Flow Rate–Modulated Dissolution of Fibrin With Clot-Embedded and Circulating Proteases

Erzsébet Komorowicz, Krasimir Kolev, István Léránt, Raymund Machovich

Abstract—The efficiency of plasmin, miniplasmin, and neutrophil leukocyte elastase in fibrin digestion is well characterized in static systems. Since in vivo the components of the fibrinolytic system are permanently exposed to flow, we have developed two in vitro models and studied the effect of shear forces on fibrin dissolution with these proteases. Cylindrical nonocclusive fibrin clots are perfused at various flow rates through their preformed axial channel, and dissolution of fibrin is followed by measuring the absorbance of degradation products released into the circulating fluid phase. In one experimental setting, fibrin surface is degraded with enzymes applied in the recirculating fluid phase; in another setting, clots containing gel-embedded proteases are perfused with enzyme-free buffer. As shear rate at fibrin surface is changed from 25 to 500 s⁻¹, the rate of product release by recirculated enzymes increases 2.8-, 2.9-, and 4-fold for plasmin, miniplasmin, and porcine pancreatic elastase, respectively. Buffer-perfused fibrin containing gel-embedded plasmin or miniplasmin is disintegrated by shear forces at a relatively early stage of dissolution, and this disassembly is related to the formation of fragment Y (150 kDa) and fragment D (100 kDa) fibrin degradation products. Fibrin clots degraded by incorporated polymorphonuclear leukocyte elastase, which yields different degradation products, do not disassemble abruptly, even at the highest shear rate (500 s⁻¹). Our results suggest that fibrin surface degradation is accelerated with increasing shear rate and that plasmin or miniplasmin embedded in the clot promotes the release of particular clot remnants into the circulating phase, whereas polymorphonuclear leukocyte elastase does not. (Circ Res. 1998;82:1102-1108.)

Key Words: polymorphonuclear leukocyte elastase ■ fibrinolysis ■ thrombus ■ protease ■ shear rate

Thrombi are composed of a fibrin gel network with embedded blood cells, and the solubilization of the fibrin matrix is considered to be the result of its proteolytic degradation by a serine protease, plasmin, formed during plasminogen activation. Platelet-rich thrombi are more resistant against tPA-induced thrombolysis than are erythrocyte-rich clots; this greater resistance is attributed to the platelet-α-subunit of factor XIII and protease inhibitors.³ In the vascular system, the molecular and cellular components of the blood coagulation–fibrinolytic system are permanently exposed to the hemodynamic forces of blood flow. Several processes of hemostasis have been investigated under well-defined flow conditions, and the regulatory role of fluid flow has been demonstrated (reviewed in Reference 3).

In an occlusive thrombus model, the lysis of a whole blood clot is ≈60 times faster when uPA is perfused through the clot compared with the lysis rate when uPA diffuses into the clot under static conditions.⁴ Besides the marked increase in the fibrinolytic rate, the pattern of fluid flow within the clot determines the spatial distribution of lytic areas.⁵ Thus, the primary determinant of the rate of occlusive clot lysis with plasminogen activators is the penetration of the activators into the clot, a process greatly accelerated by pressure-driven bulk flow.⁶-⁷ For characterization of the possible role of shear forces in the lysis of nonocclusive clots, however, only one model has been described.⁸ In this model, when nonocclusive fibrin-rich thrombi are perfused with tPA, a reduction in the rate of lysis is observed compared with the rate under static conditions, whereas lysis of whole-blood thrombi accelerates with increasing shear rates.

In addition to plasmin, PMN-elastase degrades fibrin,⁹ and the detection of fibrinogen degradation products formed by PMN-elastase in human plasma samples indicates the in vivo relevance of this protease.¹⁰ On the basis of its additional interaction with the plasminogen/plasmin system, an alternative fibrinolytic pathway has been suggested (reviewed in Reference 11). PMN-elastase cleaves plasminogen, and the product is miniplasminogen, which lacks the first four kringle domains and is more readily activated by plasminogen activators.¹¹ The physiological relevance of miniplasminogen is supported by data in literature; it has been detected in human plasma,¹² and in a canine thrombolysis model, uPA-induced clot lysis is accelerated when it is administered together with miniplasminogen, whereas supplementation with plasminogen does not improve the efficiency of uPA.¹³ Accordingly, at least three proteases may contribute to fibrinolysis, even in the presence of plasma
protease inhibitors. Their efficiency under flow conditions, however, is not known.

In the present study, we have examined the effect of shear forces on fibrin solubilization by gel-embedded enzymes and also on the lysis rate with proteases circulating at various flow rates over the surface of clots preformed with or without enmeshed platelets. We have found that an increase in shear rate generally facilitates fibrin dissolution and that fibrin degraded by incorporated plasmin or miniplasmin is abruptly disassembled by shear forces at an early stage of solubilization. Our results suggest that the presence of active plasmin or miniplasmin within the fibrin network may promote the formation of particular clot remnants under flow conditions, a phenomenon similar to the in vivo thromboembolism.

Materials and Methods

Plasminogen and Miniplasminogen Preparation

Plasminogen was purified by affinity chromatography on lysine-Sepharose 4B (Pharmacia Biotech) from fresh-frozen citrated human plasma containing 10 U/mL aprotinin and 10 mmol/L benzamidine. Miniplasminogen was prepared by limited proteolysis of plasminogen with porcine pancreatic elastase followed by inhibition of elastase with phenylmethylsulfonyl fluoride and separation of the digestion products on lysine-Sepharose according to our previously published procedure.

Plasmin and Miniplasmin Generation:

Determination of Active Enzyme Concentration

Plasminogen and miniplasminogen were activated with streptokinase (Calbiochem) at 1000 U/mg zymogen. Determination of active enzyme concentration for plasmin, miniplasmin, PMN-elastase (from Serva), and porcine pancreatic elastase (from Calbiochem) was carried out as detailed elsewhere (for the measurement of porcine pancreatic elastase amidolytic activity, the synthetic substrate methoxy-succinyl-L-alanyl-L-alanyl-L-prolyl-L-valine-p-nitroanilide [Calbiochem], \( k_{\text{cat}} = 17 \text{s}^{-1} \)) used.

Thrombin Purification

Bovine thrombin (50 NIH U/mg, Merck) was further purified by ion-exchange chromatography on sulfo-propyl-Sephadex C-50 (Pharmacia Biotech), as described, and was thereafter frozen and stored at −70°C. The specific activity of the final preparation was 1800 NIH units/mg.

Fibrinogen Purification

Fibrinogen, free of plasminogen and factor XIII, was prepared as previously described and dialyzed against HBSS containing (mmol/L) NaCl 138, KCl 5.3, CaCl₂ 0.8, MgSO₄ 0.8, KH₂PO₄ 0.34, and NaH₂PO₄ 0.3 buffered with 10 mmol/L HEPES-NaOH (pH 7.4). Fibrinogen, contaminated with factor XIII, was used for the preparation of cross-linked fibrin. The factor XIII of this fibrinogen (denoted as fibrinogen containing factor XIII) is sufficient for the formation of partially cross-linked fibrin with \( \gamma \)-\( \gamma \) dimers in 20 minutes, as evidenced by SDS gel electrophoresis of reduced samples.

Preparation of Human Washed Platelets

Platelet-rich plasma was prepared by centrifugation of freshly collected citrated human blood for 15 minutes at 155g. Thereafter, platelets were sedimented by centrifugation for 10 minutes at 300g and washed in 13 mmol/L trisodium citrate containing 120 mmol/L NaCl (pH 7.0). Finally, platelets were suspended in HBSS, and platelet count was determined by a Hycell 680 Plus electronic cell counter. To minimize centrifugation-induced platelet activation, all buffers were preequilibrated at 37°C before use, and the platelet suspension was incubated for 15 minutes at 37°C after each centrifugation step to allow the platelets to be restored to their discoid shape.

Detection of Fibrin Degradation Product Release Under Flow Conditions

For measurement of fibrinolysis with proteolytic enzymes, the following models have been developed (Figure 1).

For studying clot dissolution with proteases recirculated over the surface of preformed nonocclusive fibrin clots, fibrin formation was initiated by the addition of thrombin (final concentration, 1 NIH unit/mL) to fibrinogen (2 g/L in HBSS). The mixture was immediately cast around a polyethylene-coated needle (outer diameter, 1.2 mm), which was placed along the long axis of a cylindrical plastic syringe used as a mould. The coated needle was pulled out of the syringe after 2 minutes (at that time, clot turbidity measured at 340 nm reached its maximal value, indicating that the fibrin polymerization was complete), and in this way, a cylindrical fibrin (1.8 mL, 28 mm long) with a channel (diameter, 1.2 mm) along its long axis was prepared. The syringe containing the fibrin clot was then placed into a perfusion circuit (inner diameter of connecting tube, 1.2 mm) composed of an adjustable peristaltic pump (Pharmacia LKB Pump-1) and a UV detector with a flow cell (light path of 2 mm, Pharmacia LKB Optical Unit UV-1) connected to a recorder. Fibrinolytic enzymes in a total volume of 1.8 mL were recirculated at different flow rates through the channel in the clot. Fibrin degradation products generated on the fibrin surface by the enzymes were released into the circulating fluid phase, and their \( A_{280} \) was continuously measured and recorded. During fibrin dissolution, the absorbance was found to be proportional to the protein concentration of the circulating solution as determined according to the procedure of Lowry et al. A final value of \( A_{280} \)=0.32 (equivalent to 1 g/L protein concentration) indicated the complete dissolution of fibrin.

For studying the effect of flow on fibrin dissolution with incorporated enzymes, plasmin, miniplasmin, or PMN-elastase was mixed with fibrinogen (2 g/L) before clotting with thrombin (1 NIH unit/mL). The perfusion circuit was prepared as described above, but instead of enzyme solution, protease-free HBSS was recirculated. Degradation products of buffer-perfused fibrin formed by embedded proteases were also analyzed with SDS electrophoresis performed on 4% to 15% gradient polyacrylamide gels after treatment of samples with 0.1 mol/L Tris-HCl buffer (pH 8.2) containing 0.1 mol/L NaCl, 2% SDS, and 4 mol/L urea.

For studying the effects of platelets on fibrin dissolution, washed platelets (final platelet count, 95 000/µL) were mixed with fibrinogen (2 g/L) before clotting.

<table>
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<th>Selected Abbreviations and Acronyms</th>
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<tr>
<td>( A_{280} ) = absorbance at 280 nm</td>
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<td>PMN-elastase = polymorphonuclear leukocyte elastase</td>
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<td>( t_d ) = disassembly time</td>
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<td>tPA = tissue-type plasminogen activator</td>
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<td>uPA = urokinase-type plasminogen activator</td>
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Calculation of Shear Rate at Fibrin Surface
In our model, even the highest possible Reynolds number is an order of magnitude lower than the critical value, above which turbulence occurs,\textsuperscript{23} and since fibrin surface degradation is a layer by layer elimination process,\textsuperscript{24} the regularity of the inner surface of the cylindrical fibrin channel is preserved in the course of fibrinolysis. Assuming lamellar flow in the cylindrical channel in the fibrin clot, we determined the shear rate at the fibrin surface to be equal to 4Q/R\(^2\), where Q is the applied flow rate, and R is the radius of the channel.\textsuperscript{1} In our experimental design, R=0.6 mm, and the flow rate is varied in the range of 0.1 to 5 mL/min, resulting in a wall shear rate in the range of 10 to 500 s\(^{-1}\). Since in the course of fibrin dissolution the radius of the channel increases (fibrin consumption) and the flow rate is kept constant, the shear rate at the fibrin surface decreases according to the equation above. We always give the shear rate calculated with the initial radius of the intact fibrin channel.

Statistics
The Student two-tailed unpaired t test was used for statistical analysis. Analysis of the data in the Table, however, was performed by two-way ANOVA with a Fisher protected least significant difference test. Values are given as mean±SEM, and statistical significance was determined at a level of P<0.05.

Results
In the course of perfusion of the proteolytic enzyme–free fibrin with protease-free HBSS, the absorbance (A\(_{280}\)) of the circulating fluid phase does not increase even at the highest shear rate (500 s\(^{-1}\)). When plasmin or porcine pancreatic elastase is recirculated through the fibrin channel at any shear rate, the absorbance is a linear function of time: more and more fibrin degradation products are released into the circulatory fluid phase (Figure 2). The slope of the absorbance curve declines after 60% to 70% consumption of fibrin; thus, each curve can be characterized with the rate of product release (ΔA\(_{280}\)/h) from its initial linear portion. In the course of fibrin dissolution with circulating miniplasmin, however, a two-phase pattern of product release is found (Figure 2). The slope of the absorbance curve declines after 60% to 70% consumption of fibrin; thus, each curve can be characterized with the rate of product release (ΔA\(_{280}\)/h) from its initial linear portion. 6-Aminohexanoate modifies the kinetic pattern of soluble product release by both plasmin and miniplasmin in a manner similar to the one we have previously described in a static fibrinolytic model,\textsuperscript{15} whereas it has no effect on elastase-catalyzed solubilization (not shown). When enzymes are perfused at low shear rates (25 s\(^{-1}\)), the rate of product release does not depend on an enzyme concentration over 30 nmol/L.

Rate of Dissolution of Various Clots With Circulating Enzymes

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<th>% of Clot Dissolution/h</th>
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<td></td>
<td>Polymerized Fibrin</td>
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<tr>
<td>Plasmin (150 nmol/L)</td>
<td>27.4±0.9</td>
</tr>
<tr>
<td>Miniplasmin (150 nmol/L)</td>
<td>29.6±2.2</td>
</tr>
<tr>
<td>Porcine pancreatic elastase (1500 nmol/L)</td>
<td>20.0±0.7</td>
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Plasmin, miniplasmin, or porcine pancreatic elastase (at saturating concentration) was perfused at a wall shear rate of 500 s\(^{-1}\) on the surface of different clots. Rates of dissolution are expressed as percentage of fibrin clot solubilized per hour (mean±SEM), as estimated from the change in A\(_{280}\) of the circulating phase. The effect of clot structures on fibrinolytic rates is statistically significant (P<0.05) for all three proteases.

Figure 2. Time course of fibrin dissolution with circulating proteases. A preformed fibrin channel is perfused with 9 nmol/L plasmin (P), 9 nmol/L miniplasmin (MP), or 900 nmol/L porcine pancreatic elastase (PPE) at a wall shear rate of 25 s\(^{-1}\), and the release of degradation products is followed by continuous detection of absorbance at 280 nm in a flow cell. A\(_{280}=0.32\) represents 100% fibrin dissolution.

Figure 3. Effect of shear rate on fibrin dissolution with circulating proteases. A, Plasmin (circles), miniplasmin (inverted triangles), or porcine pancreatic elastase (triangles) is perfused at different concentrations through a preformed fibrin channel at a wall shear rate of 25 s\(^{-1}\) (solid symbols) or 500 s\(^{-1}\) (open symbols), and the rate of product release is measured and expressed as ΔA\(_{280}\)/h. Error bars represent SEM. B, A preformed fibrin channel is perfused with 150 nmol/L plasmin at a wall shear rate of 25 s\(^{-1}\), and fibrin dissolution is continuously detected at 280 nm. At the time indicated by the arrow, the shear rate is increased to 500 s\(^{-1}\).
and miniplasmin reaches its maximal value at an enzyme concentration of 120 nmol/L, and the maximal product-release rate increases 2.8-fold for plasmin and 2.9-fold for miniplasmin compared with that at 25 s$^{-1}$. At high shear rates, the release of degradation products formed by elastase is also faster: the maximal product-release rate at 500 s$^{-1}$ is 4-fold higher than at a shear rate of 25 s$^{-1}$ (Figure 4). At a shear rate of 500 s$^{-1}$, 6 to 7 hours is required for complete fibrin dissolution with circulating tPA (60 nmol/L) or streptokinase (160 U/mL), and the recorded absorbance curves decline only after 70% consumption of fibrin.

The rate of fibrin dissolution with enzymes perfused at saturating concentrations over the surface of preformed clots decreases when the substrate is cross-linked, and clots with embedded platelets are further protected against all three proteases (Table). By the time of the application of proteases (25 minutes after clotting), platelet-fibrin clots and clots prepared from fibrinogen containing factor XIII have the same partially cross-linked structure, as evidenced by SDS gel electrophoresis.

Porcine pancreatic elastase and PMN-elastase applied to the surface of preformed fibrin clots under static conditions yield similar degradation products in a similar time course, as detected with SDS gel electrophoresis (not shown). This justifies the usage of porcine pancreatic elastase instead of PMN-elastase for enzyme perfusion experiments (PMN-elastase was not available in the high amount needed to fill the perfusion circuit at concentrations in the micromolar range).

In the initial phase of fibrin dissolution with incorporated plasmin (7 nmol/L), the absorbance of circulating soluble degradation products is a linear function of time (indicated as phase 1 of curve I in Figure 5A). When $A_{280}$ reaches a certain level, an abrupt increase in $A_{280}$ is observed, followed by a

**Figure 4.** Time course of fibrin dissolution with circulating plasminogen activators. A and B, Plasminogen (135 nmol/L) is incorporated into fibrin, and tPA at indicated concentrations is recirculated at a wall shear rate of 500 s$^{-1}$ (A) or 25 s$^{-1}$ (B). C, Plasminogen (135 nmol/L) is incorporated into fibrin, and streptokinase (160 U/mL) is recirculated at the indicated shear rates. D, Zymogen-free fibrin channel is perfused with plasminogen (100 nmol/L) and tPA (3 nmol/L) at indicated shear rates. Fibrin dissolution is continuously followed as described in “Materials and Methods.”

**Figure 5.** Time course of fibrin dissolution with incorporated enzymes under flow conditions. A, Fibrin clot containing 7 nmol/L (curve I) or 3.5 nmol/L (curve II) plasmin is prepared; thereafter, protease-free HBSS is perfused through its channel at a wall shear rate of 500 s$^{-1}$. The absorbance of the perfusate is measured at 280 nm in a flow cell. The three phases of fibrin dissolution and the time elapsed until fibrin disassembly ($t_d$) are indicated. B, Fibrin clot containing 25 nmol/L (solid line) or 12.5 nmol/L (dashed line) PMN-elastase is perfused with protease-free buffer at a wall shear rate of 500 s$^{-1}$, and $A_{280}$ of the circulating fluid is continuously measured.
period of large-magnitude oscillations (phase 2 of curve I in Figure 5A). In temporal concordance with this phase, the disassembly of the solid fibrin gel and the appearance of small clot particles in the perfusate can be visualized (the light-scattering from these particles passing through the flow cell of the UV detector causes the \(A_{280}\) spikes in phase 2). The absorbance of the recirculating fluid phase is finally stabilized after complete degradation of the particular elements; the constant value of \(A_{280}\) represents the state of complete fibrin dissolution (phase 3 of curve I in Figure 5A). The time elapsed until half of this final \(A_{280}\) value is reached is arbitrarily designated \(t_d\) for the fibrin gel network. When plasmin is incorporated into fibrin at a lower concentration (3.5 nmol/L) and the same shear rate (500 s\(^{-1}\)) is applied, clot disassembly occurs later, but phase 2 of the dissolution curve starts at the same \(A_{280}\) value as for the higher plasmin concentration (curve II in Figure 5A). When miniplasmin is used instead of plasmin, similar dissolution patterns are found. When the same shear rate is applied, the disassembly time is a reciprocal function of the concentration of incorporated plasmin or miniplasmin (inset in Figure 6). At constant plasmin or miniplasmin concentrations, an increase in the shear rate results in shorter disassembly time with a marked period of large-magnitude oscillations (phase 2 of curve I in Figure 5A). Results are shown as mean±SEM.

Discussion

The role of hemodynamic forces in the lysis of nonocclusive clots is poorly characterized. Therefore, we have developed two in vitro models for the investigation of shear rate effects on fibrinolysis. In our system, both geometrical and hemodynamic conditions are simplified: a cylindrical fibrin with a cylindrical axial channel represents the nonocclusive clot, and blood circulation is modeled by laminar flow through the channel. For studying the effect of shear rate on various
processes of hemostasis, laminar flow chambers are widely accepted in vitro models. The advantage of laminar flow chambers is that fluid dynamics can be characterized while avoiding the complications introduced by branching and pulsation of the vessels, and this enables the investigators to relate an observed phenomenon to a given shear rate (reviewed in Reference 3). Geometrical and hemodynamic conditions of the formation and lysis of in vivo thrombi, however, are much more complicated; thus, limitations of these models should not be neglected in the interpretation of results.

In our first system, fibrinolytic enzymes circulate at various flow rates over the surface of a preformed cylindrical fibrin channel. This method integrates several steps, including the transport and binding of the enzyme to fibrin, the cleavage of peptide bonds in the substrate, and the release of degradation products into the circulating fluid phase. In the course of perfusion of intact fibrin with protease-free buffer, the undegraded polymerized fibrin structure is resistant against the mechanical erosive effect of fluid flow even at a wall shear rate of 500 s⁻¹, which is close to that in healthy medium-sized human arteries. In the presence of circulating proteolytic enzymes, the abrupt release of degradation products after the adjustment of the higher shear rate shows that there are certain products released at this time that have already been formed in the period of recirculation at low shear rate, but only the higher shear force can detach them from the solid-phase surface (Figure 3B). This result emphasizes the fact that in our model, the fibrinolytic rate describes the soluble product-release rate, which is not essentially equivalent to the rate of proteolysis on the fibrin surface. At all tested wall shear rates, the fibrinolytic activity of perfused plasmin is similar to that of miniplasmin in the second phase. Comparison of the maximal product-release rates achieved at a wall shear rate of 25 s⁻¹ with saturating concentrations of the various enzymes shows only a 2-fold difference between the most efficient protease, miniplasmin, and the least efficient one, elastase. As the shear rate increases up to 500 s⁻¹, this factor decreases to 1.5, and fibrin solubilization is generally facilitated (Figure 3A). Elastase and plasmin or miniplasmin yield different fibrin degradation products, which are capable of maintaining interactions of different strengths with the solid-phase clot. One could speculate that since higher shear forces can overcome stronger polymerization interactions, at higher shear rates the formation of different degradation products loses its significant role in the determination of product-release rate, and this could lead to the attenuation of differences among the fibrinolytic activities of different proteases.

Product-release patterns for perfused plasmin and miniplasmin in the absence (Figure 2) or presence of 6-aminohexanoate suggest that our previous consideration on the role of various kringle domains in fibrin degradation is valid under flow conditions as well.

In a previously described model, lysis of fibrin-rich thrombi with circulating tPA decreases with increasing shear rate, whereas that of whole-blood thrombi increases. In the same model, streptokinase-induced clot lysis is not affected by flow. In our system, soluble product release is accelerated at a high shear rate both when active enzymes are circulated (Figure 3) and when fibrin dissolution is initiated with circulating plasminogen activators, tPA or streptokinase (Figure 4). The apparent controversy may be due to the differences in clot structures or to other differences in experimental settings.

The lysis rate of fibrin clots decreases with all three proteases in the presence of enmeshed platelets, and this decrease can be attributed only in part to fibrin cross-linking (Table). Considering the protease inhibitor content of platelets and the platelet count in our system, inhibition of the enzymes by platelet-derived protease inhibitors does not seem to be a probable explanation. Ultrastructural studies on platelet-fibrin clots have shown that in the presence of platelets, fibrin strands conform to the platelet surface and platelet pseudopodia extend along fibrin bundles. As platelets constrict, fibrin strands are pulled, and force is transmitted to the clot surface, resulting in clot retraction. Although in our system macroscopic clot retraction is not observed, platelets might render the clot a more resistant structure against mechanical forces via their interaction with fibrin strands, and this could lead to a decreased rate of soluble product release under flow conditions.

In our second system, when enzymes are incorporated into the clot, fibrin digested with embedded plasmin or miniplasmin is disassembled by shear forces at a relatively early stage of fibrin dissolution, and in phase 2, circulating clot particles are present (Figure 5A). The disassembly of fibrin is related to the formation of fragment Y and D degradation products (Figure 7). Disassembly times for fibrin clots containing plasmin or miniplasmin at equivalent concentrations increase with decreasing shear rates (Figure 6), suggesting that lower shear forces disintegrate clots only at a more advanced stage of proteolytic degradation, when fibrin structure is kept together by weaker polymerization interactions. The most marked change in disassembly time is observed in the shear rate range of 0 to 50 s⁻¹, which corresponds to shear rate values measured in human large veins in vivo. When flow rates of 0.2 and 2 mL/min are switched periodically after each other, tₜ is similar to t₀ at a constant 2 mL/min. This further supports the interpretation that clot disassembly is related to that stage of its proteolytic degradation, at which wall shear forces overcome the interactions within the fibrin gel network. Abrupt clot disassembly is observed neither in the first model when enzymes recirculate over fibrin nor in the course of circulating tPA-induced lysis of fibrin containing embedded plasminogen. In both cases, the degradation of fibrin is located within a 5- to 8-μm superficial shell. The lack of clot disassembly in the course of clot lysis with circulating streptokinase suggests that its diffusion into fibrin is also slow compared with the rate of plasminogen activation. All these data indicate that clot-particle formation under flow conditions is promoted only by enzymes dispersed within the clot. Fibrin clots with embedded platelets disassemble in the course of their degradation by incorporated plasmin, and disassembly time at 500 s⁻¹ is slightly prolonged in the presence of platelets. When dissolution of a buffer-perfused plasma clot is initiated by incorporation of streptokinase into the clot, clot disassembly has also been observed, suggesting that this phenomenon is not restricted to the lysis of purified...
fibrin gel structures. The lack of clot disassembly in the course of fibrinolysis with incorporated PMN-elastase represents a different dissolution pattern, where the formation of low-molecular-weight degradation products is negligible even after 50% dissolution of fibrin (Figures 5B and 7). The relationship between the relatively early generation of fragments Y and D by plasmin and miniplasmin and the event of clot disassembly emphasizes the significance of the protease-specific cleavage pattern in the process of clot-particle formation. The fibrin dissolution pattern with clot-embedded plasmin or miniplasmin suggests that proteolytic degradation of the fibrin gel network by these incorporated proteases may promote the generation of thromboemboli under flow conditions.

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