Denaturant-Induced Stimulation of the β-Migrating Plasminogen Activator Inhibitor in Endothelial Cells and Serum

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Cultured bovine aortic endothelial cells and human serum contain plasminogen activator inhibitors (PAIs) that are immunologically related. In the present study, the electrophoretic mobilities, molecular weights (mol wt), and activities of these PAIs were compared. When fractionated by agarose zone electrophoresis, both PAIs migrated with β mobility as compared with the mobilities of human plasma/serum proteins. Two-dimensional electrophoretic analysis, employing agarose zone electrophoresis in the first dimension and sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension, indicated that these β-PAIs comigrated, both having a mol wt of approximately 50,000. The activity of the PAI in endothelial cell-conditioned medium is enhanced severalfold by treatment with either sodium dodecyl sulfate or guanidine. In preliminary experiments, we were unable to stimulate the PAI activity of undiluted serum by similar treatments. However, the PAI activities in both diluted serum and gel-filtered or electrophoretically fractionated serum were enhanced by treatment with these denaturants. The gel filtration studies also revealed that serum contains multiple forms of the β-PAI. These forms may represent polymorphic PAI and/or complexes between the PAI and other serum components. These findings indicate that the primary PAIs in bovine endothelial cells and human serum are not only immunologically related but are also biochemically similar.

THE GENERATION of plasmin from plasminogen provides an important source of proteolytic activity in tissues and biologic fluids. Precise regulation of plasminogen activator (PA) activity thus may constitute a critical feature of many biologic systems. For example, in the vascular system, the abnormal expression of PA is associated with the development of both thrombotic and bleeding disorders. The factors that regulate vascular PA activity are not entirely understood. Until recently, such control was thought to be at the level of the formation and resolution of fibrin itself rather than through the action of specific PA inhibitors (PAIs). However, the recent demonstration that PAIs not only exist in plasma but are frequently detected in complex with tissue-type PA (tPA) suggests that vascular PA activity is also regulated by these inhibitors. The observations that a PAI is also present in platelets and can be released from them by physiological stimuli and that the level of PAI in plasma may be altered in certain human diseases support this hypothesis.

Available evidence indicates that there are at least three immunologically distinct PAIs, including the endothelial cell–type inhibitor, the placental inhibitor, and plasmin. The PAI synthesized by cultured bovine aortic

endothelial cells (BAEs) has been purified and partially characterized. It inhibits both urokinase and tPA, but not plasmin. Antiserum to it has been developed and employed to demonstrate that the endothelial cell, plasma, serum, and platelet PAIs are immunologically related. This class of PAIs has a number of unusual properties that distinguish it from other cellular and blood-derived protease inhibitors. For example, it is stable under conditions that inactivate other inhibitors (eg, incubation at pH 3 or exposure to sodium dodecyl sulfate (SDS)). Moreover, the activity of the PAI from BAEs is stimulated by treatment with denaturants such as guanidine and SDS. The objective of the current study was to compare further the endothelial cell and serum PAIs. In this report, we demonstrate that both of these inhibitors have β mobility as determined by agarose zone electrophoresis, and the activity of both can be stimulated by denaturants.

MATERIALS AND METHODS

Cell culture. BAEs were isolated and maintained in vitro in modified minimum essential medium (MEM; Flow Laboratories, Inglewood, CA) containing 10% calf serum (Irvine Scientific, Santa Ana, CA or Sterile Systems, Inc, Logan, UT) as described. The cells used for these studies were cloned from a single cell and had been passaged five to 20 times. Conditioned medium (CM) was prepared by incubating washed cell monolayers in serum-free MEM for 24 hours at 37°C. This CM was collected, centrifuged at 1,000 g to remove floating cells and cellular debris, and stored in 0.01% Tween 80 (J.T. Baker Chemical Co, Phillipsburg, NJ) at −20°C until used. For some experiments, 200 μL of CM was concentrated tenfold by centrifugation in a SpeedVac concentrator (Savant, Farmingdale, NY).

Preparation of plasma and serum. Human blood was collected from healthy volunteers into polypropylene tubes containing 3.8% Na citrate, pH 7.4, and centrifuged at 1,000 rpm for 15 minutes at 23°C to obtain platelet-rich plasma. The platelet-rich plasma was chilled to 4°C and centrifuged at 3,000 rpm for 30 minutes to obtain platelet-poor plasma (hereafter referred to as plasma). Whole blood serum was prepared by collecting blood into glass tubes, allowing the blood to clot at 37°C for one hour, and then removing the clot by centrifugation at 3,000 rpm for 20 minutes at 4°C. The resulting serum was collected and stored at −70°C until used.

SDS and guanidine treatment of samples. BAE CM and human whole blood serum, either undiluted or diluted tenfold with phos-
phase-buffered saline (0.14 mol/L NaCl, 0.01 mol/L Na phosphate, pH 7.2, containing 0.01% Tween 80; PBS/Tw), were incubated with SDS (0.1% or 0.25% final concentration as indicated) for 45 minutes at 37°C. Alternatively, the samples were dialyzed against 4 mol/L guanidine-HCl (in PBS/Tw adjusted to pH 7.2) for 4.5 hours at 37°C. Dialysis was judged complete by the disappearance of the phenol red present in the CM. After treatment with SDS or guanidine, samples were dialyzed against 4,000 volumes of PBS/Tw at 4°C for 18 hours.

**Electrophoresis.** SDS-polyacrylamide slab gels and buffers were prepared as described by Laemmli.23 Samples were applied to gels consisting of 10-cm resolving gels of 9% acrylamide and 2-cm stacking gels of 4% acrylamide and then subjected to electrophoresis at room temperature for 16 hours or until the tracking dye reached the bottom of the gel.

Agarose zone electrophoresis was performed in 1% agarose gels using 0.025 mol/L Tris-tricine, pH 8.6 (Bio-Rad Laboratories, Richmond, CA), as the electrode buffer. Samples were subjected to electrophoresis at 12 mA/cm3 gel for three hours using a water-cooled horizontal electrophoresis apparatus (Bio-Rad). A sample of human plasma (4 μL) was used as a reference marker. Under the conditions employed, the albumin present in the plasma migrated in an anodal direction approximately 60 mm from the origin. The plasma proteins were stained with 0.2% Coomassie brilliant blue R250 (Bio-Rad) in 14% acetic acid and 28% isopropanol. In some experiments, lanes of the agarose gel were treated with 0.1% 2-mercaptoethanol (2-ME) and either 0.1% SDS or 4 mol/L guanidine-HCl at 37°C for 30 minutes and then washed extensively in 2.5% Triton X-100 (Bio-Rad, Richmond, CA) or PBS prior to further analysis. These treatments inactivate cellular PAIs (2-ME) or stimulate PAI activity (SDS, guanidine). In other experiments, the agarose gels were cut into 5-mm slices. The material that eluted from each slice upon extraction into 150 μL of PBS for four hours at 4°C was then analyzed for PAI activity in the tPA-binding assay (see the following material).

Two-dimensional electrophoresis was performed by using a combination of the two electrophoretic techniques just outlined. Samples were first subjected to zone electrophoresis in an agarose gel. After electrophoresis, the agarose gel was soaked in electrophoresis sample buffer (0.125 mol/L Tris, pH 6.8, containing 2.2% SDS and 20% glycerol) for 30 minutes and placed on top of a 10-cm resolving 9% acrylamide slab gel, and then SDS-polyacrylamide gel electrophoresis (PAGE) was performed as just outlined. The agarose and polyacrylamide gels were analyzed by reverse fibrin autography (RFA), immunoblotting, or autoradiography, as will be described.24

**Sephacryl S-200 chromatography of human serum.** Four and one-half milliliters of human whole blood serum were incubated with diisopropylfluorophosphate (DFP, 5 mmol/L; final concentration; Sigma Chemical Co, St Louis) for three hours at 4°C. The serum was then applied to a Sephacryl S-200 column (1.5 cm x 110 cm) that had been equilibrated in 10 mmol/L Tris-HCl, pH 7.6, 0.01% Tween 80, and 3 mol/L NaCl, and calibrated with mol wt standards (Sigma). The standards included blue dextran (mol wt, 2 x 107), bovine serum albumin (mol wt, 67,000), carbonic anhydrase (mol wt, 30,000), lysozyme (mol wt, 14,400), and phenol red (mol wt, 354). The column was eluted at 12 mL/h and 2.0-mL fractions were collected. Aliquots of the fractions were treated with either 0.25% SDS or 4 mol/L guanidine-HCl as described before, and both treated and untreated samples were dialyzed against PBS/Tw at 4°C for 18 hours. These samples were then assayed for PAI activity in the tPA-binding assay.

**Assays for PAI.** PAI was quantitated by using a variety of assays. For example, RFA was performed on samples subjected to agarose or SDS-polyacrylamide slab gel electrophoresis as outlined previously.25 Immunoblotting of material transferred from agarose gels to nitrocellulose was performed as described.26 The transferred proteins were first incubated with monospecific rabbit antiserum (diluted 1:500) against the BAE PAI,27 and the bound rabbit IgG was then detected with goat antirabbit IgG (Cooper Biomedical, Inc, Malvern, PA) that had been 125I labeled. The 125I-labeled IgG was used at a final concentration of 200,000 cpm/mL. Between incubations with the antibodies, the nitrocellulose paper was rinsed extensively with PBS containing 5% (wt/vol) nonfat dry milk, 0.01% Antifoam A (Sigma), and 0.0001% sodium ethylmercurithiosalicylate (thimerosal).28 It was then air-dried and subjected to autoradiography on Kodak X-omat film (Rochester, NY) for 24 hours at −70°C.

PAI activity was also assessed by measuring the ability of components in CM and serum samples to form SDS-stable complexes with tPA. Two approaches were taken. In the first, the samples were incubated with 125I-labeled tPA for ten minutes at 37°C. The mixtures were then fractionated by SDS-PAGE, and the gels were fixed and stained in 0.01% Coomassie brilliant blue R250/50% trichloroacetic acid, destained in 10% acetic acid, dried, and subjected to autoradiography on Kodak X-omat film for 24 hours at −70°C. High mol wt complexes were easily distinguished from free tPA by this approach. In the second method, the ability of the PAI to bind to tPA immobilized on microtiter wells was assessed in the tPA-binding assay, performed essentially as described by Schleef et al.29 The amount of bound PAI was determined by using the antiserum against the BAE PAI.

**Miscellaneous.** Plasminogen was prepared by affinity chromatography on lysine-Sepharose as described.30 Human α-thrombin was a gift from J. Fenton (New York State Department of Health, Albany). Fibrinogen (bovine, plasminogen- and thrombin-free) was purchased from Calbiochem-Behring (San Diego, CA), α2-antiplasmin from American Diagnostica Inc (Greenwich, CT), 2-ME and SDS from Bio-Rad, guanidine-HCl from Sigma, and culture supplies from Falcon Labware (Oxnard, CA). Mol wt standards used for SDS-PAGE were obtained from Bio-Rad and included carbonic anhydrase (mol wt, 31,000), bovine serum albumin (mol wt, 66,200), and β-galactosidase (mol wt, 116,250). The tPA was purified according to previously described procedures.31 Both the tPA and the goat antirabbit IgG were labeled with 125I (Amersham Corp, Arlington Heights, IL) by the Iodogen method,32 modified such that the labeling time was 3.5 minutes at 4°C. The specific activity of the 125I-labeled tPA was approximately 7.1 x 107 cpm/μg of protein. Immunodepletion experiments were performed as described previously,29,33 using nonimmune or anti-PAI IgG coupled to protein A-coated Sepharose CL-4B beads (Pharmacia Fine Chemicals, Uppsala, Sweden).

**RESULTS**

**Analysis of BAE CM by zone and two-dimensional electrophoresis.** Samples of BAE CM were subjected to agarose zone electrophoresis and then analyzed for PAI activity by RFA and for PAI antigen by immunoblotting (Fig 1). Although no PAI activity was detected in 50 μL of CM (panel B, lane 1), immunoblotting demonstrated that this same amount of CM did contain PAI antigen (panel B, lane 3). The antigen exhibited β mobility when compared with known plasma proteins (panel A). Inhibitor activity could be detected in the β region of the gel when 200 μL of CM was analyzed (panel B, lane 2). These results indicate that under the electrophoretic conditions employed, the PAI in BAE CM migrates with β mobility relative to the migration of plasma proteins.

We have shown previously that 5% or less of the total PAI in BAE CM is active, the other 95% existing in a latent, or
inactive, form.22 The latent form can be converted into the active form by treatment with denaturants. The effect of SDS on the PAI activity of BAE CM fractionated by agarose zone electrophoresis is shown in Fig 2. Again, no PAI activity was detected in any region of the gel when 50 μL of untreated CM was analyzed (Fig 2, top panel, lane 1). However, when the agarose gel containing the fractionated CM was soaked in SDS containing 2-ME, washed to remove these reagents, and then analyzed by RFA, a prominent PAI zone was detected in the β region (lane 2). This stimulation of PAI activity by SDS was quantified by using the tPA-binding assay (Fig 2, bottom panel). In this case, the SDS-treated and untreated gels were sliced, and each slice was eluted into buffer and then assessed for PAI activity. Again, no PAI activity was detected in the untreated fractions, whereas pronounced PAI activity was detected in fractions obtained from the β region of the SDS-treated gel. β-PAI activity (Fig 3, lane 1) was not detected in SDS-treated agarose gels containing CM previously incubated with anti-PAI serum bound to protein A-Sepharose beads (lane 2); however, it was apparent in SDS-treated agarose gels containing CM pretreated with nonimmune serum (lane 3). Thus, the β-PAI activity revealed by SDS treatment of CM after agarose zone electrophoresis is immunologically related to the 50,000-mol wt PAI purified from BAEs.

The electrophoretic properties of the PAI and α2-antiplasmin, the primary plasmin inhibitor in blood,233 were compared (Fig 4). Purified human α2-antiplasmin was added to BAE CM, and the mixture was fractionated by agarose zone electrophoresis, by SDS-PAGE, or by two-dimensional gel electrophoresis. As expected, no inhibitor activity was apparent in the β region of the agarose gel (panel A), in agreement with the results shown in Figs 1 and 2. However, a very prominent inhibitor zone was seen in the α2 region, presumably corresponding to α2-antiplasmin. This relationship changed dramatically when the inhibitors in the agarose gel were subsequently subjected to electrophoresis into a SDS-containing gel (panel B). Considerable inhibitor activity was now apparent in the β region, whereas the inhibitor activity in the α2 region was greatly decreased. These changes reflect the different effects of SDS on the biologic activities of the two inhibitors. Panel C shows the mixture fractionated by SDS-PAGE alone. Inhibitor activity was detected at a mol wt of 70,000 (the mol wt of α2-antiplasmin) and at a mol wt of 50,000 (the mol wt of the PAI). The proteins responsible for these activities comigrated with those shown in panel B.

Effect of SDS and guanidine on the PAI activity of serum. We have shown previously that the primary PAI in human serum is immunologically related to the BAE PAI.20 Experiments were performed to determine whether the activ-
DENATURANT-INDUCED STIMULATION OF THE β-PAI

Fig 3. Immunoadsorption of the β-PAI in BAE CM. BAE CM (50 μL, lane 1) was immunoprecipitated with anti-PAI serum (lane 2) or nonimmune serum (lane 3) as described in Materials and Methods. Each of the resulting supernatant fluids was subjected to agarose zone electrophoresis. The agarose gels were then treated with 0.1% SDS and 0.1% 2-ME as described in the legend to Fig 2 and assayed for inhibitor activity by using RFA. The mobilities of plasma proteins are indicated.

Fig 4. Analysis of BAE CM and α2-antiplasmin by two-dimensional electrophoresis. BAE CM (15 μL) was combined with α2-antiplasmin (25 μL, 500 ng) and then subjected to either agarose zone electrophoresis (panel A), agarose zone electrophoresis followed by SDS-PAGE (panel B), or SDS-PAGE alone (panel C). Each gel was then analyzed for inhibitors by using RFA. The electrophoretic mobilities of the inhibitors fractionated by agarose zone electrophoresis (panel A) were compared with those of proteins in human plasma as described in the legend to Fig 1 (arrows, bottom).

Fig 5. Analysis of BAE CM and α2-antiplasmin by two-dimensional electrophoresis. BAE CM (15 μL) was combined with α2-antiplasmin (25 μL, 500 ng) and then subjected to either agarose zone electrophoresis (panel A), agarose zone electrophoresis followed by SDS-PAGE (panel B), or SDS-PAGE alone (panel C). Each gel was then analyzed for inhibitors by using RFA. The electrophoretic mobilities of the inhibitors fractionated by agarose zone electrophoresis (panel A) were compared with those of proteins in human plasma as described in the legend to Fig 1 (arrows, bottom).
One lane of the agarose gel was untreated (lane 1) and the other lanes were treated with either 0.1% SDS (lane 2) or with 4 mol/L guanidine-HCl (lane 3), both in the presence of 0.1% 2-ME as described in Materials and Methods. All samples were then subjected to SDS-PAGE and autoradiography. The positions of the mol wt standards are indicated. The mobility of the 125I-labeled tPA remaining free in the serum (lanes 4 and 5) was distorted by the large amount of albumin present (compare with lane 1; see refs 14 and 20).

Whole blood serum was also fractionated by Sephacryl S-200 chromatography as described in Materials and Methods. Aliquots of the fractions were then treated with 0.25% SDS or 4 mol/L guanidine-HCl, dialyzed into PBS/Tw, and assayed for PAI activity in the tPA-binding assay (Fig 8). The only tPA-binding activity detected in untreated control fractions was in the material that eluted just after the void volume of the column (apparent mol wt between 150,000 and 200,000). Treatment of the fractions with either SDS or guanidine-HCl reduced this activity but induced the appearance of two additional peaks of PAI activity. The first of these migrated with an apparent mol wt of 90,000 and was stimulated equally well by both SDS and guanidine-HCl. The second peak migrated with an apparent mol wt of 30,000 and was stimulated to a greater extent by guanidine-HCl than by SDS. This latter result is consistent with our previous findings that the latent PAI in BAE CM is activated to a greater extent by guanidine-HCl than by SDS and that it migrates with an apparent mol wt of 30,000 when fractionated by Sephacryl S-200 chromatography prior to treatment with denaturant.22

To determine whether activation of the serum PAI requires the prior removal of other serum proteins or can be achieved by merely diluting the sample, BAE CM and human serum (either undiluted or diluted tenfold) were treated with 0.25% SDS or dH2O as described in Materials and Methods. All samples were then dialyzed into PBS/Tw, brought to the same final dilution, and assayed for PAI activity in the tPA-binding assay. Table 1 shows that dilution of BAE CM prior to treatment with denaturant had no effect on the enhancement of PAI activity. Untreated CM exhibited between 4 and 8 ng/mL of active PAI, whereas both undiluted SDS-treated CM and CM treated after dilution exhibited approximately 500 ng/mL of PAI activity. In contrast, when undiluted serum was SDS-treated, the amount of detectable PAI actually decreased when compared with untreated serum. However, when the serum was diluted before SDS treatment, the amount of PAI activity detected increased by a factor of three (sample 1) and 27 (sample 2).

DISCUSSION

PAIs, in addition to their role in initiating vascular fibrinolysis, have been implicated in a variety of other physiological processes including ovulation, cell migration, embryo implantation, epithelial differentiation, malignant transformation, and activation of latent collagenase.5 PAI activities have now been detected in many of the cells and fluids associated with these processes,34-37 thus emphasizing their potential role in regulating PA-mediated events. This PAI activity may result from the presence of either the endothelial cell-type PAI, the placental-type PAI, or from protease nexin. Unfortunately, most available assays cannot readily distinguish between these PAIs. This ambiguity is further emphasized by the finding that each of these PAIs appears to be widely distributed, and in some cases, one type of cell may produce more than one PAI. In fact, the human fibrosarcoma cell line, HT-1080, appears to produce all three PAIs.38,39 It is also clear that cells that do not produce one of the PAIs under one set of conditions may be induced to do so under another set of conditions.34,35,39,40 These considerations emphasize not only the ambiguity of PAI activity measure-
The results presented here identify two properties that may be unique to the endothelial-type PAls and that may aid in the development of a meaningful PAI nomenclature. First, we have demonstrated that the PAI in BAE CM (Figs 1 to 4) as well as the immunologically related PAI in human serum (Figs 6 and 7) have \( \beta \) mobility as determined by agarose zone electrophoresis. The primary PAls in human platelets and plasma have similar mobilities (Erickson and Loskutoff, unpublished observation). The placental PAI\(^1\) and most plasma protease inhibitors\(^3\) migrate in the \( \alpha \) region when fractionated by agarose zone electrophoresis. We suggest that this distinction may be used to clarify some of the ambiguities associated with PAls. The endothelial cell--type PAls may thus be termed the \( \beta \)-PAls to distinguish them from these other inhibitors. Obviously, the PAls in other samples must be analyzed immunologically and by agarose zone electrophoresis to establish this difference as a consistent and useful distinction. These experiments are in progress. Second, the BAE \( \beta \)-PAI is not inactivated by treatment at pH 3 or by incubation in the presence of SDS,\(^7\)\(^4\) treatments that rapidly inactivate protease nexin, the placental-derived PAI, and most plasma protease inhibitors. The activities of the BAE \( \beta \)-PAI (Figs 1--5; ref 22) and, as shown in this report, the human serum \( \beta \)-PAI (Figs 6 to 8; Table 1) are actually stimulated by treatment with SDS and other denaturants, a property that has been used to demonstrate that \( \beta \)-PAls exist as active and inactive, or latent, forms.\(^2\)\(^2\) Thus, both electrophoretic mobility and biochemical stability

Table 1. SDS Activation of the PAI in BAE CM and in Human Serum

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Control</th>
<th>SDS</th>
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</thead>
<tbody>
<tr>
<td>BAE CM</td>
<td></td>
<td>3.9</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8.5</td>
<td>519</td>
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<tr>
<td>Serum sample 1</td>
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<td>21.8</td>
<td>18.7</td>
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<tr>
<td></td>
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<td>5.4</td>
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<tr>
<td></td>
<td>10</td>
<td>2.2</td>
<td>59.6</td>
</tr>
</tbody>
</table>

Aliquots of BAE CM or of human whole blood serum from two different donors (samples 1 and 2), were diluted tenfold with PBS/Tw and treated with either dH\(_2\)O (control) or 0.25% SDS as described in Materials and Methods. The samples were then analyzed in the tPA-binding assay. The results are compared with those obtained with undiluted samples. The data are presented as nanograms of PAI per milliliter of undiluted sample and were obtained by direct comparison with a standard curve constructed using samples of purified BAE PAI.
may be useful parameters for distinguishing between the various PAIs.

In the case of the \( \beta \)-PAI in CM from cultured BAEs, the latent form may represent greater than 95% of the total potential PAI activity. Human endothelial cells and platelets (our unpublished observations) as well as HTC rat hepatoma cells also contain active and latent forms of \( \beta \)-PAI. The latency of the BAE \( \beta \)-PAI seems to be a property of the molecule itself and not the result of the presence of another molecule in CM that masks our ability to detect PAI activity. Whether the PAI is actually synthesized in a latent form or made as an active molecule that is then converted into the latent form either by the cells or upon exposure to the cell culture environment cannot be established from available information. We have shown that the active BAE \( \beta \)-PAI is rapidly inactivated by chloramine T and other oxidants, thus raising the possibility that at least part of the latency of this molecule may result from oxidants in the culture medium. These considerations also may apply to the \( \beta \)-PAI elaborated by other cells or found in platelets, plasma, and serum. The \( \beta \)-PAI in serum was stimulated with SDS only after the serum had been diluted or fractionated chromatographically or electrophoretically (compare Fig 5 with Figs 6 to 8). Since activation is dose-dependent with respect to SDS concentration, we hypothesize that our inability to stimulate the PAI activity of undiluted serum is a consequence of the high protein concentration of serum as compared with CM. These serum proteins may bind the SDS and thus lower its effective concentration with respect to the PAI. The fact that the PAI activity of diluted serum is stimulated by SDS (Table 1) is consistent with this conclusion. The exact mechanism of activation by SDS and guanidine-HCl is not known. However, since these denaturants appear to act directly on the latent PAI, they may induce a conformational change in the molecule that exposes a previously masked reactive site. Activation of plasma/serum \( \beta \)-PAI activity might thus represent an additional means of regulating vascular PA activity.

The results obtained upon gel filtration of serum (Fig 8) revealed three additional characteristics of the serum \( \beta \)-PAI. First, PAI activity was detected in untreated samples only in those fractions that corresponded to a mol wt between 150,000 and 200,000. This is in agreement with the results previously reported by Wiman et al with human plasma. These authors have suggested that this high mol wt component may represent tetrameric PAI or a complex between the PAI and another plasma/serum component. It is not clear why this activity is apparently destroyed by SDS and guanidine. However, this finding is consistent with the fact that no PAI is detected in these fractions by RFA (data not shown). Thus, it is possible that this component may represent a form of active PAI that is SDS sensitive. Others have previously shown that the active PAI in human endothelial cell CM is more sensitive to inactivation by a variety of treatments than is the latent form. Second, both SDS and guanidine-HCl treatments revealed PAI activity in fractions that corresponded to a mol wt of approximately 90,000. This high mol wt form may reflect PAI in complex with another serum component, a complex that is dissociated by denaturants, or may represent aggregates of PAI (Hekman and Loskutoff, unpublished observations). Third, both SDS and guanidine treatments also revealed PAI activity in fractions corresponding to a mol wt of approximately 30,000. This finding is in agreement with our previous report concerning the analysis of the PAI in BAE CM by gel filtration. The difference between this mol wt estimation for the \( \beta \)-PAI and that obtained by SDS-PAGE (ie, 50,000) cannot be fully explained at present. Because most, if not all, of the PAI in serum is derived from platelets, these various mol wt forms of the PAI may also exist in platelets. This possibility is currently being examined.

Although it is not at all clear what function the \( \beta \)-PAI actually serves in normal and pathophysiological situations, the finding of elevated levels of PAI activity in certain individuals with thrombotic disease suggests a possible relationship between such conditions and an overall reduction of vascular PA activity. Further examination of this issue awaits the purification of sufficient quantities of human \( \beta \)-PAI.

ACKNOWLEDGMENT

The authors acknowledge the excellent technical assistance of K. Roegner and thank P. Tayman, C. Dike, and J. Obreiter for typing the manuscript. This is publication number 8383-1MM from the Scripps Clinic and Research Foundation.

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