The Extracellular Matrix Proteins Laminin and Fibronectin Contain Binding Domains for Human Plasminogen and Tissue Plasminogen Activator

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This study describes the binding of plasminogen and tissue-type plasminogen activator (t-PA) to the extracellular matrix proteins fibronectin and laminin. Plasminogen bound specifically and saturably to both fibronectin and laminin immobilized on microtiter wells, with \( K_{\text{app}} \) values of 115 and 18 nM, respectively. Limited proteolysis by endoproteinase V8 coupled with ligand blotting analysis showed that both plasminogen and t-PA preferentially bind to a 55-kDa fibronectin fragment and a 38-kDa laminin fragment. Amino acid sequence analysis demonstrated that the 55-kDa fragment originates with the fibronectin amino terminus whereas the laminin fragment was derived from the carboxyl-terminal globular domain of the laminin A chain. Ligand blotting experiments using isolated plasminogen domains were also used to identify distinct regions of the plasminogen molecule involved in fibronectin and laminin binding. Solution phase fibronectin binding to immobilized plasminogen was mediated primarily via lysine binding site-dependent interactions with plasminogen kringle 1-4. Lysine binding site-dependent binding of soluble laminin to immobilized plasminogen kringle 1-5 as well as an additional lysine binding site-independent interaction between mini-plasminogen and the 38-kDa laminin A chain fragment were also observed. These studies demonstrate binding of plasminogen and tissue-type plasminogen activator to specific regions of the extracellular matrix glycoproteins laminin and fibronectin and provide further insight into the mechanism of regulation of plasminogen activation by components of the extracellular matrix.

The extracellular matrix is composed of a variety of protein components including laminin, fibronectin, and type IV collagen which contribute to the formation of the basement membrane. Laminin, the major noncollagenous component of the basement membrane, is a large multidomain glycoprotein composed of three distinct disulfide-linked polypeptide chains. Binding sites for a variety of extracellular matrix molecules including glycosaminoglycans, type IV collagen, and nidogen are contained within the laminin molecule. In addition, diverse biological activities have been attributed to laminin including stimulation of cellular adhesion, growth, and migration and promotion of neurite outgrowth and tumor metastasis (for a review, see Martin and Timpl (1987)). The adhesive glycoprotein fibronectin is found in plasma as well as in interstitial connective tissues and basement membranes. Fibronectin is composed of two nearly identical polypeptide chains linked by a pair of disulfide bonds near the COOH termini. Each fibronectin subunit is organized into a variety of structural domains containing specific binding sites for cells, collagen, fibrin and glycosaminoglycans (for a review, see Hynes (1990)). Fibronectin also participates in complex interactions with other macromolecules to influence cellular properties including adhesion, morphology, migration, hemo-ostasis and oncogenic transformation (Hynes and Yamada, 1982).

Plasminogen (Pg), the zymogen of the serine proteinase plasmin, has been implicated in numerous physiological and pathological processes involving extracellular matrix remodeling including inflammation (Reich et al., 1988), wound healing (Highsmith, 1981), and tumor migration (Ossowski and Vassalli, 1978). Although plasmin functions primarily as a fibrinolytic enzyme, evidence suggests that basement membrane proteins including laminin and fibronectin are also susceptible to degradation by plasmin (Solen et al., 1985; Liotta et al., 1981a). Numerous studies have demonstrated that macromolecules such as fibrin, which simultaneously bind Pg and Pg activators, stimulate the activation reaction leading to enhanced plasmin formation (Ramby, 1982; Nieuwenhuizen et al., 1983). In addition to fibrin, intact extracellular matrix synthesized in vitro by endothelial cells also bind Pg and stimulate activation by tissue-type plasminogen activator (t-PA) (Knudsen et al., 1986). We previously demonstrated that several intact extracellular matrix components, most notably type IV collagen, stimulate t-PA-catalyzed Pg activation (Stack et al., 1990). The current study investigates the interaction of both Pg and t-PA with the extracellular matrix glycoproteins fibronectin and laminin. Evidence is provided for a kringle-dependent interaction of Pg with laminin and fibronectin. Furthermore, using limited proteolysis coupled with ligand blot binding assays, we have identified and sequenced specific domains of laminin and fibronectin which bind both Pg and t-PA. These studies provide additional information regarding regulation of Pg activation by protein components of the extracellular matrix and suggest the presence of additional previously unidentified functional epitopes of laminin and fibronectin.

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1 The abbreviations used are: Pg, plasminogen; t-PA, tissue-type plasminogen activator; 6-AIA, 6-aminohexanoic acid; BCP, 5-bromo-4-chloro-3-indolyl phosphate; NBT, nitro blue tetrazolium.
EXPERIMENTAL PROCEDURES

Materials—Bovine serum albumin, murine Engelbreth-Holm-Swarm tumor-derived plasminogen activator (6-AHA), fragments Tyr<sub>4</sub>Val<sub>5</sub> (kringle 3-1), lysine binding site 2), Val<sub>5</sub>Ala<sub>6</sub> (kringle 4, lysine binding site 2), benzylamine, anti-(goat IgG) alkaline phosphatase conjugate, anti-(rabbit IgG) alkaline phosphatase conjugate, dichlororesorcinol, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), nitro blue tetrazolium (NBT) grade III, and endoproteinase Glu-C from Staphylococcus aureus strain V8 (endoproteinase V8) were purchased from Sigma. Rabbit anti-(laminin) affinity-purified IgG antibody and anti-(fibronectin) purified IgG were purchased from Collaborative Research, Inc., Bedford, MA. Goat anti-(human melanoma t-PA) affinity-purified IgG and Val<sub>5</sub>Ala<sub>6</sub>-t-PA (mini-Pg) were purchased from Dr. Henry Berger at Wellcome Research Laboratories, Research Triangle Park, NC. Radioiodination of proteins was performed with Dr. Henry Berger at Wellcome Research Laboratories, Research Triangle Park, NC. Radioiodination of proteins was performed with [125I]iodine in a Packard Tri-Carb 3170TR liquid scintillation counter.

ELISA—ELISA binding studies were performed with proteins passively adsorbed on medium polystyrene(a) 96-well flat bottom culture plates (Costar, Cambridge, MA). Plates were used in a 200 μl of 10 mg/ml laminin or fibronectin in 0.1 M sodium carbonate, pH 9.6, containing 0.02% NaN<sub>3</sub> and incubated overnight at 5 °C. Optimal coating concentrations were determined by coating plates with increasing concentrations of [125I]-labeled fibronectin or laminin. Under optimal conditions, the amount of protein adsorbed was 193 ng of fibronectin/well and 762 ng of laminin/well. Prior to use in ELISA assays, nonspecific sites were blocked by incubating with 10 mM Tris-HCl, 0.15 M NaCl, 0.05% Tween 80, 0.02% bovine serum albumin, pH 7.5 (TSN/Tween), to room temperature for 1 h. For binding studies, increasing amounts of Pg (0-200 nM) were added in a 200 μl final volume of TSN/Tween in the presence or absence of 100 mM 6-AHA as described by Stenn et al. (1992). Absorbance was monitored at 405 nm using a Molecular Devices Thermomax kinetic plate reader. All experiments were repeated a minimum of eight times. Apparent binding affinities were calculated from double-reciprocal plots of the binding data.

RESULTS

ELISA of Pg Binding to Immobilized Fibronectin and Laminin—An ELISA was developed to examine the interaction between Pg and immobilized fibronectin and laminin. Binding of Pg with fibronectin or laminin passively adsorbed to microtiter wells led to specific saturable binding of the zymogen to immobilized fibronectin (Fig. 1A) and laminin (Fig. 1B). Data shown are corrected for 6-AHA noncompetitive binding. No significant binding of Pg was observed (results not shown). The apparent dissociation constants (K<sub>d</sub>) calculated from the binding data are 115 nm and 18 nm for fibronectin and laminin, respectively. Addition of 100 mM 6-AHA resulted in greater than 90% inhibition of Pg binding (data not shown), indicating that the interaction of Pg with laminin and fibronectin is primarily a lysine binding-site dependent reaction. Pg binding to fibronectin and laminin immobilized on microtiter wells was also analyzed using [125I]-labeled Pg to determine rates of association and dissociation. Association rates (k<sub>a</sub>) of Pg with fibronectin- and laminin-coated wells were 0.02 μM<sup>-1</sup> min<sup>-1</sup> and 0.019 μM<sup>-1</sup> min<sup>-1</sup>, respectively. Dissociation of Pg from fibronectin- or laminin-coated wells occurred at a rate of k<sub>d</sub> = 1.2
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FIG. 1. ELISA of plasminogen binding to immobilized fibronectin and laminin. Plasminogen was added in increasing concentrations to 96-well microtiter plates coated with (A) fibronectin or (B) laminin. Binding was analyzed by ELISA as described under "Experimental Procedures." Apparent dissociation constants ($K_d,pp$) were determined from double-reciprocal plots of the binding data.

binding of $P_g$ and t-PA to Fibronectin and Fibronectin Fragments—Because ELISA experiments indicated a specific interaction between $P_g$ or t-PA and immobilized fibronectin, fibronectin was subjected to limited proteolysis with endoproteinase V8, electrophoresed, blotted onto Immobilon, and incubated with $P_g$ or t-PA. Both $P_g$ and t-PA bound to intact immobilized fibronectin (Fig. 2, C and D). In addition, although greater than 20 fibronectin fragments were generated by limited proteolysis, $P_g$ interacts predominantly with only 3 of these fragments, most notably a 55-kDa polypeptide (Fig. 2C, arrow). As observed with $P_g$, t-PA also bound strongly to the identical 55-kDa fragment (Fig. 2D, arrow). $P_g$ and t-PA also bound to the identical fibronectin fragment under non-reducing conditions, although the mobility of fibronectin and fibronectin fragments varied in the absence of reducing agent (data not shown). Binding by $P_g$ was significantly inhibited by 6-AHA (Fig. 2E) but only slightly affected by benzylamine (data not shown), whereas binding by t-PA was unaltered by 6-AHA (Fig. 2F). In control experiments in which blots were incubated with either anti-$P_g$ or anti-t-PA without prior incubation with the respective ligand, no binding was detected (data not shown). Furthermore, no nonspecific binding to endoproteinase V8 and molecular weight standards was observed.

To identify which region of the fibronectin molecule was represented by this 55-kDa fragment, amino acid sequence analysis was performed on the polypeptide. NH₂-terminal sequence analysis suggested that the NH₂ terminus of the fragment was blocked. Since fibronectin is reported to contain pyroglutamate as the NH₂ terminus (Garcia-Pardo et al., 1983), the 55-kDa fragment was subjected to further proteolysis with trypsin followed by microsequence analysis of purified peptides. Amino-terminal sequencing of two tryptic peptides revealed single sequences of Ile-Gly-Asp-Thr-X-Arg and Ile-Gly-Asp-Thr-X-Ser-Lys, representing amino acids 119-124 and 211-217, respectively of the NH₂-terminal region of fibronectin (Garcia-Pardo et al., 1983). Based on the size of the original 55-kDa fragment, the $P_g$- and t-PA-binding fibronectin fragment comprises the NH₂-terminal heparin binding domain of fibronectin as well as approximately half of the gelatin binding domain (Hynes, 1990). The sequence of the 50-kDa $P_g$ and t-PA-binding fragment located immediately below the 55-kDa fragment (Fig. 2, C and D) was AEETCFDKYTGYNTYR, which corresponds to residues 62-
76 of the fibronectin molecule, demonstrating that this fragment is derived from the same region of fibronectin as is the 55-kDa fragment.

To ensure that the interaction between Pg and the 55-kDa fibronectin fragment did not reflect an artifact of adsorption of fibronectin to a surface, binding of Pg to intact and endoproteinase V8-digested fibronectin was also analyzed in solution. 

\[ ^{125} \text{I-Pg} (30 \text{ pg}) \] was incubated at 4 °C for 18 h with either intact fibronectin (300 \text{ pg}) or fibronectin (300 \text{ pg}) digested with endoproteinase V8 (0.075 mg/ml) as described above. The reaction mixture (400 \text{ μl}) was then passed over a column containing 1.0 ml of gelatin-agarose (Sigma), washed with 10 column volumes of 50 mM Tris, pH 7.4, and eluted with 10 column volumes of 4 M urea, pH 7.4. Wash and eluate fractions were lyophilized and analyzed by Western blotting using anti-fibronectin or anti-plasminogen antibodies as described above. Fractions were also analyzed by \( \gamma \)-counting to detect \( ^{125} \text{I-Pg} \). Results from this experiment indicated that although intact fibronectin bound to the gelatin-agarose column, \( ^{125} \text{I-Pg} \) was present only in the unbound fractions, indicating that Pg does not interact strongly with solution phase intact fibronectin (data not shown). When the experiment was repeated using endoproteinase V8-digested fibronectin, four major gelatin binding fibronectin fragments of molecular masses 270, 181, 90, and 55 kDa were eluted from gelatin agarose (data not shown). In addition, \( ^{125} \text{I-Pg} \) was also present in the gelatin-agarose eluate. Since control experiments indicated that \( ^{125} \text{I-Pg} \) alone does not bind gelatin-agarose, this result directly demonstrates binding of Pg to a fibronectin fragment(s) in solution.

**Binding of Pg and t-PA to Laminin and Laminin Fragments**—The interaction of Pg and t-PA with laminin was also determined using ligand blot binding assays between soluble Pg or t-PA and immobilized laminin. Laminin was subjected to limited proteolysis with endoproteinase V8, electrophoresed, blotted onto Immobilon, and incubated with Pg or t-PA. Both Pg and t-PA bound to intact immobilized laminin (Fig. 3, C and D) and several laminin fragments, predominantly an identical 38-kDa polypeptide (Fig. 3, C and D, arrows). Pg and t-PA also bound to the identical laminin fragment under nonreducing conditions, although the mobility of laminin and laminin fragments was altered in the absence of reducing agent (data not shown). The majority of Pg binding to intact laminin and laminin fragments was completely inhibited by 6-AHA (Fig. 3E) while binding to the 38-kDa fragment remained detectable in the presence of either 6-AHA (Fig. 3E) or benzylationmine (data not shown) and is characterized further below. Binding of t-PA was unaltered by 6-AHA (Fig. 3F). In control experiments in which blots were incubated with either anti-(Pg) or anti-(t-PA) without prior incubation with the respective ligand, no binding was detected (data not shown). Furthermore, no nonspecific binding to endoproteinase V8 and molecular weight standards was observed. The amino-terminal sequence of the 38-kDa peptide was Leu-X-Ala-Val-Asp-Thr-Ala-Pro-Gly-Tyr-Val-Ala-Gly-Ala-His which corresponds to residues 2709-2723 of the laminin A chain (Sasaki et al., 1988). Based on molecular weight, this fragment is derived from the COOH-terminal globular domain of the laminin A chain comprising the heparin/type IV collagen binding domain (Martin and Timpl, 1987).

**Binding of Fibronectin and Laminin to Immobilized Pg and Pg Fragments**—To identify the region of the Pg molecule which was interacting with fibronectin and laminin, isolated Pg domain fragments were electrophoresed on 11% gels under nonreducing conditions, blotted onto Immobilon, and incubated with laminin or fibronectin (Fig. 4). Development of the blots with an anti-(Pg) antibody (Fig. 4B) indicates that similar amounts of protein were transferred during the blotting procedure. Binding of fibronectin to intact Pg or the fragments consisting of kringle 1-3 (Fig. 4C, lanes 1 and 2, respectively) or kringle 4 (Fig. 5D, lane 2) was much stronger than the binding to the miniPg fragment (consisting of kringle 5 and the serine proteinase domain of Pg; Fig. 4C, lane 3). No inhibition of fibronectin binding was observed in the presence of 6-AHA (Fig. 4D, lanes 1 and 2) or benzylamine (data not shown). In similar experiments, laminin bound to intact Pg as well as the Pg fragments consisting of kringle 1-3, (Fig. 4E, lanes 1 and 2, respectively), miniPg (Fig. 4E, lane 3), and kringle 4 (Fig. 5C, lane 2). Binding of laminin to kringle 1-3 (Fig. 4F, lane 2) or kringle 4 (data not shown) was moderately inhibited by either 100 mM 6-AHA or benzylamine, whereas laminin binding to miniPg (Fig. 4F, lane 3) was significantly inhibited by either 6-AHA or benzylamine (data not shown).

**Binding of MiniPg to Laminin and Laminin Fragments**—Because the results described above suggested a specific interaction between laminin and miniPg, ligand blot binding studies were repeated using immobilized laminin and soluble miniPg. Laminin was subjected to limited proteolysis as previously described, blotted onto Immobilon and incubated with miniPg (2.5 \text{ μg/ml}). MiniPg bound to intact laminin as well as several laminin fragments (Fig. 6B), including the 38-kDa fragment identified above (Fig. 6B, arrow). As previously

![Fig. 3. Binding of Pg and t-PA to laminin and laminin fragments.](image-url)
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Fig. 4. Binding of fibronectin and laminin to isolated Pg domain fragments. Intact Pg (lane 1), Pg kringle 1–3 fragment (lane 2), and miniPg (lane 3) were electrophoresed on 11% SDS-polyacrylamide gels under nonreducing conditions and transferred to Immobilon as described under “Experimental Procedures.” A, Immobilon membrane stained with Coomassie Blue; B, Immobilon membrane incubated with anti-(Pg) antibody; C, Immobilon incubated with 15 ng/ml fibronectin; D, Immobilon membrane incubated with fibronectin in the presence of 100 mM 6-AHA. E, Immobilon incubated with 15 ng/ml laminin; F, Immobilon incubated with laminin in the presence of 100 mM 6-AHA.

observed with intact Pg (Fig. 3), the majority of miniPg binding to intact laminin and laminin fragments was inhibited by benzylamine, whereas binding to the 38-kDa laminin fragment was unaffected (Fig. 6C). These data suggest that the interaction between miniPg and the 38-kDa laminin fragment may be mediated in part via the serine proteinase domain of Pg.

DISCUSSION

The role of plasmin in physiological and pathological processes involving modification of the extracellular matrix suggests that conversion of Pg to the active enzyme plasmin may be regulated under conditions which differ from those on the surface of the fibrin clot. Previous studies from our laboratory using isolated extracellular matrix protein components demonstrated that several extracellular matrix proteins, most notably type IV collagen, enhanced the rate of t-PA-catalyzed Pg activation (Stack et al., 1990). The extracellular matrix adhesive glycoproteins laminin and fibronectin had a differential effect on Pg activation by t-PA. Current evidence regarding the regulation of physiological and pathological fibrinolysis suggests that Pg activation is a surface-associated process (Miles and Plow, 1988; Saksela and Rifkin, 1988). Since the adhesive glycoproteins fibronectin and laminin are intimately associated with both the basement membrane layer and the cell surface, we have investigated the interaction of Pg and t-PA with these extracellular matrix proteins.

ELISA studies were initially utilized to probe the interaction of Pg with intact laminin and fibroenectin passively adsorbed to microtiter wells. Incubation of Pg with fibronectin- or laminin-coated microtiter wells led to concentration dependent, saturable binding of Pg which was greater than 90% reversible by 6-AHA. The $K_{d(app)}$ calculated from the binding data indicate that Pg binds more strongly to laminin than fibronectin. Furthermore, these values are similar to those reported for Pg binding to other immobilized matrix proteins including thrombospondin (35 nM), fibronectin (91 nM), and type IV collagen (12 nM) (Silverstein et al., 1985; Salonen et al., 1985; Stack et al., 1992).

Since initial binding studies demonstrated specific interactions between Pg and intact fibronectin or laminin, we performed limited proteolysis of these proteins followed by ligand blot binding assays with solution phase Pg to determine which isolated domains interact with Pg. Endoproteinase V8, which cleaves Asp-X or Glu-X bonds (Houmard and Drapeau, 1972), was chosen for these experiments to avoid generation of fragments with COOH-terminal lysine residues which would be expected to bind Pg (Castellino, 1981). Ligand blotting studies using proteolyzed fibronectin demonstrated that Pg bound strongly to several fibronectin fragments, most notably a 55-kDa polypeptide comprising the NH$_2$-terminal
hemin, electrophoresed on 5-15% gradient SDS-polyacrylamide gels under "Experimental Procedures." They were later stained with Coomassie Blue; the presence of 100 mM benzylamine. Molecular mass standards are plasma cryoprecipitate (Ruoslahti et al., 1981), suggesting the occurrence of plasma fibronectin proteolysis in vivo.

Pg contains a series of five triple-loop disulfide-bonded structures referred to as kringle (Sottrup-Jensen et al., 1978) coupled to the serine proteinase domain which are involved in targeting of the zymogen to the fibrin clot surface via several distinct lysine binding sites (Thorsen, 1975). To determine whether the Pg kringle domains were involved in the interaction with fibronectin polypeptides, the effect of 6-AHA and benzylamine on Pg binding was determined. Pg kringle 1–4 contain binding sites for lysine and lysine analogues such as 6-AHA (Christensen, 1984), whereas kringle 5 interacts with benzylamine and benzamidine (Varadi and Patthy, 1981; Thewes et al., 1990). Although t-PA binding to fibronectin polypeptides was unaffected by the addition of lysine analogues, addition of 6-AHA inhibited the interaction between Pg and fibronectin fragments, suggesting lysine binding site-dependent binding. Similar results were obtained by ELISA which demonstrated greater than 90% 6-AHA inhibitable binding. Only slight inhibition was observed in the presence of benzylamine, indicating that kringles 1–4, rather than kringle 5, are responsible for the interaction.

Similar ligand blotting experiments were performed with intact laminin and laminin fragments generated by limited proteolysis with endoproteinase V8. Pg bound strongly to intact laminin as well as several laminin fragments, particularly a 38-kDa polypeptide. Amino acid sequence analysis localized this peptide to the COOH-terminal globular domain of the laminin A chain containing the heparin-type IV collagen binding domain (Martin and Timpl, 1987). t-PA also bound specifically to the identical 38-kDa A chain fragment suggesting that, as observed with fibronectin, this laminin domain may also mediate Pg-t-PA interactions. Ligand blotting experiments demonstrated that binding of Pg to intact laminin was inhibited by both 6-AHA and benzylamine. Similar results were obtained by ELISA which showed greater than 90% 6-AHA inhibitable binding. However, little inhibition of Pg binding to the 38-kDa fragment was observed with either compound. These data suggest that the interaction of Pg with intact laminin is mediated via lysine binding sites located on Pg kringles 1–5, whereas the Pg-38-kDa laminin A chain fragment interaction may be lysine binding site-independent.

Since these studies suggested that Pg binding to fibronectin and laminin may be mediated via Pg kringle domains, the reverse ligand blot binding experiments were performed to characterize binding interactions between intact solution phase fibronectin or laminin and immobilized Pg fragments. Fibronectin bound strongly to fragments containing kringle 1–3 and kringle 4, with minimal binding to kringle 5 containing miniPg. This is in agreement with the results described above demonstrating that binding of soluble Pg by immobilized fibronectin is unaffected by benzylamine. Together these data suggest that Pg binding to fibronectin is mediated primarily via kringles 1–4. However, with Pg as the immobilized ligand, 6-AHA had no effect on the binding interaction. Similar results were obtained in our laboratory using type IV collagen (Stack et al., 1992) and by DePoli et al. (1989) with thrombospondin; namely that lysine analogues inhibit the interaction between Pg and extracellular matrix proteins only when the extracellular matrix protein is the immobilized ligand and Pg is in soluble form. This discrepancy may result from conformational restriction of immobilized Pg which would prevent lysine-induced conformational changes that may mediate interaction of Pg with macromolecular ligands (Castellino, 1981; Mangel et al., 1990).

In similar experiments, solution phase laminin was incubated with immobilized Pg and Pg fragments. As observed above with fibronectin, laminin bound strongly to Pg fragments containing kringles 1–3 and kringle 4. However, in contrast to fibronectin, binding of laminin to miniPg was also observed. To elucidate further the interaction between miniPg and laminin, the reverse ligand blot binding experiments were performed using solution phase miniPg and immobilized laminin. As observed with intact Pg, miniPg also bound to intact laminin and several laminin fragments including the 38-kDa laminin A chain fragment interaction may be lysine binding site-independent.
A chain fragment. However, incubation of miniPg with laminin in the presence of benzylamine inhibited the interaction of miniPg with all but the 38-kDa A chain fragment. Together these data suggest that Pg binding to intact laminin is mediated primarily via interactions between laminin and Pg kringles 1–5. However, interaction between miniPg and the 38-kDa A chain globular domain fragment is lysine binding site-independent and may therefore involve the serine proteinase domain of Pg.

In summary, we have identified fragments of laminin and fibronectin which bind specifically to both Pg and t-PA. Previous investigators have reported that fibronectin and laminin are readily cleaved by proteinases such as plasmin, thrombin and elastase into fragments that retain biological activity (Beck et al., 1990; Ruoslahti et al., 1981; Liotta et al., 1981b). Interestingly, Pg and t-PA bound to domains of both laminin and fibronectin known to be involved in binding of heparin and collagen/gelatin (Martin and Timpl, 1987; Hynes, 1990). Although the significance of this observation remains unclear, numerous studies have demonstrated that macromolecules which simultaneously bind Pg and t-PA enhance the efficiency of the activation reaction (Ranby, 1982; Nieuwenhuis et al., 1983). Together these data suggest the presence of additional previously unidentified functional epitopes of laminin and fibronectin with the potential to regulate Pg activation by t-PA. It is interesting to speculate that proteolysis of fibronectin or laminin under physiological or pathological conditions may result in exposure or release of Pg- and t-PA-binding epitopes, which in turn may provide a mechanism for localized regulation of plasmin generation in the extracellular matrix.

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