Behavior of Plasminogen at the Luminal Surface of the Normal and Deendothelialized Rabbit Aorta in vivo and in vitro

By Mark W.C. Hatton, Susan L. Moar, and Mary Richardson

The behavior of purified rabbit plasminogen at the luminal surface of the uninjured and deendothelialized rabbit aorta has been studied in vivo and in vitro. After intravenous injection, $^{125}$I-plasminogen associated rapidly with the endothelium (approximately 0.1 pmol/cm$^2$ at saturation) and passed through to accumulate in the subendothelium. Of the adsorbed plasminogen (radioactivity), 9.1 pmol/cm$^2$ was associated with the adherent platelet monolayer. Uptake of $^{125}$I-plasminogen by the deendothelialized vessel was not significantly inhibited by $\epsilon$-aminohexanoic acid whether injected before or after the $^{125}$I-plasminogen. No evidence of plasmin activity at the aorta surface was found from either transmission electron microscopy studies or from amidolytic assays of plasminogen-saturated deendothelialized aorta samples before or after urokinase treatment. Balloon catheter treatment in vivo, however, generated significant antiplasmin activity of the deendothelialized aorta surface. We conclude that plasmin formed in vivo is probably inactivated by the antiplasmin activity that is associated with the subendothelium.

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MATERIALS AND METHODS

Arvin (also known as ancond), a purified fibrinogen-coagulating enzyme from the venom of Agkistrodon rhodostoma (Boie), was kindly donated by Dr J. Burton (Berk Pharmaceuticals, High Wycombe, England). S-2251 was purchased from KabiVitrum (Stockholm) and urokinase from Leo Pharmaceuticals (Copenhagen) or Calbiochem-Behring (San Diego).

Enzymobeads were bought from Bio-Rad Laboratories, (Mississauga, Ontario); minimum essential (Eagle's) medium from Gibco (Grand Island, NY); rabbit albumin, HEPES, heparin, heparitinase and chondroitinase ABC from Miles Laboratories (Elkhart, IN); and bovine albumin from Sigma Chemical Co (St Louis). Sepharose 4B was purchased from Pharmacia (Dorval, Quebec), Na$_2$CO$_3$ from Amersham Corp, (Oakville, Ontario), and glutaraldehyde from Can-Em (Guelph, Ontario). Sepharose (cellulose acetate) chromatographic strips were from Gelman Sciences, Inc (Ann Arbor, MI).

Preparation of rabbit plasminogen and plasmin. Rabbit blood was taken by cannulation of the carotid artery into acid-citrate-dextrose (ACD) (ratio [vol/vol] of blood:ACD was 8:5:1:5) and the plasma recovered by centrifugation (1,800 g, ten to 15 minutes). Plasma was treated with arvin$^+$ to remove fibrinogen as described previously.$^5$ Arvinized plasma was used to isolate plasminogen by affinity chromatographic procedure of Deutsch and Mertz$^8$ or by an adaptation of this method. The adapted method used a gradient of $\epsilon$-aminohexanoic acid ($\epsilon$AHX) buffered by 0.1 mol/L Na phosphate, pH 7.4, to separate the two plasminogen subforms, I and II. The plasminogen preparations were concentrated by pressure dialysis against 0.1 mol/L Na phosphate containing 0.05 mol/L $\epsilon$AHX (pH 7.4) and stored at –40°C. Protein purity was assessed by polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate.$^9$ At loads of 10 to 20 μg, all preparations of plasminogen contained a single stained band (with 0.1% Coomassie Blue R250) of molecular weight 88 to 90 kd. Reduction with
binding and desorption was measured at either 37°C or at 2°C. In all experiments, pretreatment, binding, and desorption stages were conducted in a medium consisting of either MEM containing 4% (wt/vol) rabbit albumin (RSA-MEM) or arvin-treated recalcified plasma. The tissue, after treatment, was measured for surface area and radioactivity as described above.

**Determination of plasmin and antiplasmin activities associated with the aorta wall.** A device that consisted of a linear series of eight open-ended wells supported by a Perspex base had been constructed from a 96-well microtitert plate by Dr A. Fagiotti (Department of Pathology, McMaster University) for the measurement of leukocyte behavior at the surface of the vessel wall. This simple apparatus was used for measuring plasmin and antiplasmin activities of the aorta surface. Each well measured 6 mm (diameter) and had a maximum capacity of 0.30 mL. The thoracic aorta was slit longitudinally between the paired intercostal arteries and laid flat on the Perspex base with the intimal surface uppermost. By placing carefully and clamping the modified microtitert plate firmly over the vessel surface, six to eight intact wells were usually obtained per aorta. Plasmin activity was measured directly by adding 100 µL Tyrode’s solution containing rabbit albumin (0.35% wt/vol) and buffered by 0.015 mol/L HEPES, pH 7.5 (RSA-HBT); this was followed by adding 150 µL S-2251 to each well. Absorbance measurements (405 nm) were taken over a 60-minute incubation period at 23°C. Any adsorbed plasmin was determined by first adding 5 µL urokinase (12 Ploug units) to 150 µL RSA-HBT at the aorta surface before adding 150 µL S-2251 for the measurement of released plasmin.

Antiplasmin activity was measured by exposing the aorta surface to RSA-HBT containing a known quantity (0.0015 to 0.003 CU, 0.5 to 1.0 pmol) of rabbit plasmin. After a timed interval (ten to 60 minutes), a sample (100 µL) from the well was added to a solution containing 150 µL S-2251 and 50 µL RSA-HBT. Plasmin activity was measured (405 nm) during a 60-minute incubation at 23°C.

**Morphological evaluation.** Degradation of extracellular connective tissue was evaluated by morphological examination of treated thoracic aorta segments by using transmission electron microscopy (TEM). Segments were fixed by immersion in 2.5% glutaraldehyde in 0.1 mol/L Na cacodylate. The aorta was cut into 1-mm full-circumference rings, rinsed in 0.2 mol/L Na cacodylate wash buffer containing ruthenium red (0.75 mg/mL) for 18 hours, and postfixed in OsO4 containing ruthenium red (0.75 mg/mL). The tissue was then dehydrated and embedded in the normal manner.

Aortic segments that had been deendothelialized in vivo or ex vivo were examined after incubation in RSA-MEM, either without or with urokinase (final concentration, 20 to 400 Ploug U/mL), for five or 20 minutes at 37°C. The urokinase-treated segments were compared with untreated segments incubated at the same time and from the same aorta.

Sections of each segment of aorta were coded and examined by TEM. Micrographs, at an instrumental magnification of 25,000, were taken at random from the intimal connective tissue and smooth muscle cells luminal to the internal elastic lamina. The amount of stained proteoglycan present in the intima was evaluated on a (+) to (++++) basis.

**RESULTS**

**Uptake of 125I-plasminogen by the uninjured aorta in vivo and in vitro.** Adsorption of 125I-plasminogen by the aorta wall in vivo is illustrated in Fig 1. The association of 125I-plasminogen with the endothelium was rapid (equivalent to 0.11 ± 0.09 pmol/cm² at saturation) and did not increase.
adsorption of '25I-plasminogen in arvin-treated plasma by the endothelial and intimamedia layers prepared for radioactivity measurements. For uninjured aorta segments: endothelium, O—O; intimamedia, A—A. For the deendothelialized aorta segments: intimamedia, Δ—Δ. Values are given as the mean (±SEM/ n = 3 to 6) percentage bound per square centimeter per milliliter of plasma; for all other data points, n = 2.

Fig 1. Adsorption of rabbit plasminogen by the luminal surface of the rabbit aorta in vivo. 125I-plasminogen (approximately 5 to 10 μg) was injected IV, and blood samples were taken five minutes after injection and one minute before exsanguination and recovery of the aorta. Some rabbits received a balloon catheter injury (indicated as B) to the aorta through the left femoral artery 13 (±2) minutes after injection. Each aorta segment was slit longitudinally and opened flat, with the luminal surface uppermost. After measuring the surface area, the endothelial layer (O) and the underlying intimamedia layer (Δ) of the uninjured vessel segments or the entire intimamedia layer of the balloon catheter-injured vessel segments (Δ) were separated for radioactivity measurement along with blood plasma samples. Each data point represents the mean (±SD) percentage of plasminogen bound per square centimeter by eight segments of one aorta per milliliter of blood at exsanguination.

significantly with time (Table 1). The transendothelial passage of 125I-plasminogen into the subendothelium, which progressed slowly over a 16 hour period, was equal to 0.07% to 0.1% of plasminogen/cm²/mL of blood at exsanguination and was approximately 11- to 15-fold greater than that associated with the endothelium.

When using an unstimulated incubation system in vitro, adsorption of 125I-plasminogen in arvin-treated plasma by the endothelium was maximal at ten to 20 minutes at 37°C (Fig 2).

Table 1. Transendothelial Passage of 125I-Plasminogen in Uninjured Rabbit Thoracic Aorta In Vivo

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Bound Plasminogen (pmol/cm²)</th>
<th>Endothelium</th>
<th>Intimamedia</th>
<th>Ratio I-M/E</th>
</tr>
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<tbody>
<tr>
<td>0.25</td>
<td>0.35 ± 0.14</td>
<td>0.40 ± 0.16</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.11 ± 0.04</td>
<td>0.15 ± 0.04</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.06 ± 0.02</td>
<td>0.16 ± 0.04</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>0.08 ± 0.04</td>
<td>0.27 ± 0.12</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>2.3</td>
<td>0.04 ± 0.03</td>
<td>0.46 ± 0.22</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>0.04 ± 0.02</td>
<td>0.54 ± 0.23</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>5.2</td>
<td>0.05 ± 0.04</td>
<td>0.64 ± 0.25</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>10.4</td>
<td>0.08 ± 0.06</td>
<td>0.89 ± 0.12</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>15.3</td>
<td>0.12 ± 0.09</td>
<td>1.75 ± 0.24</td>
<td>14.6</td>
<td></td>
</tr>
</tbody>
</table>

Each rabbit was injected IV with 125I-plasminogen, and the protein was allowed to circulate for the times stated. A blood sample was taken immediately before exsanguination. From each thoracic aorta, eight 1-cm segments were measured for surface area. Radioactivity contents of the endothelium (denoted as E), subendothelium (intimamedia, I-M), and plasma samples were measured. Bound plasminogen was calculated from the distribution of radioactivity between the vessel wall and blood at exsanguination. Each value is expressed as the mean (±SD) of eight segments for a single aorta.
Table 2. Distribution of 125I-plasminogen Between the Adherent Platelet-Leukocyte Layer at the Deendothelialized Surface and the Underlying Intimamedia Layer of the Aorta Wall

<table>
<thead>
<tr>
<th>Circulation Time (h)</th>
<th>Platelet-Leukocyte Layer</th>
<th>Blotted Intimamedia</th>
<th>Whole Intimamedia</th>
<th>Ratio I-M/P-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>0.14 ± 0.03</td>
<td>6.31 ± 0.51</td>
<td>6.90 ± 2.22</td>
<td>45.1</td>
</tr>
<tr>
<td>1.1</td>
<td>0.21 ± 0.06</td>
<td>9.01 ± 1.37</td>
<td>8.97 ± 1.00</td>
<td>42.9</td>
</tr>
<tr>
<td>1.6</td>
<td>0.31 ± 0.02</td>
<td>10.53 ± 3.15</td>
<td></td>
<td>34.0</td>
</tr>
<tr>
<td>1.7</td>
<td>0.33 ± 0.07</td>
<td>9.89 ± 1.30</td>
<td>11.19 ± 0.85</td>
<td>30.0</td>
</tr>
<tr>
<td>3.0</td>
<td>0.36 ± 0.10</td>
<td>8.47 ± 2.15</td>
<td></td>
<td>23.5</td>
</tr>
<tr>
<td>3.0</td>
<td>0.41 ± 0.07</td>
<td>11.06 ± 1.68</td>
<td></td>
<td>27.0</td>
</tr>
</tbody>
</table>

125I-plasminogen was injected IV ten minutes after injury to the thoracic aorta by balloon catheter and was allowed to circulate for the times stated. For three aortas, segments 2, 4, 6, and 8 were blotted with cellulose acetate paper to remove the platelet-leukocyte (P-L) layer from the surface of the subendothelium before the intimamedia layer was removed. Segments 1, 3, 5, and 7 were not treated with cellulose acetate paper; instead, the entire intimamedia layer was removed. Of the other three vessels, all eight segments were blotted with cellulose acetate. All layers, including cellulose acetate blots, were counted; bound plasminogen (± SD) was calculated as described in Table 1.

Deendothelialized by a balloon catheter after exsanguination. Uptake of 125I-plasminogen continued almost linearly with time until saturation, at approximately 0.5% to 0.6% plasminogen/cm² (Fig 2, curve c). The quantity of plasminogen bound by the deendothelialized segments in vitro was comparable with although adsorbed more slowly than uptake in vivo. Uptake by uninjured vessel segments in vitro indicated that the endothelium acted as a leaky barrier as shown by the accumulation of plasminogen by the subendothelial tissue (Fig 2, curve b).

Attempts to influence the uptake of 125I-plasminogen by the deendothelialized subendothelium in vitro were made to characterize the plasminogen binding site(s). Pretreatment of the subendothelium with either thrombin (10 to 20 IU/mL) with a glycosaminoglycan-degrading enzyme, ie, heparitinase (5 U/mL) or chondroitinase ABC (5 U/mL), did not affect the uptake of 125I-plasminogen compared with that of control segments from the same aorta. In addition, no significant change in plasminogen binding by the subendothelium was observed if either heparin (10 U/mL) or 10 mmol/L sodium citrate was present during incubation. The addition of 28 mmol/l eAhx to the incubate caused only a small decrease (20% to 30%) in adsorption of 125I-plasminogen. By contrast, the presence of 28 mmol/L eAhx in arvinized plasma prevented the adsorption of plasminogen by lysine-Sepharose during chromatography.

Desorption of 125I-plasminogen bound by the subendothelium. Segments of deendothelialized aorta containing adsorbed 125I-plasminogen were obtained either from rabbits injected with 125I-plasminogen in vivo or from aortas that had been deendothelialized by balloon catheter treatment after exsanguination and the aorta segments exposed subsequently to 125I-plasminogen in vitro. Incubation of the aorta segments at 37°C in either 4% RSA-MEM, with or without eAhx (1.0 to 10 mmol/L) present, or in arvinized plasma did not encourage a significant loss of radioactivity from the intimamedia (Fig 4). By exposing deendothelialized segments to 125I-plasminogen at 2°C, however, desorption of much (ap-
proximately 50%) of the bound protein from the subendothelium was observed after incubation of the segments at 2°C in 4% RSA-MEM or in arvinized plasma (Fig 4). The presence of 10 mmol/L εAHx did not increase desorption significantly at 2°C compared with 4% RSA-MEM alone.

** Fate of plasminogen adsorbed by the aorta.** Evidence of plasmin production at the surface of or within the vessel was sought by direct measurement using a plasmin-selective substrate, S-2251, and from signs of degradation of the extracellular matrix observed by TEM of aorta samples taken from uninjured vessels and from vessels that had been deendothelialized in vivo or ex vivo and incubated with RSA-MEM, with or without urokinase.

All measurements made at room temperature (23°C) at the surface of uninjured aortas and deendothelialized vessels (ex vivo or in vivo) indicated that plasmin activity was not detectable over a 60-minute incubation period with S-2251. Pretreating these vessels with urokinase also failed to release measurable plasmin activity; however, by exposing precooled aorta subendothelium (deendothelialized ex vivo) at 2°C first to purified plasminogen (0.38 to 1.5 μmol/L) at 2°C and then rinsing and incubating with urokinase (8.5 Plough units) at 2°C, plasmin activity, in direct proportion to the plasminogen concentration, was released from the vessel surface as shown by the amidolytic activity.

To determine whether antiplasmin activity was associated with the vessel, the intimal surface was exposed to small quantities of plasmin (0.5 to 1.0 pmol) by using the well technique. The decrease in plasmin activity at each aorta surface was compared with plasmin activity recovered from intact plastic wells after incubation for a similar time (Fig 5, curve A). Compared with the endothelial surface of the uninjured vessel where plasmin was slowly inactivated (Fig 5, curve B), the aorta surface after deendothelialization in vivo rapidly inactivated plasmin (Fig 5, curve D). By incubating with different quantities of plasmin, the capacity of the aorta surface, deendothelialized in vivo, to inactivate plasmin was calculated to be 1.5 to 2.0 pmol/cm². In contrast, the aorta surface that was deendothelialized ex vivo (curve C) caused a loss of plasmin activity that resembled more closely that of the uninjured vessel than that of the aorta deendothelialized in vivo.

Regarding the proteoglycan granules (PG) that were visible by TEM after ruthenium red staining (Fig 6), no significant differences in PG content were observed between aorta samples that had been deendothelialized in vivo (rabbit exsanguinated 20 minutes after balloon catheter injury) or deendothelialized ex vivo when compared with samples from an uninjured aorta. Furthermore, treatment of segments from these deendothelialized aortas with urokinase (400 Ploug U/mL) for 20 minutes did not cause obvious degradation of the extracellular matrix (cf, Fig 6A with 6B and 6C). In contrast, PG of the extracellular matrix were completely degraded after incubation for 20 minutes in excess plasmin in vitro (Fig 6D).

**DISCUSSION**

In this work we have attempted to understand the relationships between circulating plasminogen and the aorta wall before and after deendothelialization injury.

Plasminogen was not strongly attracted to the normal (ie, uninjured) endothelium but, in keeping with previous reports of the interaction of plasma proteins with the vessel wall,20,21 passed through the endothelium into the subendothelial compartment. At two to five hours after injection, equilibrium with 125I-plasminogen in the circulation was attained. The permeability of the endothelium to plasminogen was noticeably more variable in vitro than in vivo (cf, Fig 2, curve B, with Fig 1). The quantity of endothelium-bound plasminogen did not appear to increase, however, as the subendothelial content increased. The transendothelial route taken by plasminogen to reach the subendothelium, ie, whether intracellular or intercellular, and the fate of this protein in the subendothelium of the uninjured vessel wall has not been determined; however, there was no evidence of resident plasmin activity associated with the endothelium.

In comparison with the endothelium of the uninjured vessel, removal of the endothelium in vivo allowed plasminogen to be bound rapidly and in a greater quantity by the exposed subendothelium (Figs 1 and 3), with a minor amount (2% to 3%) being adsorbed by the platelet-leukocyte surface layer (Table 2). Our present investigations of the bound protein have not allowed us to make strong conclusions about the quality or location of binding; however, neither the rate of uptake nor the quantity of absorbed plasminogen by the subendothelium was affected significantly by εAHx in the circulation (Fig 3). From this lack of inhibition, we conclude that the lysine binding site of plasminogen was not involved in the mechanism of adsorption by the subendothelium. The conclusion was supported by the failure of εAHx to strongly influence binding by or displacement from the subendothelium in vitro. This observation may conflict with the suggestion of Silverstein et al20,21 that thrombospondin, which is present in the extracellular matrix synthesized by cultured endothelial cells,22 may bind plasminogen selectively through the lysine-binding site of thezymogen. Also, Salonen et al23 have reported that fibronectin, another protein component of
Fig 6. Transmission electron micrographs of the intimal connective tissue that show the presence of extracellular PG (indicated by large arrowheads) after incubation with urokinase in incubation in plasmin. (A) A segment of aorta deendothelialized ex vivo and incubated (37°C) in RSA-MEM for 20 minutes. (B) A segment from the same aorta incubated with urokinase (400 Ploug U/mL) for 20 minutes. (C) A segment of aorta deendothelialized in vivo (20 minutes before exsanguination) and incubated with urokinase (400 U/mL) for 20 minutes. (D) A segment of aorta deendothelialized ex vivo and incubated with excess plasmin (2.9 CU/mL) for 20 minutes. In C, platelets (P) are associated with the luminal surface; even in areas where the platelet monolayer is incomplete, there is no evidence of degradation of the PG of the connective tissue. L, lumen; C, collagen; E, elastin; (bar = 100 nm. (Current magnification ×40,000.)
the extracellular matrix and, particularly, basement membranes, binds plasminogen through a lysine-dependent binding site. In the system used by Knudsen et al.,\textsuperscript{24} 10 mmol/L eAHx strongly inhibited binding of human Glu-plasminogen to the extracellular matrix synthesized by cultured endothelial cells, although 10% to 20% of plasminogen binding was lysine independent and due to an interaction with the gelatin coating used for cell cultures.

In contrast to antithrombin III,\textsuperscript{17,18} binding of plasminogen to the ballooned vessel surface in vitro was not inhibited by pretreating the subendothelium was either glycosaminoglycan-degrading enzyme or thrombin. Thus, neither the galactosaminoglycan moieties of the proteoglycans present in the subendothelial extracellular matrix, which are destroyed by chondroitinase ABC,\textsuperscript{19,25,30} nor the proteoheparan sulphate components of the basement membrane of smooth muscle cells, which are degraded by heparitinase\textsuperscript{17} or by thrombin,\textsuperscript{12,27} are likely to bind plasminogen.

The reluctance of plasminogen, bound by the subendothelium in vivo or in vitro at 37°C, to be displaced by either arvinized plasma, RSA-MEM, or eAHx indicated that reversible binding by static components of the extracellular matrix was an unlikely event. Furthermore, results from the TEM study of deendothelialized aorta samples that were incubated with urokinase in vitro (Fig 6A, B, C) suggested that most of the plasminogen adsorbed by the vessel in vivo was probably not available for conversion to plasmin. This conclusion was supported by the absence of measurable plasmin activity associated with these vessel surfaces after exposure at 23°C to urokinase in vitro. From previous observations,\textsuperscript{17,27} treatment of balloon-injured aorta segments with excess, purified plasmin in vitro caused damage, particularly to the extracellular matrix, by selectively degrading the large PG; the small PG of the smooth muscle cell basement membrane were not degraded significantly.

Thus, if adsorbed plasminogen is not accessible for activation by urokinase and is not displaced from the deendothelialized aorta, we conclude that plasminogen is tightly complexed by a binding site that is located, possibly, at the smooth muscle cell membrane. Plausibly, binding of plasminogen would be followed by endocytosis and lysosomal degradation of the protein. Evidence in favor of this idea was obtained by exposing the ballooned aorta surface to plasminogen at 2°C. Adsorption of the protein at 2°C would not be followed by endocytosis, and desorption or activation by urokinase at 2°C was possible.

We reasoned that antiplasmin activity, resident in the subendothelium,\textsuperscript{28} may control the level of any plasmin released by activation of plasminogen within the tissue as a result of the rapid uptake of the zymogen after balloon injury in vivo. As shown in Fig 5, plasmin activity was lost relatively slowly by exposing a small quantity of purified plasmin to the intimal surface of either the uninjured vessel or the deendothelialized vessel (ex vivo). Presumably, of the activity lost, a proportion was due to direct adsorption by the vessel surface and the remainder due to inactivation by any antiplasmin activity from the vessel.\textsuperscript{29} Considerably greater plasmin inactivation was associated with the deendothelialized surface of the aorta that had been balloon injured in vivo. The quantity of antiplasmin activity contained by the deendothelialized aorta 20 minutes after balloon injury in vivo was estimated to be 1.5 to 2.0 pmol/cm², assuming that a 1:1 complex was formed between plasmin and the inhibitor. This quantity of antiplasmin was considerably less than the theoretical amount of plasmin that could possibly have been generated from 7 to 11 pmol plasminogen/cm² 20 minutes after balloon catheter injury, but whether this recovered antiplasmin activity represents that quantity remaining after inactivation of any plasmin generated is not yet known.

The origin of the antiplasmin activity of the subendothelium, ie, whether synthesized by the cells that contribute to the subendothelium matrix or adsorbed from adherent platelets or from plasma, is not fully understood. In a preliminary experiment, we exposed the intimal surface of the deendothelialized aorta (ex vivo) to plasma in vitro for ten minutes before rinsing and measurement of antiplasmin activity. This pretreatment increased the antiplasmin activity of the vessel, which indicated that some of the antiplasmin activity of the vessel wall injured in vivo may be of plasma or platelet origin, although the slower, progressive antiplasmin activity of the uninjured vessel and the vessel ballooned ex vivo may represent another source of antiplasmin, possibly that released by smooth muscle cells of the intima-media as proposed by Noordhoek-Heg\textsuperscript{29} and by Bernik and Kwaan.\textsuperscript{31}

At present we are investigating whether α2-plasmin inhibitor from plasma\textsuperscript{32} is adsorbed in an active state by the exposed subendothelium. Such behavior of a plasma protease inhibitor is reminiscent of the properties of another plasma protease inhibitor, antithrombin III, which has affinity for the deendothelialized rabbit aorta and which, in the adsorbed state, actively inhibits thrombin in vitro.\textsuperscript{17,18} The availability of these and possibly other protease inhibitors may well determine the dominant hemostatic mechanism, eg, thrombosis or fibrinolysis, at the exposed subendothelial surface of the injured vessel wall.

ACKNOWLEDGMENT

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