TAFI, or Plasma Procarboxypeptidase B, Couples the Coagulation and Fibrinolytic Cascades through the Thrombin-Thrombomodulin Complex

Laszlo Bajzar‡, John Morser§, and Michael Nesheim‡

From the 1Departments of Biochemistry and Medicine, Queen’s University, Kingston, Ontario K7L 3N6, Canada and 2Berlex Biosciences, Richmond, California 94804-0099

TAFI (thrombin-activatable fibrinolysis inhibitor) is a recently discovered plasma protein that can be activated by thrombin-catalyzed proteolysis to a carboxypeptidase B-like enzyme that inhibits fibrinolysis. This work shows that the thrombin-thrombomodulin complex, rather than free thrombin, is the most likely physiologic activator. Thrombomodulin increases the catalytic efficiency of the reaction by a factor of 1250, an effect expressed almost exclusively through an increase in \( k_{cat} \). The kinetics of the reaction conform to a model whereby thrombin can interact with either TAFI (\( K_m = 1.0 \mu \text{M} \)) or thrombomodulin (\( K_m = 8.6 \text{ nm} \)), and either binary complex so formed can then interact with the third component to form the ternary thrombin-thrombomodulin-TAFI complex from which activated TAFI is produced with \( k_{cat} = 1.2 \text{ s}^{-1} \). This work also shows that activated TAFI down-regulates tPA-induced fibrinolysis half-maximally at a concentration of 1.0 nm in a system of purified components. This concentration of TAFI is about 2% of the level of the zymogen in plasma, which indicates that ample activated TAFI could be generated to very significantly modulate fibrinolysis in vivo. Therefore, TAFI in vitro and possibly in vivo defines an explicit molecular connection between the coagulation and fibrinolytic cascades, such that expression of activity in the former down-regulates the activity of the latter.

The coagulation and fibrinolytic cascades comprise a series of zymogen to enzyme conversions which terminate in the proteolytic enzymes thrombin and plasmin, respectively (1–3). These enzymes catalyze the deposition and removal of fibrin. A proper balance between the activities of the two cascades is required both to protect the organism from excessive blood loss upon injury and to maintain blood fluidity within the vascular system. Imbalances are characterized by either bleeding or thrombotic tendencies, the latter of which are manifested as heart attacks and strokes.

Thrombomodulin is a component of the blood vessel wall which binds thrombin and changes its specificity from fibrinogen to protein C, yielding anticoagulant rather than procoagulant activity (4). The thrombin-thrombomodulin complex catalyzes cleavage of protein C to activated protein C, which then down-regulates the coagulation cascade by proteolytically inactivating the essential cofactors Factor Va and Factor VIIIa (5). These events are essential in the regulation of the coagulation cascade (4).

Early studies suggested that activated protein C is not only anticoagulant but also profibrinolytic, both in vitro and in vivo (6–9). Subsequent work from our laboratory showed that activated protein C only appears profibrinolytic because it prevents the thrombin-catalyzed activation of a previously unknown fibrinolysis inhibitor (10). The precursor of this inhibitor was isolated from plasma (11) and was designated TAFI (thrombin-activatable fibrinolysis inhibitor). The zymogen is activated by thrombin to an enzyme with carboxypeptidase B-like activity. This enzyme, designated TAFIa, inhibits plasminogen activation and thereby prolongs fibrinolysis (11), presumably by removing C-terminal lysines from partially degraded fibrin, thereby attenuating the cofactor activity of fibrin and preventing the accelerated phase that ordinarily occurs during plasminogen activation (12).

Our initial studies suggested that thrombin is a weak activator of TAFI. Thus, the present work was initiated to analyze the effects of a soluble form of thrombomodulin (Solulin, Ref. 13) on the reaction. The results presented below comprise a report of both the activation of TAFI by the thrombin-thrombomodulin complex and the attenuation of fibrinolysis by TAFIa.

**EXPERIMENTAL PROCEDURES**

Proteins and Reagents—The human proteins fibrinogen, plasminogen, prothrombin, and antithrombin III and TAFI were isolated from plasma as described previously (11). Recombinant tissue plasminogen activator (Activase) was a generous gift of Dr. Gordon Vehar of Genentech (South San Francisco, CA). Recombinant soluble thrombomodulin (Solulin) was obtained as a generous gift from Berlex Biosciences (Richmond, CA). Recombinant human \( \alpha_2 \)-antiplasmin was isolated from culture supernatants of baby hamster kidney cells transfected with the human cDNA and grown in serum-free medium, as described previously (11). The synthetic carboxypeptidase B substrate, hippuryl-arginine, was obtained from Sigma. All other reagents were of analytical quality.

Analysis of Thrombin-Thrombomodulin-dependent TAFI Activation by SDS-PAGE and Activity Measurements—TAFI (1.92 \( \mu \text{g} \)), thrombin (1.0 \( \text{nm} \)), and soluble thrombomodulin (50 \( \text{nm} \)) were incubated at 22 °C in 0.02 M HEPES, 0.15 M NaCl, 5.0 mM CaCl\(_2\), pH 7.4. At regular intervals samples were removed and added to the irreversible thrombin inhibitor \( \alpha \)-phenylalanine-prolyl-arginyl chloromethyl ketone (PAPK), the final concentration of which was 5.0 \( \mu \text{M} \). One aliquot was then immediately assayed for carboxypeptidase B activity by adding it to hippuryl-arginine (400 \( \mu \text{M} \)) in 0.02 M Tris-HCl, 0.10 M NaCl, pH 7.4, and measuring the time course of increase in absorbance at 254 nm in a Perkin-Elmer \( \lambda\)-4 spectrophotometer. Another aliquot was then prepared for SDS-PAGE in a 5–15% gradient gel under nonreducing conditions.

* This work was supported by the National Institutes of Health, the Medical Research Council of Canada, and the Heart and Stroke Foundation of Ontario. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 613-545-2957; Fax: 613-545-2987; E-mail: nesheimm@qucdn.queensu.ca.

‡ Berlex Biosciences, Richmond, California 94804-0099

§ Departments of Biochemistry and Medicine, Queen’s University, Kingston, Ontario K7L 3N6, Canada

¶ To whom correspondence should be addressed. Tel.: 613-545-2957; Fax: 613-545-2987; E-mail: nesheimm@qucdn.queensu.ca.

The abbreviations used are PAGE, polyacrylamide gel electrophoresis; PAPK, \( \alpha \)-phenylalanine-prolyl-arginyl chloromethyl ketone; DAPA, dansylarginine N-3-(ethyl-1,5-pentanediyl)amide.
according to the method of Neville (14). The gel was stained with Coomassie Blue, destained, and scanned with an LKB densitometer. One sample, taken at 5 min and added to PPack, was assayed at regular intervals for the next 2 h to determine the rate of spontaneous activity loss (no deactivation occurs in this interval). The kinetics of loss were first order with a half-life of 70 min. Activity measurements on samples taken at 5 min and added to PPack, at the 2-h interval and at 4-h interval were recorded both as raw data and as data corrected for spontaneous decay up to the time of sampling and assay. To make this correction, the sequence of reactions TAFI → TAFIa → TAFI was determined in separate experiments to determine the kinetics parameters of hydrolysis of hippuryl-arginine by TAFI, as described by analysis of densitometry scans of the TAFI band on the Coomassie Blue, destained, and scanned with an LKB densitometer. For each band, a time course of the intensity of fluorescence was determined by adding aliquots of the reaction mixtures to 400 μM hippuryl-arginine in 0.2 M Tris-HCl, 0.01% Tween 80, pH 7.4, and measuring the time courses of absorbance at 254 nm. The activated TAFI concentrations were calculated from the kinetic parameters for hydrolysis of hippuryl-arginine by TAFI determined in separate experiments (kcat = 144 μM s−1), k0 = 21 s−1).

Kinetics of the Thrombomodulin-dependent Activation of TAFI—Because TAFI, at concentrations ranging from 0.26 to 2.08 μM, was incubated at 22°C with thrombin (1.0 μM) and soluble thrombomodulin at various concentrations ranging from 1.56 to 50 μM in 0.02 M HEPES, 0.15 M NaCl, 5.0 mM CaCl2, pH 7.4, after 10 min PPack was added to halt the reactions, and activated TAFI levels were assayed by adding aliquots of the reaction mixtures to 400 μM hippuryl-arginine in 0.2 M Tris-HCl, 0.01% Tween 80, pH 7.4, and monitoring the time courses of absorbance at 254 nm. The activated TAFI concentrations were calculated from the kinetic parameters for hydrolysis of hippuryl-arginine by TAFI determined in separate experiments (kcat = 144 μM s−1), k0 = 21 s−1).

RESULTS

The proteolytic activation of TAFI by thrombin-thrombomodulin was analyzed by both SDS-PAGE (Fig. 1) and measurements of carboxypeptidase B activity with hippuryl-arginine (Fig. 2). The zymogen (58 kDa) is progressively cleaved to a 44-kDa, free component, and to a 54-kDa, bound component (Fig. 1). The samples in the wells of the plate then were monitored for turbidity at 405 nm, at 2.5-min intervals 37°C, in a Titertek plate reader operated in the kinetics mode. Under these conditions, clotting, marked by increased turbidity, occurs within 1–2 min, and fibrinolysis, marked by decreased turbidity, occurs within about 30–90 min, depending on the contents of the well. A parameter, denoted lysis time, is defined as the time, after adding the sample to the well, at which the turbidity is one-half the difference between the plateaus reached after clotting and the baseline value achieved at complete lysis. In these experiments the TAFI concentration was varied from 0 to 50 μM. Controls were performed by executing identical experiments without TAFI.

The proteolytic activation of TAFI by thrombin-thrombomodulin was analyzed by both SDS-PAGE (Fig. 1) and measurements of carboxypeptidase B activity with hippuryl-arginine (Fig. 2). The zymogen (58 kDa) is progressively cleaved to yield major and minor components, with respective masses of 35 and 25 kDa. Although direct measurements of activity do not correlate with the appearance of the 35-kDa product, values corrected for the intrinsic instability of TAFI (t1/2 = 70 min) correlate very well (Fig. 2).

Initial rates of TAFI activation show saturation in the concentrations of both TAFI (Fig. 3) and thrombomodulin (Fig. 4). Km values are independent of the thrombomodulin concentration, and Vmax values show saturation in it (Table 1). These patterns were analyzed for conformity to one or more of the seven equilibrium models described by Boskosic et al. (18) for three component systems consisting of enzyme, substrate, and cofactor. Two of the models conform to the present data. Both models have the rate equation: r = kcat[T-M][TAFI]/[Km + [TAFI]]. In this equation the concentration of the thrombin-thrombomodulin complex, [T-M], is given by [T-M] = 0.5([K2 + [TM]b + [TM]a] – ([K2 + [TM]b + [TM]a]0)2 - 4[TM]b[TM]a)/2). The definitions used are: r = initial rate of TAFI activation; T = thrombin; TM = thrombomodulin; and D = the dissociation con-
stant for the T-TM complex; and [T]₀ and [TM]₀ the total concentrations of the respective species. Since both models have the same rate equation, they cannot be distinguished by measurements of steady-state kinetics alone. They do have different physical interpretations, however. Although both models predict the thrombin-thrombomodulin complex, one predicts the existence of thrombomodulin-TAFI, but not thrombin-TAFI, on the reaction pathway, whereas the other predicts the opposite. Because of these differences, the models can be distinguished by characterizing the binary interactions between the three components.

In order to confirm the existence of the thrombin-thrombomodulin complex and to determine which one of the other two possible binary complexes exist and thereby distinguish between the two models, the binding of thrombin to thrombomodulin was characterized by $K_d = 2.3 \pm 1.4 \text{ nM}$ (Fig. 5). Thrombin binding to thrombomodulin was characterized by $K_d = 2.3 \pm 1.4 \text{ nM}$ (Fig. 5), and competition kinetics indicated 50% inhibition at 1.2 µM TAFI (Fig. 6). These values are in very good agreement with

FIG. 1. SDS-PAGE analysis of cleavage of TAFI by thrombin-thrombomodulin. TAFI (1.92 µM), thrombin (1.0 nM) and soluble thrombomodulin (Solulin), (50 nM) were incubated at 22 ℃ in 0.02 M HEPES, 0.15 M NaCl, 5.0 mM CaCl₂, pH 7.4. At intervals indicated below each lane, samples were removed and added to the thrombin inhibitor PPAck. Half of each sample was prepared for SDS-PAGE (nonreduced) on a 5–15% gradient gel, and the other half was assayed for carboxypeptidase B-like activity with hippuryl-arginine (0.4 mM) at 22 ℃, pH 7.65, in 0.02 M Tris, 0.10 M NaCl. The lane designated 5 + 115 is from a sample treated with PPAck at 5 min and incubated a further 115 min prior to preparation for SDS-PAGE. This specimen spontaneously lost substantial activity over this interval, but no additional cleavage occurred.

FIG. 2. Time course of cleavage and appearance of carboxypeptidase B activity. Densitometry of the gel of Fig. 1 yielded the time courses of zymogen (58 kDa, ○) and the 35 kDa product (●). Carboxypeptidase activity measurements, uncorrected (▲) and corrected for spontaneous decay (○), also were made.

FIG. 3. Michaelis-Menten plots of TAFI activation. TAFI at the concentrations indicated by the horizontal axis was incubated with thrombin (1.0 nM) and thrombomodulin at concentrations (nM) of 1.56 (▲), 3.12 (○), 6.25 (●), 12.5 (▲), 25 (○), and 50 (●). Incubations were performed for 10 min at 22 ℃ in 0.02 M HEPES, 0.15 M NaCl, 5.0 mM CaCl₂, pH 7.4, and then thrombin was inhibited with PPAck, and TAFI activity was measured with hippuryl-arginine. The lines represent the results of nonlinear regression of the data to the Michaelis-Menten equation.

FIG. 4. Thrombomodulin concentration dependence of initial rates of TAFI activation. The concentration of thrombomodulin was varied as indicated in solutions of TAFI at concentrations (from bottom to top) of 0.26, 0.52, 0.69, 1.04, 1.5, and 2.08 µM. The thrombin concentration was 1.0 nM and conditions were as described in the legend of Fig. 3. The lines are the results of a global fit of the data by nonlinear regression to the rate equation given in the text under “Results.”

TABLE I

| TM            | $K_m$ (µM) | $k_{cat(app)}$ (s⁻¹) |
|---------------|------------|----------------------|
| 1.56          | 0.83 ± 0.14| 0.17 ± 0.01          |
| 3.13          | 1.01 ± 0.20| 0.32 ± 0.03          |
| 6.25          | 1.06 ± 0.11| 0.52 ± 0.03          |
| 12.50         | 1.37 ± 0.13| 1.37 ± 0.14          |
| 25.00         | 0.84 ± 0.10| 0.83 ± 0.04          |
| 50.00         | 1.03 ± 0.08| 1.07 ± 0.04          |
the Kₐ and Kₚ values inferred independently from the kinetics of TAFI activation in both the absence and presence of thrombomodulin (Table II).

These observations support the model for TAFI activation shown in Fig. 7. According to this model, the formation of the ternary TAFI-thrombin-thrombomodulin complex can proceed via two parallel paths involving, respectively, the binary thrombin-thrombomodulin and TAFI-thrombin complexes. All of the data were fit globally to the above rate equation by nonlinear regression analysis with Kd, Km, and kcat as fit parameters. The lines shown on Fig. 4 are the regression lines and the fits appear to be excellent. The results, along with those obtained in the absence of thrombomodulin, are summarized in Table II. The effect of thrombomodulin is expressed almost exclusively through an increase in Kcat. The catalytic efficiency (kcat/Km) of thrombin-thrombomodulin is 1250-fold greater than that of thrombin.

In order to assess the impact of TAFIa on fibrinolysis, samples of it at increasing concentrations were added to a system of purified fibrinolytic components in which initial clotting (induced by thrombin) and subsequent fibrinolysis (induced by tissue plasminogen activator) are monitored over time by turbidity at 405 nm. The formation of fibrin is accompanied by a rapid increase in turbidity to a plateau value, and subsequent fibrinolysis is marked by a corresponding decrease (Fig. 8). As the TAFIa concentration increases, the time required for fibrinolysis to occur increases. The relationship between the TAFIa concentration and time required for the turbidity value to fall to one-half the maximum value is shown in Fig. 9. The value increased in a saturable manner from 30 to about 90 min, and the half-maximal effect was achieved at a TAFI concentration of 1.0 nM. Since the zymogen TAFI is present in plasma at a concentration of about 50 nM (11), TAFIa attenuates the dissolution of fibrin via the fibrinolytic cascade at a concentration considerably below that which potentially could be generated by thrombin-thrombomodulin in vivo.

**TABLE II**

| Kₚ, Kₚm, and kcat values for TAFI activation | Kₚ, Kₚm, and kcat values for TAFI activation |
|--------------------------------------------|--------------------------------------------|
| Kₚ, Kₚm, and kcat values for TAFI activation | Kₚ, Kₚm, and kcat values for TAFI activation |
| Km, µM | kcat, sec⁻¹ | Kd, µM | kcat/Km, sec⁻¹ |
|---|---|---|---|
| IIa | 2.14±0.59 | 0.0021±0.0004 | NA* | 0.00098 |
| IIa·TM | 1.01±0.09 | 1.24±0.06 | 8.6±0.5 | 1.23 |

*NA, not applicable.

**FIG. 7. Enzyme central model of TAFI activation.** This model is from Boskovic et al. (16) and implies that the enzyme, thrombin (T), can interact with either TAFI or thrombomodulin (TM), and the resulting binary complexes interact further to form the ternary T-TM-TAFI complex, from which TAFIa is formed. Independent evidence for the existence of the binary complexes is given in Figs. 5 and 6.

**FIG. 6. TAFI binding to thrombin.** Initial rates of hydrolysis of the chromogenic substrate S-2266 (250 µM) by thrombin (5.0 nM), measured by absorbance at 405 nm, were determined in the presence of various concentrations of TAFI (0–1.9 µM) in 20 mM HEPES, 150 mM NaCl, 5.0 mM Ca²⁺, pH 7.4 at 37°C.

**FIG. 5. Thrombin binding to thrombomodulin.** Binding of thrombin to thrombomodulin was determined by titrating 1.5 ml of a solution composed of thrombin (20 nM) and DAPA (20 nM) in 20 mM Tris-HCl, 150 mM NaCl, 5.0 mM Ca²⁺, 0.01% Tween 80 with 1.54 µM thrombomodulin in an identical solution. Fluorescence intensity was measured (λₑₓ = 280 nm, λₘₜ = 545 nm).

**Discussion**

The present work shows that TAFI is activated to an inhibitor of fibrinolysis by the thrombin-thrombomodulin complex. Although thrombin at high concentrations, such as those which could potentially be generated upon complete conversion of the prothrombin in plasma to thrombin, can activate TAFI in plasma (11), the catalytic efficiency is increased about three orders of magnitude in the presence of thrombomodulin. Thus, the thrombin-thrombomodulin complex is most likely the physiologic activator of TAFI. In addition, TAFI is now the second known macromolecular substrate for the thrombin-thrombomodulin complex, the other of which is protein C, the precursor of the anticoagulant enzyme, activated protein C. The existence of these two substrates implies that the thrombin-thrombomodulin complex may contribute to the down-regulation of not only the coagulation cascade but also the fibrinolytic cascade.

In some respects the mechanisms by which thrombomodulin enhances activation of TAFI and protein C appear similar. The Kₚ and kcat values are similar at saturating thrombomodulin (Kₚ = 1.0 µM, kcat = 1.2 s⁻¹ for TAFI, and Kₚ = 5.9 µM, kcat =...
1.3 s⁻¹ for protein C (17)). In addition, the enhanced catalytic efficiency is clearly expressed through an effect in kcat in TAFI activation. This is probably so also in protein C activation, although this conclusion is obscured somewhat by the complex dependence of reaction kinetics on the concentration of Ca²⁺. At the level of structure, however, some subtle differences in the two substrates are apparent. The sequence of amino acids corresponding to P7-P5' peptide surrounding the thrombin activating cleavage in protein C site is EDQVDPRLIDGK (19). Le Bonniec et al. (17) and Le Bonniec and Esmon (19) provided convincing arguments with mutants of thrombin that the aspartic acid residues at the P3 and P3' positions of this sequence contribute substantially to resistance to cleavage by thrombin in the absence of thrombomodulin. In TAFI, however, the corresponding P7 to P5' sequence is NDTVSPRASAY (11, 19). Thus, in TAFI the negatively charged P3 and P3' residues are not present. Determining whether the elements of structure of TAFI and protein C, which both limit cleavage by freethrombin and allow it by thrombin-thrombomodulin, are similar or grossly different clearly will require further work. In addition, TAFI provides another tool by which to gather further insights into the means by which thrombomodulin alters the activity of thrombin toward macromolecular substrates.

TAFI was discovered independently in three different laboratories. It initially appeared as an unstable carboxypeptidase B-like entity in human serum and was described by Hendriks et al. (20). Then Eaton et al. (21) discovered it as a contaminant in preparations of α₂-antiplasmin; they cloned the cDNA, deduced the amino acid sequence, described its activation by trypsin, and analyzed its enzymatic properties toward synthetic carboxypeptidase B substrates. They designated the protein pCPB, for plasma carboxypeptidase B. Wang et al. (22) independently isolated the activated material and named it carboxypeptidase U, where "U" indicates unstable. In addition, our group discovered it, showed that it is both activated by thrombin and inhibits fibrinolysis, and that it accounts for the apparent profibrinolytic effects of activated protein C. Consequently, we named it TAFI (11). Subsequently, Tan and Eaton (23) studied the trypsin activated enzyme and renamed the protein plasma procarboxypeptidase B (pro-pCPB). The identity of TAFI and pro-pCPB is established by their behavior in affinity chromatography on plasminogen Sepharose and the amino acid sequences at the activation cleavage site (11, 21). In addition, Redlitz et al. (24) recently demonstrated that activated pro-pCPB and carboxypeptidase N diminish tPA-induced plasminogen binding to U937 cells and that fibrinolysis occurs more rapidly in carboxypeptidase depleted, clotted plasma than in controls.

Our previous results indicate that TAFI couples the coagulation and fibrinolytic cascades in vitro, such that the operation of the former down-regulates the activity of the latter (10–11). The present work confirms this and further suggests that this connection is most likely established in vivo through the thrombin-thrombomodulin complex on the endothelial cell lining of the blood vessel. Because TAFI is exquisitely sensitive to activation by thrombin-thrombomodulin and, when activated, potently suppresses fibrinolysis, it very likely plays a fundamental role in mediating "cross-talk" between the coagulation and fibrinolytic cascades. Because of this, TAFI should be considered in the future, both in studies of biochemical defects that lead to bleeding or thrombosis and in efforts to therapeutically accomplish thrombolysis through the use of components of the fibrinolytic cascade.

Acknowledgments—The technical support of Tom Abbott and Reginald Manuel are greatly appreciated, as are numerous fruitful discussions with Dr. Anton Horrevoets and Willem Stevens. In addition, we wish to express our gratitude to our colleagues at Berlex Biosciences for their splendid efforts in producing Solulin.

REFERENCES
1. Mann, K. G., Bovill, E. G., and Krishnaswamy, S. (1991) Ann. N.Y. Acad. Sci. 614, 63–75
2. Collen, D., and Lijnen, H. R. (1991) Blood 78, 3114–3124
3. Astrup, T. (1991) Semin. Thromb. Hemostasis 17, 161–174
4. Esmon, C. T. (1993) FASEB J. 9, 946–955
5. Esmon, C. T., Johnson, A. E., and Esmon, N. L. (1999) Ann. N.Y. Acad. Sci. 860, 30–43
6. Taylor, P. B., and Lockhart, M. S. (1985) Thromb. Res. 37, 639–649
7. Burdick, M. D., and Schaub, R. G. (1987) Thromb. Res. 45, 413–419
8. de Fouw, N. J., Haverkate, F., and Bertina, R. M. (1990) Adv. Exp. Med. Biol. 281, 235-243
9. Bajzar, L., Frondeburgh, J. C., and Nesheim, M. (1990) J. Biol. Chem. 265, 16948–16954
10. Bajzar, L., and Nesheim, M. (1993) J. Biol. Chem. 268, 8868–8861
11. Bajzar, L., Manuel, R., and Nesheim, M. E. (1995) J. Biol. Chem. 270, 14477–14484
12. Suenson, E., Lutzen, O., and Thorsen, S. (1984) Eur. J. Biochem. 140, 513–522
13. Glaser, C. B., Monier, J., Clarke, J. H., Blasko, E., McLean, K., Kuhn, I., Chang, R. J., Lin, J. H., Vilander, L., Andrews, W. H., and Light, D. R. (1992) J. Clin. Invest. 90, 2565–2573

Fig. 8. The effect of TAFIa on clot lysis. TAFIa, generated by incubating TAFI (2.0 μM) with thrombomodulin (50 nM) and thrombin (20 nM) at 22 °C for 15 min, was serially diluted in a solution containing thrombin and thrombomodulin. The TAFIa solutions (1 volume) were mixed with solutions (9 volumes) of fibrinogen (3.36 μg/ml), plasminogen (0.89 μg/ml), α₂-antiplasmin (0.56 μg/ml), and antithrombin III (1.11 μg/ml) in 0.02 M HEPES, 0.15 M NaCl, 0.01% Tween 80, pH 7.4. Aliquots (100 μl) were immediately added to the wells of a microtiter plate containing 0.02 M HEPES, 0.15 M NaCl, 0.01% Tween 80, pH 7.4. Aliquots (100 μl) were immediately added to the wells of a microtiter plate containing 0.02 M HEPES, 0.15 M NaCl, 0.01% Tween 80, pH 7.4. Aliquots (100 μl) were immediately added to the wells of a microtiter plate containing 0.02 M HEPES, 0.15 M NaCl, 0.01% Tween 80, pH 7.4. Aliquots (100 μl) were immediately added to the wells of a microtiter plate containing 0.02 M HEPES, 0.15 M NaCl, 0.01% Tween 80, pH 7.4.
Activation of TAFI

14. Neville, D. M. (1971) J. Biol. Chem. 246, 6328–6334
15. Nesheim, M. E., Prendergast, F. G., and Mann, K. G. (1979) Biochemistry 18, 996–1003
16. Hibbard, L. S., Nesheim, M. E., and Mann, K. G. (1982) Biochemistry 21, 2285–2292
17. Le Bonniec, B. F., MacGillivray, R. T. A., and Esmon, C. T. (1991) J. Biol. Chem. 266, 13796–13803
18. Boskovic, D. S., Giles, A. R., and Nesheim, M. E. (1990) J. Biol. Chem. 265, 10497–10505
19. Le Bonniec, B. F., and Esmon, C. T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7371–7375
20. Hendriks, D., Wang, W., Scharpe, S., Lommaert, M. P., and van Sande, M. (1990) Biochim. Biophys. Acta 1034, 96–92
21. Eaton, D. L., Malloy, B. E., Tsai, S. P., Henzel, W., and Drayna, D. (1991) J. Biol. Chem. 266, 21833–21838
22. Wang, W., Hendriks, D. F., and Scharpe, S. S. (1994) J. Biol. Chem. 269, 15937–15944
23. Tan, A. K., and Eaton, D. L. (1995) Biochemistry 34, 5811–5816
24. Redlitz, A., Tan, A. K., Eaton, D. L., and Plow, E. F. (1995) J. Clin. Invest. 96, 2534–2538