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Rearrangements of the Fibrin Network and Spatial Distribution of Fibrinolytic Components during Plasma Clot Lysis

STUDY WITH CONFOCAL MICROSCOPY*

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Binding of components of the fibrinolytic system to fibrin is important for the regulation of fibrinolysis. In this study, decomposition of the fibrin network and binding of plasminogen and plasminogen activators (PAs) to fibrin during lysis of a plasma clot were investigated with confocal microscopy using fluorescein-labeled preparations of fibrinogen, plasminogen, tissue-type PA (t-PA), and two-chain urokinase-type PA (tcu-PA).

Lysis induced by PAs present throughout the plasma clot was accompanied by a gradual loss of fibrin content of fibers and by accumulation of plasminogen onto the fibers. Two sequential phases could be distinguished: a phase of prelysis, during which the fibrin network remained immobile, and a phase of final lysis, during which fibers moved with a tendency to shrink and eventually disappeared.

The two phases occurred simultaneously but in different locations when lysis was induced by PAs present in the plasma surrounding the clot. The zone of final lysis was located within a 5–8-μm superficial layer, where fibers were mobile, and surface-associated fibrin agglomerates appeared. Plasminogen accumulated in these agglomerates up to 30-fold as compared with its concentration in the outer plasma. t-PA was also highly concentrated in the agglomerates, and tcu-PA bound to them slightly. The zone of prelysis, where plasminogen was moderately accumulated on the immobile fibers, was located deeper in the clot. This zone was much thinner in the case of t-PA-induced lysis than in the case of tcu-PA-induced lysis, reflecting the difference in penetration of the two PAs into the clot.

We conclude that under conditions of diffusional transport of fibrinolytic enzymes from outside a plasma clot, extensive lysis is spatially restricted to a zone not exceeding 5–8 μm from the clot surface. In this zone the structure of the fibrin network undergoes significant changes, and strikingly high accumulation of fibrinolytic components takes place.

Fibrin represents the insoluble matrix of thrombi and the eventual target of fibrinolysis, either physiologic or therapeutic (1–3). In fibrinolysis, fibrin plays a much more complicated role than that of a mere insoluble substrate for proteolysis. It is involved in numerous interactions with components of both fibrinolytic (4–9) and antifibrinolytic (10, 11) systems, thus actively participating in the regulation of the self-destruction.

Binding of plasminogen to partially degraded fibrin is an important mechanism of positive feedback regulation of fibrinolysis (12–17). Using conventional fluorescence microscopy, we have recently demonstrated that plasminogen accumulates severalfold in a thin superficial layer of a plasma clot during its lysis (18).

Here, we report on the fibrin architecture in a lysing plasma clot as studied with confocal laser-scanning fluorescence microscopy. Using this technique, we were able to follow the structural rearrangements of the fibrin network and to quantitate the spatial microdistribution of plasminogen and plasminogen activators (PAs)[1] near the surface of the plasma clot during its lysis. The study reveals specific fibrin structures associated with the surface of the lysing clot that provide a scaffold for an extremely high superficial accumulation of fibrinolytic components.

EXPERIMENTAL PROCEDURES

Preparations—Glu plasminogen was a product of Biofine (Leiden, The Netherlands). No contamination by the Lys form of plasminogen or by other proteins was found in the preparation by SDS-polyacrylamide gel electrophoresis. The t-PA preparation (Actilyse) was supplied by Boehringer Ingelheim (Ingelheim, Germany), and human urinary tcu-PA (Ukidan) was obtained from Serono (Aubonne, Switzerland). Human fibrinogen from Kabi (Stockholm, Sweden) was made plasminogen-free by lysine-Sepharose chromatography. Fluorescein isothiocyanate (FITC) was from Sigma. Fluorescein 5-maleimide was from Pierce. We also used thrombin (Leo, Ballerup, Denmark) and hirudin (Pentapharm, Basel, Switzerland). Experiments were performed with pooled citrated platelet-poor plasma from healthy volunteers.

Labeling of Proteins with Fluorescein Derivatives—Fibrinogen (0.3 ml, 20 μM) was gel-filtered in a centrifuge using a 2-ml microcolumn with Sephadex G-25 (fine) equilibrated with a buffer containing 30 mM sodium borate, 140 mM NaCl, pH 8.0. FITC (1 mg/ml in Me_2SO) was added to the fibrinogen solution under intensive stirring to a final concentration of 50 μg/ml. After 1 h of incubation in the dark at room temperature, unreacted FITC was removed by gel filtration on the same column with Sephadex G-25 reequilibrated with Tris-buffered saline containing 20 mM Tris-HCl, 140 mM NaCl, pH 7.4. The preparation was stored frozen in small portions, which were thawed only once before each experiment. The ratio A_{495}/A_{280} in preparations of FITC-fibrinogen was about 0.2, corresponding to a molar fluorescein:fibrinogen ratio of 2. The fluorescent signal of the FITC-fibrinogen was neither quenched upon polymerization with thrombin nor dequenched upon cleavage with plasmin (data not shown), as was described for more heavily labeled preparations of FITC-fibrinogen (19). The procedures for labeling plasminogen and tcu-PA with FITC were described in detail elsewhere (18).

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[1] The abbreviations used are: PA, plasminogen activator; t-PA, tissue-type plasminogen activator; tcu-PA, two-chain urokinase-type plasminogen activator; FITC, fluorescein isothiocyanate.

TABLE I

Accumulation of fibrinolytic components in surface-associated agglomerates and in different layers of a plasma clot during lysis induced by PAs in plasma surrounding the clot

The data presented are the result of three independent experiments similar to those presented in Fig. 3. Values represent accumulation factors (mean ± S.D.), defined as the ratios of local concentration of labeled fibrinolytic components in the areas indicated to their concentration in the plasma surrounding the clot. Note: labeled plasminogen was added both to the surrounding plasma and inside the clots in equal concentrations; labeled PAs were added only to the surrounding plasma.

Location in a plasma clot	Plasminogen		t-PA	tcu-PA
	t-PA-induced lysis	tcu-PA-induced lysis		
Individual agglomerates	26.2 ± 9.1	29.7 ± 11.2	25.2 ± 6.9	1.9 ± 0.6
0–3-μm layer, comprising agglomerates	8.6 ± 2.3	9.6 ± 1.2	9.2 ± 1.6	1.1 ± 0.1
3–20-μm layer, not including agglomerates	1.9 ± 0.2	1.8 ± 0.3	1.6 ± 0.2	ND[a,b]
20–150-μm layer	0.9 ± 0.1	0.9 ± 0.1	ND[c]	ND[a,b]

[a] ND, not determined; values could not be determined accurately.
[b] The values were <1; data were dependent on the time of incubation and were strongly affected by local stirring near the boundary of the clot.
[c] The value was ≪1, and the specific fluorescence signal was weak as compared with plasma autofluorescence.

t-PA was labeled with fluorescein 5-maleimide, presumably attached to the sulfhydryl group in position 83, as described by others (20). As reported earlier (18), the labeling did not significantly disturb functional properties of the fibrinolytic enzymes.

Design of the Experimental System—The experimental system for visualization of the spatial distribution of FITC-labeled molecules inside a plasma clot has been described elsewhere (18, 21). In brief, plasma was clotted with thrombin (final concentration 1.4 NIH units/ml) between two parallel glass slides separated by a spacer about 0.2 mm high. After 3 min a clot with an approximate diameter of 2–3 mm was formed firmly attached to the parallel glass slides, and then the remaining volume of the chamber was filled with plasma containing 5 antithrombin unit/ml of hirudin. Plasminogen activators and/or tracer fluorescent molecules were added either to plasma before clotting or to the outer plasma after clotting, as indicated. Lysis was performed at room temperature.

Confocal Fluorescence Microscopy—The confocal microscopy experiments were performed using an MRC-600 Laser Sharp system (Bio-Rad Microscience Ltd., Hemel Hempsted, United Kingdom) linked to a Zeiss IM 35 inverted microscope (Carl Zeiss, Oberkochen, FRG). The microscope was equipped with a Zeiss 63× Planapo oil immersion objective (numerical aperture 1.3). An argon laser was used in combination with a 488-nm band pass filter for the excitation and a 520-nm-long pass filter for the emission. The optical resolution (about 0.5 μm in the *x-y* direction and about 0.7 μm in the *z* direction) allowed the reliable measurement of the fluorescence in structures with characteristic dimensions of 1 μm and more. Thinner structures (*i.e.* fluorescence-labeled fibrin fibers) were visible, although neither their dimensions nor the concentration of a fluorescence label associated with the fibers could be measured quantitatively.

The design of the system allowed images to be collected periodically during ongoing lysis. Bleaching of fluorescence in the specimens was reduced by using optical filters attenuating laser intensity to 3 or 10% of the full intensity. To exclude the disturbing influence of clot/glass interface, all images were taken at a distance of 40–50 μm from the surface of the glass.

A computer equipped with standard Bio-Rad software (Comos 6.03) was used for operating the system and for processing of images. Images were collected in a format of 768 × 512 pixels, with 256 gradations of intensity. Kalman filtering (averaging of images to reduce noise) was used for improvement of image quality. Collecting a single complete image took 5 s. Images were printed using either a Sony Video Graphic Printer UP-850 (black and white) or a Mitsubishi CP50–1 color printer.

The software was used to determine the intensity of the fluorescence signal in selected areas of the images obtained. Calibration curves (not shown) establishing correlations between concentration of labeled proteins in the chamber and the intensity of fluorescence were linear and were not affected significantly by the presence of the plasma clot. The accumulation of labeled fibrinolytic components in different layers of the clot (see Table I) was measured as a ratio of intensity of fluorescence in the selected layer and the intensity of fluorescence in the plasma surrounding the lysing clot. It should be noted that this technique does not allow discrimination of intact labeled proteins (Glu plasminogen, PAs, and fibrin) and their derivatives, which might be generated during fibrinolysis (like Lys plasminogen, plasmin, complexes of plasmin or PAs with inhibitors, and fibrin degradation products).

In order to judge the mobility of the fibrin network, consecutive images were taken at 30-s intervals. The fibers were considered to be immobile if their positions in such images coincided. Merging of two consecutive images into one was used to distinguish mobile and immobile fibers.

RESULTS

Confocal fluorescence microscopy allows visualization of the fibrin network of a plasma clot, monitoring of changes in its architecture during lysis, and quantitation of the spatial microdistribution of fluorescence-labeled components inside the clot. Two different experimental setups were used: 1) lysis induced by a PA present throughout the plasma clot, and 2) lysis induced by a PA added to plasma surrounding the clot.

Lysis of a Plasma Clot with PAs Present throughout the Clot—Fig. 1, *A–D*, shows the dynamics of the FITC-labeled fibrin network of a plasma clot during its lysis induced by t-PA present inside the clot. The structure of the clot during the first phase of lysis (*B*) was similar to that of the intact clot in the absence of PA (*A*) and, in general, resembled the images published recently by Blombäck *et al.* (22, 23). It was a network consisting of fibers that formed nodes in places of intersections. During the first phase of lysis, the fibrin lattice remained immobile, and some loss of the fibrin content of fibers took place, manifested by a reduction of fiber-associated fluorescence. Then the network changed its geometry, fibers started to move with a tendency to shrink (*C* and *D*), and very soon thereafter the network was completely dissolved.

Parallel experiments performed in the presence of tracer amounts of FITC-plasminogen are presented in Fig. 1 (*E–H*). The binding of plasminogen to the intact fibrin network was very weak, hardly allowing the visualization of the fibrin lattice of a clot without the addition of a PA (*E*). In the presence of t-PA, plasminogen accumulated gradually onto the immobile fibers during the first stage of lysis (*F*) and remained bound to the mobile and partially disconnected lattice until its complete lysis during the second phase (*G*, *H*). In the very last moments of lysis, both fibrin and plasminogen appeared to be concentrated in the remnants of the lattice (*D*, *H*), possibly as a result of shrinkage and/or aggregation of disconnected pieces of the fibrin network. The sequence of events illustrated in Fig. 1 was basically the same when lysis was induced by tcu-PA under the same conditions (not shown).

Lysis of a Plasma Clot with PAs Added to the Surrounding Plasma—Fig. 2*A* represents four consecutive views of the boundary of a plasma clot that was lysed by the addition of t-PA to the surrounding plasma. Fibrin fibers were mobile in close proximity to the moving surface of the lysing clot. They adhered to the surface, bent along with its movement, and eventually merged with it. Further away from the surface, the fibrin network remained immobile.

The regions with either mobile or immobile network can be easily discriminated in Fig. 2*B*, in which a pair of consecutive images from Fig. 2*A* are combined into one image. The network

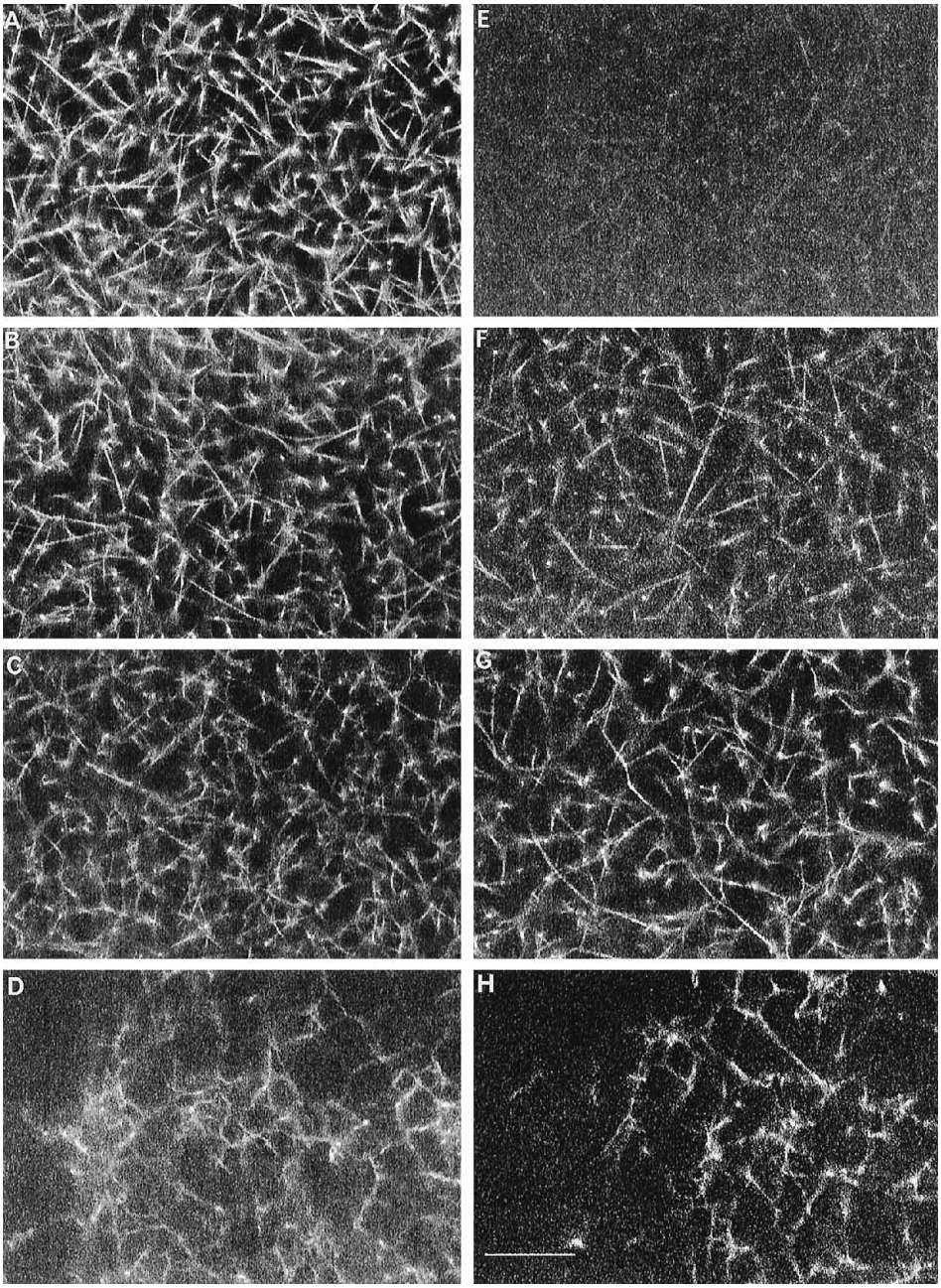

FIG. 1. **Rearrangements of the fibrin network and redistribution of plasminogen during lysis of a plasma clot induced by t-PA present inside the clot.** Either tracer FITC-fibrinogen (final concentration 1 μM, *panels A–D*) or FITC-plasminogen (final concentration 0.3 μM, *panels E–H*) was added to plasma before clotting. Then t-PA was added to plasma to a final concentration of 1.5 μg/ml (*panels B–D* and *F–H*), followed immediately by the addition of thrombin (final concentration 1.4 NIH units/ml). Images were made 3 min (*B* and *F*), 7 min (*C* and *G*), and 10 min (*D* and *H*) after the addition of thrombin. *Panels A* and *E* show the distribution of FITC-fibrin and FITC-plasminogen, respectively, in an intact plasma clot with no PA added, 10 min after the addition of thrombin. *Bar*, 20 μm.

of the second and third images of Fig. 2*A* is presented in *green* and *red*, respectively, resulting in a *yellow color* in places where the two images coincide. Based on this typical image, we estimated the width of the layer in which the network was mobile (the layer in which the "red" fibers are split from the "green" ones) as approximately 5–8 μm.

The surface bore agglomerates of fibrin (2–4 μm in diameter), which mainly appeared in places where the moving surface came in contact with the nodes of the fibrin fibers. The concentration of fibrin in these agglomerates appeared to be 3–7-fold higher than the average concentration of fibrin in the original plasma clot (about 10 μM). Thus, the concentration of fibrin in the agglomerates was estimated as 30–70 μM. Once an agglomerate appeared, it moved together with the reducing surface of the clot for several minutes and eventually disappeared. The dynamics of changes of the fibrin network were essentially the same when the lysis of a plasma clot was induced by tcu-PA (not shown).

The patterns of the spatial distribution of plasminogen during lysis induced by t-PA (1.5 μg/ml) and by tcu-PA (300 IU/ml) in the surrounding plasma are presented in Fig. 3, *A* and *B*, respectively. In both cases, the high accumulation of plasminogen in a thin superficial layer of the lysing clot was observed. The highest concentration of plasminogen was found in the surface-associated fibrin agglomerates described above. Deeper into the clot, plasminogen bound moderately to fibrin fibers, making them visible. As in Fig. 1*F*, this binding reflects exposure of new plasminogen binding sites on the fibrin fibers,

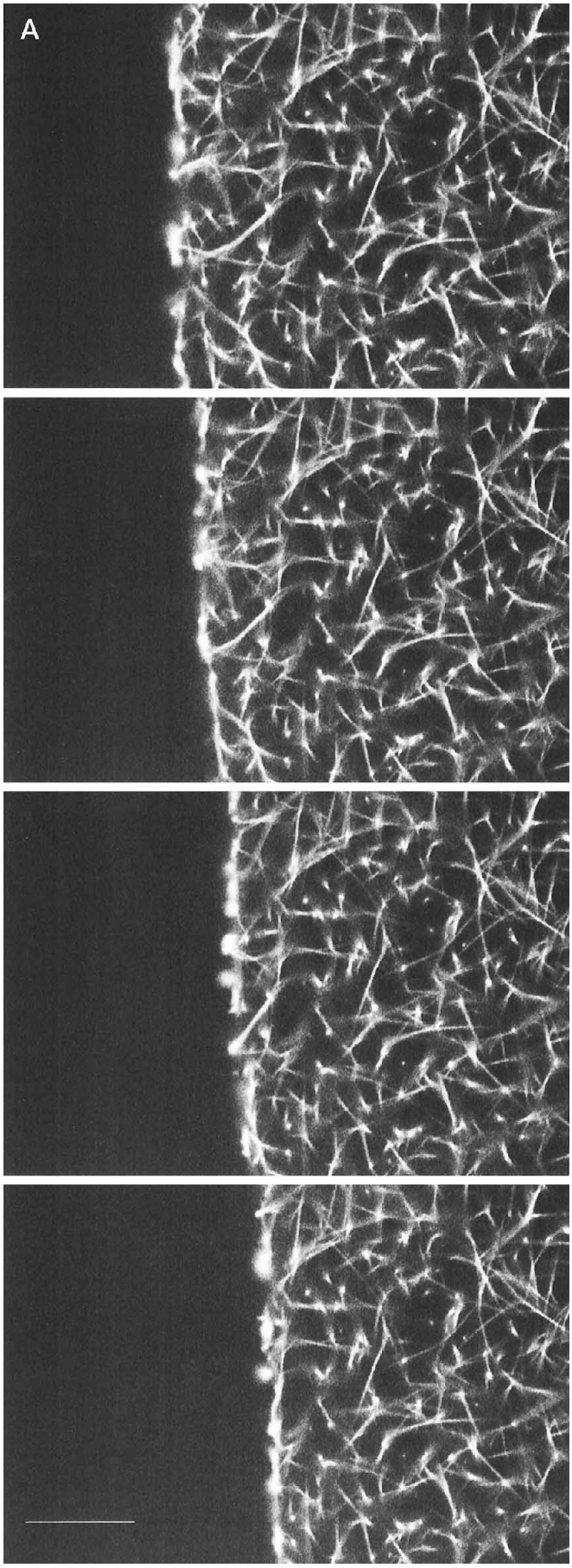

FIG. 2

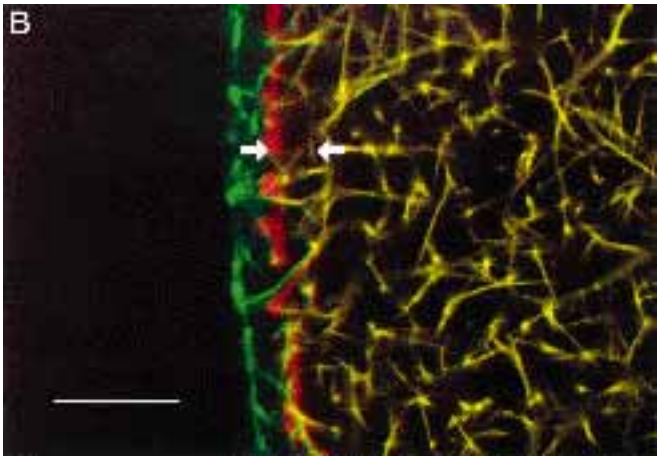

FIG. 2. **Rearrangements of the fibrin network of a plasma clot during lysis induced by t-PA present in plasma surrounding the clot.** Tracer FITC-fibrinogen (final concentration 1 μM) was added to plasma before clotting. *A*, four consecutive images were made 20 min after the addition of t-PA (1.5 μg/ml) with 30-s intervals between the images (from *top* to *bottom*). *B*, second (*green*) and third (*red*) images from Fig. 2*A* merged into one image. The regions with an immobile network (*green* and *red* images coincide) appear *light yellow*. The *arrows* indicate the zone where the network is mobile (*red* splits from *green*). *Bars*, 20 μm.

generated as a result of their nicking with plasmin. The depth of this zone of "prelysis" depended on the plasminogen activator used. In the case of t-PA, this zone was restricted to a 20-μm layer adjacent to the surface. The fibrin lattice remained invisible beyond this layer (Fig. 3*A*). The zone of prelysis was much deeper when the lysis was induced by tcu-PA, as illustrated by Fig. 3*B*, where the fibrin matrix is visible at least up to the depth of 150 μm inside the clot.

Quantitative data on the accumulation of fibrinolytic components at different distances from the surface of the lysing clot are summarized in Table I. They demonstrate that plasminogen (during lysis induced by either t-PA or tcu-PA) was concentrated up to 30-fold in individual surface-associated agglomerates in comparison with its concentration in the surrounding plasma (1.5 μM (1)). Thus, the local concentration of plasminogen in the agglomerates could be as high as 45 μM. On average, plasminogen was concentrated about 10-fold within a 3-μm layer comprising these agglomerates. The average concentration of plasminogen was also somewhat elevated (about 2-fold) in a 3–20-μm layer contiguous to the surface but not including the surface-associated agglomerates. Binding of plasminogen to fibers in deeper layers of the clot (20–150 μm) in case of tcu-PA-induced lysis was not associated with an overall increase of the plasminogen concentration within the area. Therefore, the observed binding seems to be a result of local redistribution of plasminogen between the soluble and the fibrin-bound phases within this clot region rather than a result of recruiting of plasminogen from other areas of the clot or from the surrounding plasma.

Experiments with fluorescence-labeled derivatives of PAs indicated a considerable difference in penetration of the two PAs into the clot during its lysis (Fig. 3, *C* and *D*). The distribution of labeled t-PA (*C*) was very similar to that of plasminogen in the case of the t-PA-induced lysis. t-PA was highly concentrated in the surface-associated agglomerates and was moderately bound to immobile fibers within a 3–20-μm layer near the surface not including these agglomerates (see Table I). This pattern of binding of both plasminogen and t-PA was apparently not dependent on the time passed after the beginning of lysis (data not shown). These results suggest that the penetration of t-PA into the clot was restricted, supposedly, as

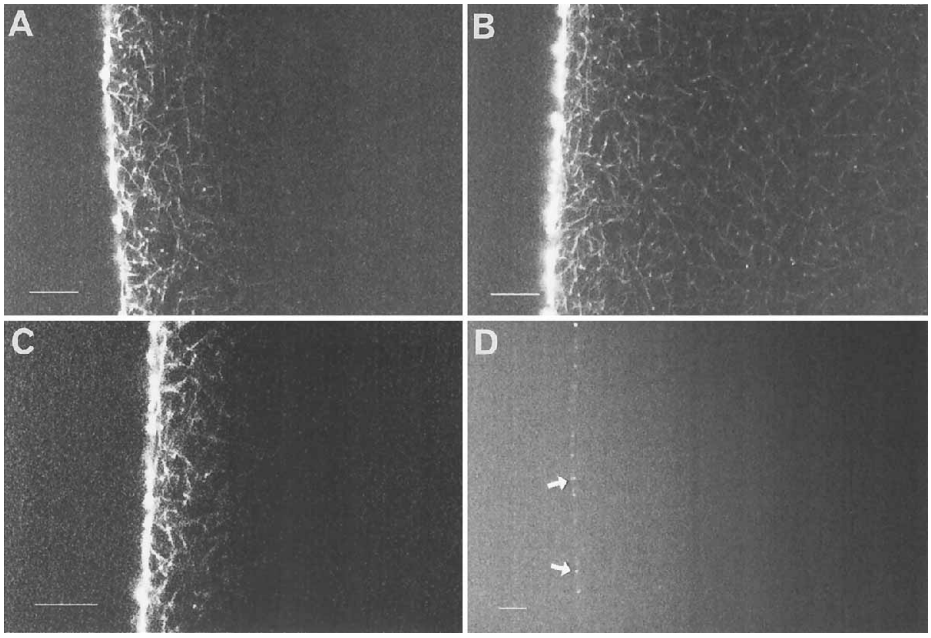

FIG. 3. **Spatial distribution of fluorescein-labeled fibrinolytic components during lysis of a plasma clot induced by PAs added to plasma surrounding the clot.** *A* and *B*, the distributions of FITC-plasminogen during lysis induced by t-PA (1.5 μg/ml) and tcu-PA (300 IU/ml), respectively. The speed of lysis was similar in the two cases. Tracer FITC-plasminogen (0.3 μM) was present both inside the clot and in the outer plasma. *C* and *D*, the distributions of fluorescein 5-maleimide-t-PA (10 μg/ml), and FITC-tcu-PA (1200 IU/ml) only added to the outer plasma. *Arrows* in *panel D* indicate surface-associated agglomerates, slightly accumulating FITC-tcu-PA. All images were taken 20 min after the addition of the PAs. *Bars*, 20 μm. Note: in *panel D*, a lower magnification was used than in *panels A–C* (compare the *scale bars*).

a result of its affinity to fibrin (5, 8). In contrast, labeled tcu-PA diffused into the clot freely, although a weak binding was detected in the surface-associated agglomerates (*D*). tcu-PA did not bind detectably to fibers inside the clot and penetrated deeper into the clot as compared with t-PA. The distance of the tcu-PA penetration increased during the course of lysis (data not shown). In control experiments, fluorescence-labeled bovine serum albumin bound neither to surface-associated agglomerates nor to the fibrin network inside the lysing clot (not shown).

DISCUSSION

The *in vitro* model of a noncontracted plasma clot, obtained as a result of thrombin-induced clotting of blood plasma, is commonly used for studying fibrinolysis. Depending on the aim of the investigation, lytic agents are added to plasma before clotting (see, for example, Refs. 13 and 24–26) or, alternatively, are added to the surrounding medium after the preparation of the clot (see, for example, Refs. 27–29). Lysis either proceeds throughout the clot or from the boundary to the inside of the clot, respectively.

The two variants roughly correspond to two different modes of transport of therapeutic fibrinolytic agents into a thrombus: pressure-driven permeation and diffusional transport. Both modes of transport are, presumably, realized *in vivo* during thrombolytic therapy. Pressure-driven permeation is known to accelerate the lysis (30–33) and is expected to play a role *in vivo* when a considerable drop in blood pressure exists across the thrombus (*i.e.* in occluding arterial thrombi). The fibrinolytic components rapidly delivered to the inner parts of the thrombus by pressure-driven flow lyse these parts of the thrombus from inside (34, 35). The diffusional mode of transport is expected to be dominant when there is no pressure applied across the thrombus or when this pressure is insignificant (*i.e.* nonoccluding thrombi, remainders of partially lysed occluding thrombi after reperfusion, or large venous thrombi (30, 34, 35). In this case, lysis proceeds from the surface to the inner parts of the thrombus.

Although many data on functional activities of different fibrinolytic and antifibrinolytic components have been obtained in these models, little is known about the changes in fibrin architecture occurring during lysis. Fibrin acts as an important regulator of the lytic process, exposing binding sites for components of both fibrinolytic and antifibrinolytic systems (for review see Ref. 3). Thus, lysis-associated changes in fibrin organization may be important for the regulation of fibrinolysis. In a previous study (18), we observed accumulation of plasminogen in a thin superficial layer of a plasma clot during its lysis, using conventional fluorescence microscopy. We have supposed that not only plasmin-mediated generation of C-terminal lysines on the fibrin fibers (12–14) but also structural changes of the fibrin network close to the surface of the lysing clot contribute to this strictly superficial plasminogen binding. Here, we investigate the phenomenon using confocal fluorescence microscopy, allowing individual fibrin fibers to be visualized.

During clot lysis induced by PAs present throughout the clot, we distinguished two sequential phases. During the first phase (prelysis), plasminogen gradually accumulated onto the relatively immobile fibrin lattice of the clot. Clearly, this accumulation is a consequence of the gradual increase of plasminogen binding sites appearing on the fibers as a result of fibrin nicking by plasmin. It took a considerable time for this partial degradation to start affecting the general architecture of the clot. Fragmentation of the plasminogen-loaded fibrin lattice took place during the second phase (final lysis). The fibrin network became mobile and collapsed, and soon after this was completely dissolved.

During the lysis of a plasma clot induced by PAs added to the surrounding plasma, the two phases of lysis occurred simultaneously but in different locations within the clot; the zone of the final lysis was localized within a 5–8-μm superficial layer, and the zone of prelysis was located farther away from the moving surface of the clot.

In the zone of prelysis, FITC-plasminogen moderately accumulated to immobile fibers, making them visible on the confo-

cal images. The depth of this zone was dependent on the fibrin-binding properties of the PA used and corresponded to the depth of penetration into the clot of the PA itself. The penetration of t-PA was restricted to a 20-μm zone near the surface of the lysing clot (Fig. 3*C*), presumably due to its high affinity to fibrin (5, 8). FITC-plasminogen appeared to be bound to the fibrin network in the same zone in the case of t-PA-induced lysis (Fig. 3*A*). Urokinase-type PA did not bind detectably to the fibrin network and penetrated deeper inside the clot (Fig. 3*D*). The depth of the penetration increased with time. Correspondingly, the zone of prelysis spread deeper into the clot, as revealed by FITC-plasminogen binding to fibrin fibers in areas lying much deeper than 20 μm from the surface (Fig. 3*B*).

In the zone of final lysis (5–8-μm layer near the surface of the clot) fibers loaded with plasminogen became mobile, adhered to the moving surface, and formed surface-associated agglomerates. Using differential interference contrast microscopy, Wu *et al.* (32) have recently observed agglomerated material on the surface of a lysing coarse fibrin clot. In this study, we were able to follow transformations of fibrin fibers leading to formation of these agglomerates. We found that the agglomerates accumulate plasminogen to an extremely high level (up to 45 μM) that is about 30-fold higher than its concentration in the surrounding plasma. The overall concentration of fibrin within the agglomerate volume (30–70 μM) appeared to be severalfold higher than its average concentration in the plasma clot (about 10 μM) but was considerably lower than in normally packed fibrin fibers, where the fibrin concentration is about 800 μM (36).

Plasminogen is capable of bridging fibrin molecules (37) with a tendency to form 1:1 complexes with polymerizing fibrin or with fibrin degradation products (38, 39). The roughly equimolar concentrations of fibrin and plasminogen found in the surface-associated agglomerates allowed us to suppose that plasminogen bridging contributes to the formation of the observed agglomerates. Disintegration of fibers into thin filaments during lysis as described by Bauer *et al.* (40) may facilitate the plasminogen-mediated agglomeration of fibrin.

t-PA was also highly accumulated in the surface-associated fibrin agglomerates, explaining the earlier results of Krause and Deutsch (41), who observed spots of fluorescence-labeled t-PA on the surface of a model thrombus. Even tcu-PA, which has no apparent affinity to fibrin, appeared to accumulate weakly in these agglomerates, in line with our earlier results (21).

One of the interesting aspects of the results obtained is the demonstration of a very sharp front of lysis (5–8-μm layer between completely lysed and virtually nonlysed areas of a clot). Probably this is a result of the extremely high accumulation of fibrinolytic components, strictly localized to the thin superficial zone of the lysing clot, where the structure of the fibrin network undergoes significant changes. We hypothesize that this mechanism can provide an accurate, layer-by-layer elimination of thrombi *in vivo* without releasing partially lysed but still insoluble material into the bloodstream.

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