and \( K \) for all FDPs. The data show that the intrinsic catalytic efficiency of t-PA is higher than that of DSPA1 when the cofactor is either FDPs or TAFIa-treated FDPs. With FDPs as the cofactor, the intrinsic catalytic efficiency of t-PA is increased by a factor of 3(1) to 10(3) compared with fibrin and approaches that seen with fibrin when the substrate is Lys-plasminogen (3). Although the intrinsic catalytic efficiency of DSPA1 with FDPs was the same as that found by others (1) with fibrin, the effect of TAFIa on DSPA1-mediated fibrinolysis (Fig. 6) shows that the intrinsic catalytic efficiency of DSPA1 with FDPs is higher than that seen with fibrin (see “Discussion”). Although TAFIa treatment of the FDPs markedly decreases the catalytic efficiencies of t-PA (90-fold) and DSPA1 (210-fold), to values below those for intact fibrin, TAFIa-treated FDPs are still superior cofactors for both t-PA (30-fold) and DSPA1 (50-fold) in comparison to fibrinogen.

| Cofactor       | t-PA     |           |           |           | DSPA1    |           |           |
|----------------|----------|-----------|-----------|-----------|----------|-----------|-----------|
|                | \( k_{cat} \) | \( K_m \) | \( k_{cat}/K_m \) | \( k_{cat} \) | \( K_m \) | \( k_{cat}/K_m \) |
| FDPs           | 0.075 ± 0.003 | 43 ± 4   | 17.4 ± 1.7 | 0.30 ± 0.10 | 500 ± 200 | 6.0 ± 2.2  |
| Fibrin\(^{a}\) | 0.058 ± 0.001 | 410 ± 30 | 1.4 ± 0.1  | 0.26 ± 0.07 | 380 ± 40  | 6.8 ± 2.0  |
| Fibrin \( ^{b} \) | 0.068 ± 0.006 | 130 ± 10 | 5.3 ± 0.6  | 2.69 ± 2.6  | 32 ± 3  | 0.028  |
| Fibrin (Lys-Pgn)\(^{b}\) | 0.086 ± 0.002 | 32 ± 3  | 2.69 ± 2.6  | 32 ± 3  | 0.028  |
| Fibrinogen\(^{b}\) | 0.0015 ± 0.0001 | 2400 ± 300 | 0.0064 ± 0.0009 | 0.00086 ± 0.00023 | 16300 ± 7500 | 0.00053 ± 0.00028 |

\( ^{a} \) From Horrevoets et al. (3).
\( ^{b} \) From Bringmann et al. (1).

**DISCUSSION**

The Catalytic Properties of t-PA and DSPA1 with FDPs as Cofactors—The intrinsic catalytic efficiency \( (k_{cat}/K_m) \) of t-PA exceeds that of DSPA1 by ~3-fold when FDPs are used as cofactors and 5AF-Pgn is the substrate. t-PA and DSPA1 are qualitatively similar, however, in that their intrinsic \( k_{cat} \) and \( K_m \) values, and thus their intrinsic catalytic efficiencies are not dependent on the molecular weight of the FDP (Table III). The similarity in the \( k_{cat} \) values with the different cofactors shows that the conversion of fibrin into FDPs and, by analogy, to partially degraded fibrin does not substantially alter the influence of the cofactor on the turnover of the ternary complex with either activator. Nonetheless, because three components are involved in the reactions, the reaction rates with both activators are sensitive to the FDP size when the reactions are not saturated with respect to the concentrations of FDP and 5AF-Pgn. Both activators showed decreased rates with decreasing FDP \( M_w \). The dependence is modest with t-PA but substantial with DSPA1 (Figs. 3 and 4). With both activators, the decrease in reaction rate with decreasing FDP \( M_w \) can be accounted for by a decrease in the binding affinity of the activator for the smaller FDPs. With t-PA the decrease in affinity is modest, whereas with DSPA1 the decrease is large. For example, the \( K_A \) values for the binding of t-PA to the two smallest FDPs (\( M_w = 0.48 \) and \( 1.08 \times 10^6 \) g/mol) are only 4.5- and 1.5-fold greater, respectively, than the average \( K_A \) value for FDPs with \( M_w \approx 1.93 \times 10^6 \) g/mol (182 nm). In contrast, with DSPA1, the corresponding \( K_A \) values are 43- and 4-fold higher, respectively, than the average value for the FDPs with \( M_w \approx 1.93 \times 10^6 \) g/mol (432 nm).

These differences can be rationalized on the basis of differences in structure between t-PA and DSPA1. t-PA interacts with fibrin by both its finger domain (\( K_f = 260 \) nM (4)) and its kringle-2 domain (\( K_f = 690 \) nM (4)). DSPA1, which lacks the kringle-2 domain, interacts with intact fibrin with high affinity via the finger domain (\( K_f = 150 \) nM (1, 2)). Light scattering measurements by Stewart et al. (2) of the binding of t-PA to fragment DD/E, the terminal product of fibrin degradation by plasmin, indicated high affinity binding (\( K_f = 20 \) nM). The authors concluded that the binding was mediated by kringle-2 since the lysine analogue e-amino caproic acid abolished the binding, and no binding of DSPA1 to the fragment was detected (\( K_f \gg 3000 \) nM). Thus, we infer that for the smallest FDPs used in our studies the predominant mode of binding of t-PA involves the kringle-2 domain and the lack of DSPA1 binding reflects its lack of a kringle-2 domain. This inference is supported by the quantitative agreement between the \( K_A \) values for the binding of t-PA to the smallest FDP sample (885 nm), as determined by kinetics, and the direct measurement of the binding of a fingerless t-PA mutant to intact fibrin (690 nm (4)). The interactions between DSPA1 and the larger FDPs must be mediated, therefore, by its finger domain, and the modest increase seen in the affinity of t-PA for the larger FDPs is consistent with the ability of the larger FDPs to support both finger domain and kringle-2 domain-dependent binding. As a corollary, the smallest FDP does not contain the elements of structure necessary for finger-mediated binding, whereas the large FDPs do.
Comparison of Cofactor Activities of FDPs and Fibrin—The cofactor activities of FDPs and fibrin with t-PA can be quantitatively compared because prior measurements with t-PA (3) were made using an experimental approach identical to that used in the present study. Under these conditions, with 5AF-Pgn (Glu-plasminogen) as the substrate, the $k_{cat}$ value with fibrin (0.058 s$^{-1}$) is very similar to the globally measured value for the FDPs (0.075 s$^{-1}$, Table V). The $K_m$ value with Glu-plasminogen and fibrin is much greater than that observed with FDPs as the cofactor (410 versus 43 nm, Table V). Thus, with Glu-plasminogen as the substrate, the intrinsic catalytic efficiency of t-PA with FDPs exceeds that of intact fibrin by a factor of 12.4 (Table V). With fibrin as the cofactor and Lys-plasminogen as the substrate, the intrinsic catalytic efficiency of t-PA with FDPs exceeds that of intact fibrin by a factor of 19.2, an effect that is also reflected by a large $K_m$ difference between the substrates. Thus, proteolytic modification of the substrate by plasmin (the conversion of Glu- to Lys-plasminogen) has the same impact on the catalytic efficiency as proteolytic modification of the cofactor (the conversion of intact fibrin to large FDPs).

The catalytic efficiency of DSPA1 with FDPs from the current data cannot be compared quantitatively to those obtained by Bringmann et al. (1) with intact fibrin as the cofactor because the conditions under which the data were obtained are not identical. In addition, the model used for the kinetic analysis in the present study and in the work of Horrevoets et al. (3) involved a template mechanism, whereas that used by Bringmann et al. (1) involves a bimolecular mechanism. Thus, despite the apparent identity of the intrinsic catalytic efficiency of DSPA1 measured here with FDPs ($6.0 \times 10^5 M^{-1} s^{-1}$) and that measured by Bringmann et al. (1) with intact fibrin ($6.8 \times 10^5 M^{-1} s^{-1}$), FDPs are undoubtedly more active than fibrin because TAFIa suppresses the up-regulation of fibrinolysis (Fig. 6), a phenomenon shown to be due to the suppression of the up-regulation of cofactor activity that occurs upon plasmin-catalyzed modification of fibrin (8).

The value of the parameter $K$ in the template model indicates the FDP concentration at which the reaction rate will be half-maximal at saturating plasminogen concentration. With both t-PA and DSPA1 this value is essentially independent of FDP $M_a$ (Table III). The average value for t-PA (158 nm) is considerably lower than that for DSPA1 (1990 nm). Since the physiologic concentration of plasminogen (2–3 $\mu$M) is near saturation for both activators, the lower value of $K$ for t-PA indicates that plasminogen activation would be saturated at much lower levels of FDPs with t-PA than with DSPA1. Thus, FDPs of all sizes are superior cofactors for t-PA compared with DSPA1, in that at subsaturating concentrations of FDPs more activity will be elicited with t-PA than with DSPA1.

The Effect of TAFIa on the Cofactor Activity of FDPs—In the presence of TAFIa, the up-regulation of plasminogen activation, through the formation of modified fibrin by plasmin, is eliminated by the removal of the lysine and arginine residues from the modified fibrin (8). FDPs (and degraded fibrin) increase the intrinsic catalytic efficiency of t-PA and DSPA1. TAFIa attenuates this up-regulation, decreasing the intrinsic catalytic efficiency of both t-PA (0.20 $\times 10^5 M^{-1} s^{-1}$) and DSPA1 (0.028 $\times 10^5 M^{-1} s^{-1}$) by 90- and 210-fold, respectively. Although TAFIa-treated FDPs are much less active than intact fibrin, they are considerably more active than fibrinogen. Interestingly, when the TAFIa-treated FDPs are cofactors, the size dependence seen with DSPA1 is markedly attenuated and that seen with t-PA is absent. Since DSPA1 has only finger binding, one would expect the TAFIa-treated FDPs to exhibit the same size dependence as the native FDPs. In addition, since TAFIa treatment removes carboxyl-terminal lysines, one would expect the t-PA kringle-2 interaction to be affected and thus the size dependence seen with t-PA should mirror that seen with DSPA1. That the expected results did not occur can be explained by a comparison of the DSPA1 activity to DSPA2a and DSPA4, highly homologous relatives of DSPA1 (89 and 91% identity, respectively) which lack either the finger domain (DSPA), or the finger and epidermal growth factor domains (DSPA) (12). Although neither activator binds to fibrin with high affinity (1), the intrinsic catalytic efficiencies are increased 1650- (DSPA2a) and 800-fold (DSPA) in the presence of fibrin (1), indicating the existence of a finger-independent, low affinity interaction with fibrin. Given the shared identity among the DSPAs, the same interaction likely occurs with DSPA1. The effect of this interaction on plasminogen activation is not observed with intact fibrin and FDPs not treated with TAFIa due to the overwhelming contribution of finger-bound DSPA1 to the kinetics of plasmin generation. However, with TAFIa-treated FDPs the finger-independent, low affinity interaction of DSPA1 contributes a significant fraction of the total plasmin generating activity. Therefore, the total activity of DSPA1 is a composite of the activities of DSPA1 bound by both finger-dependent and finger-independent modes. Since the larger FDPs contain more finger-binding sites, the small observed dependence of the rate on FDP $M_a$ results from the low activity, due to the loss of the high affinity plasminogen binding, of the finger-bound DSPA1. This interpretation receives support from the similarity of the intrinsic catalytic efficiency of DSPA1 with the TAFIa-treated FDPs (0.028 $\times 10^5 M^{-1} s^{-1}$) to the catalytic efficiencies of both DSPA1 (0.099 $\times 10^5 M^{-1} s^{-1}$) and DSPA (0.035 $\times 10^5 M^{-1} s^{-1}$) (1) with intact fibrin. Similarly, the lack of size dependence with t-PA and the TAFIa-treated FDPs reflects catalysis through a mode independent of finger binding of t-PA to FDPs. Thus, finger-bound activator loses the ability to form a ternary complex due to the loss of the plasminogen-binding site. That t-PA interacts with TAFIa-treated FDPs via a finger-independent mode indicates that t-PA binding is not strictly dependent on the lysine residues that are the targets of plasmin. The contribution of t-PA interacting through this mode to the overall rate of plasmin generation masks that of finger bound t-PA with TAFIa-treated FDPs. This interpretation receives support from the fibrin-dependent intrinsic catalytic efficiency of a recombinant t-PA lacking the finger domain (0.17 $\times 10^5 M^{-1} s^{-1}$) (3). In cleaving the carboxyl-terminal lysines from FDPs, TAFIa does not simply convert the high affinity sites back to low affinity sites, it removes the sites altogether.

Implications for the Fibrin Specificity of Plasminogen Activators—Recently, Stewart et al. (2) demonstrated that plasminogen activation catalyzed by t-PA, but not DSPA1, is effectively stimulated by the fibrin degradation product DD/E. The authors speculated that this property may increase the fibrin selectivity of DSPA1, relative to t-PA, as the DD/E fragments released from the clot would continue to stimulate the t-PA-catalyzed but not the DSPA1-catalyzed plasminogen activation, and thus, the release of DD/E into the circulation would promote fibrinogenolysis in the presence of t-PA but not DSPA1. This speculation rests on the assumption that DD/E is the primary product of plasmin-catalyzed fibrin degradation. We, however, recently demonstrated that FDPs produced in a perfused, cross-linked clot vary in molecular weight from 250 (the mass of DD/E) to 10,000 kDa, with the majority of the mass
contained in fragments larger than DD/E (9). This suggests that FDPs much larger than DD/E are released into circulation during fibrinolysis, and indeed FDP complexes of at least \(2.0 \times 10^6\) Da have been observed in the plasma of patients with disseminated intravascular coagulation (13, 14) and chronic subdural hematoma (15). Whereas a large difference in activity between t-PA and DSPA1 would be expected with DD/E, this difference disappears with larger FDPs. Therefore, the relative fibrin specificity of DSPA1 compared with t-PA would be attenuated in the presence of larger FDPs.

Considerations on the Use of Plasminogen Activation to Measure Soluble Fibrin and Fibrin Degradation Products in Plasma—The t-PA and DSPA1 cofactor activity of FDPs larger than DD/E can be attenuated by the action of TAFIa. Our studies have implications regarding the use of plasminogen activation assays for the measurement of soluble fibrin and/or FDPs in plasma. The rate of plasminogen activation in plasma will be determined by the relative concentrations of soluble fibrin, native FDPs, FDPs exposed to TAFIa, and fibrinogen. Since the \(K_m\) of the reactions with the FDPs is 10-fold lower and the intrinsic catalytic efficiency is 10-fold greater than with fibrin, and therefore presumably with soluble fibrin, small amounts of soluble FDPs could compromise the measurement of soluble fibrin. Furthermore, TAFIa treatment attenuates the cofactor activity of FDPs by ~100-fold. In determinations of FDPs, the rate of plasmin generation in the assay will essentially be a measure of the concentration of the native FDPs, which may or may not reflect the total concentration, the value of which will be underestimated according to the extent of FDP exposure to TAFIa. Thus, measurements using plasminogen activation represent the composite cofactor activity of plasma, which may or may not be a good measure of the species in question. The complexity of the cofactor mixture and differences in the kinetics associated with each component in the mixture may explain the differences observed with antibody-based and cofactor activity-based assays in the determination of the concentrations of both soluble fibrin and FDPs (16–18).

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A Kinetic Analysis of the Tissue Plasminogen Activator and DSPAα1 Cofactor Activities of Untreated and TAFIa-treated Soluble Fibrin Degradation Products of Varying Size

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