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Binding of human plasminogen to basement-membrane (type IV) collagen

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Plasminogen, the zymogen form of the serine proteinase plasmin, has been implicated in numerous physiological and pathological processes involving extracellular-matrix remodelling. We have previously demonstrated that the activation of plasminogen catalysed by tissue plasminogen activator is dramatically stimulated in the presence of basement-membrane-specific type IV collagen [Stack, Gonzalez-Gronow & Pizzo (1990) Biochemistry **29**, 4966–4970]. The present paper describes the binding of plasminogen to type IV collagen. Plasminogen binds to both the α1(IV) and α2(IV) chains of basement-membrane collagen, with binding to the α2(IV) chain preferentially inhibited by 6-aminohexanoic acid. This binding is specific and saturable, with $K_{d,app.}$ values of 11.5 and 12.7 nM for collagen and gelatin respectively. Although collagen also binds to immobilized plasminogen, this interaction is unaffected by 6-aminohexanoic acid. Limited elastase proteolysis of plasminogen generated distinct collagen-binding fragments, which were identified as the kringle 1–3 and kringle 4 domains. No binding of collagen to mini-plasminogen was observed. These studies demonstrate a specific interaction between plasminogen and type IV collagen and provide further evidence for regulation of plasminogen activation by protein components of the extracellular matrix.

INTRODUCTION

The extracellular matrix (ECM) is composed of a variety of protein and proteoglycan components, which contribute to the formation of the basement membrane. The major structural protein of the basement membrane is type IV collagen (Kefalides, 1973), which differs from interstitial collagens by the presence of globular domains interspersed within the triple-helical segments (Glanville, 1987). This unique structural feature gives collagen type IV the flexibility to assemble into sheet-like networks characteristic of basement membranes. Degradation of basement-membrane proteins occurs under a variety of physiological and pathological circumstances including embryogenesis (Strickland *et al.*, 1976), wound healing (Highsmith, 1981), inflammation (Reich *et al.*, 1988) and neoplasia (Ossowski & Vassalli, 1978; Reich *et al.*, 1988). Previous studies have demonstrated the involvement of numerous enzymes in basement-membrane proteolysis, including matrix metalloproteinases 1–3 (Okada *et al.*, 1986, 1990), plasminogen activators and plasmin (Reich *et al.*, 1988; Liotta *et al.*, 1981; Mackay *et al.*, 1990).

The serine proteinase plasmin is formed by cleavage of the zymogen plasminogen by proteins that function as plasminogen activators. Although plasmin functions primarily as a fibrinolytic enzyme, evidence suggests that basement-membrane proteins such as collagen type IV (Liotta *et al.*, 1981; Mackay *et al.*, 1990), fibronectin (Salonen *et al.*, 1985) and laminin (Liotta *et al.*, 1981) are also susceptible to plasmin degradation. The efficiency of the plasminogen activation reaction is enhanced when both the zymogen and its activator are immobilized by binding to a macromolecular protein surface such as fibrin (Ranby, 1982; Nieuwenheuizen *et al.*, 1983). This binding is facilitated by a series of five triple-loop disulphide-bonded bridge structures referred to as kringles (Sottrup-Jensen *et al.*, 1978), which are involved in targeting of the zymogen to the surface of the fibrin clot. In addition to fibrin, specific binding of plasminogen to isolated ECM components such as thrombospondin (DePoli *et al.*, 1989), fibronectin (Salonen *et al.*, 1985) and vitronectin (Preissner, 1990) has also been reported. Intact ECMs synthesized and secreted by endothelial cells were shown to bind plasminogen as well as enhance the efficiency of plasminogen activation (Knudsen *et al.*, 1986). Previous kinetic studies in our laboratory with isolated ECM proteins indicated that native and denatured type IV collagen were responsible for this stimulatory effect (Stack *et al.*, 1990). This study demonstrates that plasminogen binds specifically and saturably to both type IV collagen and gelatin. Evidence is provided for a specific interaction between the plasminogen kringle domains and type IV collagen.

MATERIALS AND METHODS

Materials

Plasminogen fragments Tyr-79–Val-353 (kringles 1–3), Val-354–Ala-439 (kringle 4) and Val-442–Asn-790 (mini-plasminogen), human placental type IV collagen, BSA, 6-aminohexanoic acid (6-AHA), benzylamine, anti-(rabbit IgG) antibody–alkaline phosphatase conjugate, anti-(mouse IgG) antibody–alkaline phosphatase conjugate and pig pancreatic elastase were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Anti-(type IV collagen) affinity-purified IgG was purchased from Heyltex Corporation, Houston, TX, U.S.A. Purity of collagens was assessed by electrophoresis on SDS/7.5% polyacrylamide gels under reducing conditions (Laemmli, 1970). Gelatin was produced by thermal denaturation of collagen by heating for 20 min at 60 °C (Murphy *et al.*, 1985). All other chemicals were of reagent-grade quality.

Proteins

Plasminogen was purified from human plasma by affinity chromatography on L-lysine–Sepharose (Deutsch & Mertz, 1970) and separated into isoforms 1 and 2 by affinity chromatography on concanavalin A–Sepharose (Gonzalez-Gronow & Robbins, 1984). Affinity-chromatography-purified form 2 (which contains a single *O*-linked carbohydrate chain) was utilized for all experiments. Protein concentrations were determined spectrophotometrically at 280 nm by using an $A^{1\%}_{1\,cm}$ value of 16.8 and molecular mass of 92 kDa for Glu^1-plasminogen (Castellino,

Abbreviations used: ECM, extracellular matrix; 6-AHA, 6-aminohexanoic acid.

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1981). Radioiodination of plasminogen was performed with Enzymobeads (Bio-Rad Laboratories) according to the manufacturer's specifications. Radioactivity was measured in an LKB 1272 γ-radiation counter. Limited proteolysis of plasminogen with pig pancreatic elastase was carried out as described by Sottrup-Jenssen *et al.* (1978). Briefly, plasminogen was incubated with elastase in a 50:1 molar ratio in 20 mM-Hepes buffer, pH 7.4, at 37 °C. Samples were removed at various time intervals, boiled immediately and electrophoresed on SDS/5–15%-gradient polyacrylamide gels.

Blot-binding assay

Collagen type IV and plasminogen were electrophoresed on quadruplicate SDS/polyacrylamide gels (Laemmli, 1970) and electroblotted on to poly(vinyl difluoride) membranes (Immobilon) as described by Matsudaria (1987). After electroblotting, one membrane was stained with Coomassie Blue for protein identification. Transfer of plasminogen and plasminogen fragments was complete in 20 min, whereas 30–40 min was required for optimal transfer of collagen. Before binding studies, blots were incubated in 20 ml of 10 mM-Tris/HCl buffer, pH 7.5, containing 0.15 M-NaCl, 0.05% Nonidet P40 and 1% BSA (TSN buffer) at room temperature for 30 min to block non-specific sites. All blot-binding studies were performed a minimum of three times.

In studies examining the binding of collagen to immobilized plasminogen, reduced and non-reduced samples of plasminogen and plasminogen fragments were electrophoresed in quadruplicate on SDS/polyacrylamide gels and transferred to Immobilon. After being blocked with TSN buffer, blots were incubated with collagen type IV (15 μg/ml) in 20 ml of TSN buffer overnight at 5 °C. To examine the effect of 6-AHA or benzylamine on collagen binding, collagen was also incubated with immobilized plasminogen in the presence of 100 mM-6-AHA or benzylamine in TSN buffer (adjusted to neutral pH after 6-AHA or benzylamine addition, if necessary). After being washed three times with TSN buffer, collagen bound to immobilized plasminogen was detected with rabbit anti-(type IV collagen) IgG using the immunoblotting procedure described by Enghild *et al.* (1989). Anti-(collagen type IV) IgG was added at a dilution of 1:1000 followed by the addition of goat anti-(rabbit IgG) antibody–alkaline phosphatase conjugate (1:1000 dilution). After being washed, blots were developed with 5.0 mg of 5-bromo-4-chloroindol-3-yl phosphate and 5.0 mg of Nitro Blue Tetrazolium in 20 ml of 0.1 M-Tris/HCl buffer, pH 9.5, containing 0.1 M-NaCl and 5 mM-$MgCl_2$. Development was stopped with 10 mM-Tris/HCl buffer, pH 8.5, containing 5 mM-EDTA.

In studies examining the binding of plasminogen to immobilized collagen type IV, 30 μg of protein was electrophoresed on SDS/7.5% polyacrylamide gels and blotted on to Immobilon as described above. After being blocked with TSN buffer, blots were incubated overnight with ^{125}I-labelled plasminogen (8 μg; 1.2×10^5 c.p.m./μg) in 20 ml of TSN buffer in the presence or absence of 100 mM-6-AHA or benzylamine. Blots were washed four times for 5 min in TSN buffer and allowed to dry before autoradiography.

E.l.i.s.a.

E.l.i.s.a. binding studies were performed with proteins passively adsorbed on 96-well culture plates. Plates were coated with 200 μl of 5 μg of collagen type IV, gelatin type IV or fibrinogen/ml in 0.1 M-sodium carbonate, pH 9.6, containing 0.02% NaN_3 and incubated overnight at 4 °C. After being coated, plates were washed with 200 μl of 0.01 M-sodium phosphate buffer, pH 7.4, containing 0.1 M-NaCl, and 0.05% Tween 80 (PBS/Tween) to remove unbound protein. With ^{125}I-labelled collagen, approx. 1.5 ng of collagen/well was bound under these conditions. Non-specific sites were blocked by incubating with PBS/Tween containing 2% BSA (PBS/Tween + BSA) at room temperature for 60 min. Plates were washed again with 200 μl of PBS, air-dried and stored at 4 °C. For binding studies, increasing amounts of plasminogen (0–200 nM) were added in quadruplicate samples in a 200 μl final volume of PBS/Tween + BSA and incubated for 2 h at 37 °C. Plates were then washed three times with PBS/Tween and incubated with goat anti-(human plasminogen) antibody (1:400 dilution) at room temperature for 60 min. After being washed, plates were incubated with anti-(goat IgG) antibody–alkaline phosphatase conjugate (1:400 dilution) for 30 min. Plates were again washed and 200 μl of alkaline phosphatase substrate (1 mg of *p*-nitrophenyl phosphate/ml) in 0.1 M-glycine buffer, pH 10.4, containing 1 mM-$MgCl_2$ and 1 mM-$ZnCl_2$ was added to the plate and absorbance was monitored at 405 nm with an Anthos Labtec kinetic plate reader. Bound plasminogen was expressed as ΔA_{405}/min. Apparent binding affinities ($K_{d,app.}$) were calculated from double-reciprocal plots of the binding data.

RESULTS

Binding of plasminogen to immobilized collagen type IV

The interaction of type IV collagen with plasminogen was determined by blot binding between soluble plasminogen and immobilized collagen type IV. In these experiments, collagen type IV was electrophoresed under denaturing conditions to separate the α1(IV) and α2(IV) chains, blotted on to Immobilon and incubated with ^{125}I-labelled plasminogen (Fig. 1). Although both the α1(IV) and α2(IV) chains of collagen bound ^{125}I-labelled plasminogen (Fig. 1*a*), binding of soluble plasminogen to the α2(IV) chain was preferentially inhibited by both 6-AHA and benzylamine (Figs. 1*b* and 1*c*), suggesting both a lysine-binding-site-dependent and a lysine-binding-site-independent component of binding.

Binding of collagen type IV to immobilized plasminogen and plasminogen fragments

Blot-binding assays were also utilized to study the interaction between soluble collagen and immobilized plasminogen and plasminogen fragments. Fig. 2 demonstrates that collagen type

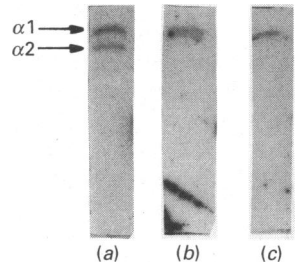

Fig. 1. Binding of ^{125}I-labelled plasminogen to immobilized type IV collagen

Type IV collagen (30 μg) was electrophoresed under denaturing conditions on SDS/7.5% polyacrylamide gels and electroblotted on to Immobilon as described in the Materials and methods section. Blots were incubated with ^{125}I-labelled plasminogen (8 μg; 1.2×10^5 c.p.m./μg) followed by autoradiography. (*a*) Autoradiograph of blot incubated with ^{125}I-labelled plasminogen. (*b*) Autoradiograph of blot incubated with ^{125}I-labelled plasminogen in the presence of 100 mM-6-AHA. (*c*) Autoradiograph of blot incubated with ^{125}I-labelled plasminogen in the presence of 100 mM-benzylamine. The positions of the α1 and α2 chains of collagen type IV are shown.

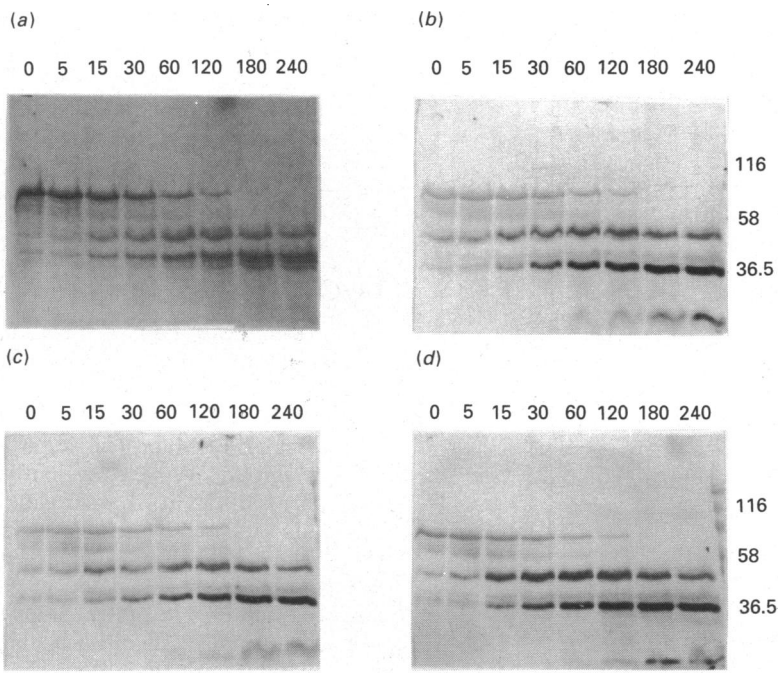

Fig. 2. Binding of type IV collagen to plasminogen fragments

Plasminogen was subjected to limited proteolysis with elastase for increasing times (min) as indicated followed by electrophoresis on SDS/5–15 %-gradient polyacrylamide gels and transfer to Immobilon as described in the Materials and methods section. (*a*) Immobilon membrane stained with Coomassie Blue. (*b*) Immobilon incubated with collagen type IV. Collagen binding was detected by using an anti-(type IV collagen) antibody followed by an alkaline phosphatase-conjugated secondary antibody as described in the Materials and methods section. (*c*) Immobilon incubated with collagen type IV in the presence of 100 mM-6-AHA. (*d*) Immobilon incubated with collagen type IV in the presence of 100 mM-benzylamine. Molecular-mass standards are: β-galactosidase, 116 kDa; pyruvate kinase, 58 kDa; lactate dehydrogenase, 36.5 kDa.

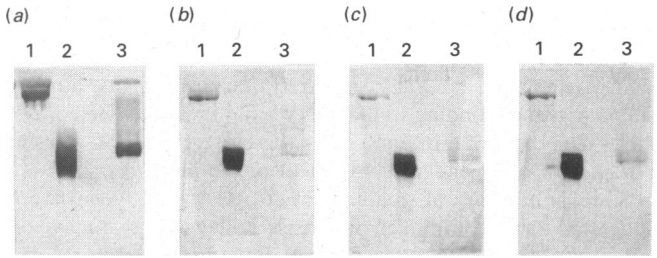

Fig. 3. Binding of type IV collagen to isolated plasminogen domains

Intact plasminogen (lane 1), plasminogen kringle 1–3 fragment (lane 2) and mini-plasminogen (lane 3) were electrophoresed on SDS/11 % polyacrylamide gels and transferred to Immobilon as described in the Materials and methods section. (*a*) Immobilon membrane incubated with anti-plasminogen antibody followed by an alkaline phosphatase-conjugated secondary antibody as described in the Materials and methods section. (*b*) Immobilon incubated with collagen type IV. Collagen binding to plasminogen was detected as described in Fig. 2. (*c*) Immobilon incubated with collagen type IV in the presence of 100 mM-6-AHA. (*d*) Immobilon incubated with collagen type IV in the presence of 100 mM-benzylamine.

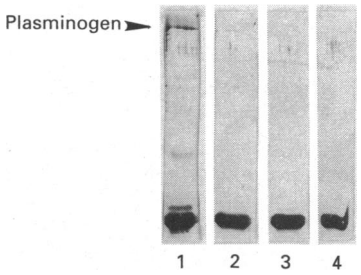

Fig. 4. Binding of collagen type IV to plasminogen kringle 4

Plasminogen kringle 4 was electrophoresed on an SDS/15 % polyacrylamide gel and transferred to Immobilon as described in the Materials and methods section. Lane 1, Immobilon incubated with an anti-plasminogen antibody followed by an alkaline phosphatase-conjugated secondary antibody as described in the Materials and methods section. Lane 2, Immobilon incubated with collagen type IV. Collagen binding to plasminogen was detected as described in Fig. 2. Lane 3, Immobilon incubated with collagen type IV in the presence of 100 mM-6-AHA. Lane 4, Immobilon incubated with collagen type IV in the presence of 100 mM-benzylamine.

IV interacts specifically with immobilized intact plasminogen as well as with plasminogen fragments generated by limited proteolysis with elastase. The binding is primarily localized to a 36 kDa plasminogen fragment (Fig. 2*b*). Collagen binding to plasminogen was not significantly decreased in the presence of 6-AHA or benzylamine (Figs. 2*c* and 2*d*). In order to identify which region of the plasminogen molecule was represented by the 36 kDa collagen-binding fragment, isolated plasminogen domains were electrophoresed, blotted on to Immobilon and incubated with collagen type IV (Figs. 3 and 4). Development of the blots with an anti-plasminogen antibody (Figs. 3*a* and Fig. 4, lane 1) indicates that similar amounts of protein were transferred during the blotting procedure. However, binding of collagen type IV to the plasminogen fragments consisting of kringles 1–3 (Fig. 3*b*, lane 2) and kringle 4 (Fig. 4, lane 2) is much stronger than binding to either the intact molecule (Fig. 3*b*, lane 1) or the mini-plasminogen fragment (consisting of kringle 5 and the serine proteinase domain of plasminogen) (Fig. 3*b*, lane 3). Identical results were obtained when plasminogen and plasminogen fragments were electrophoresed under non-reducing

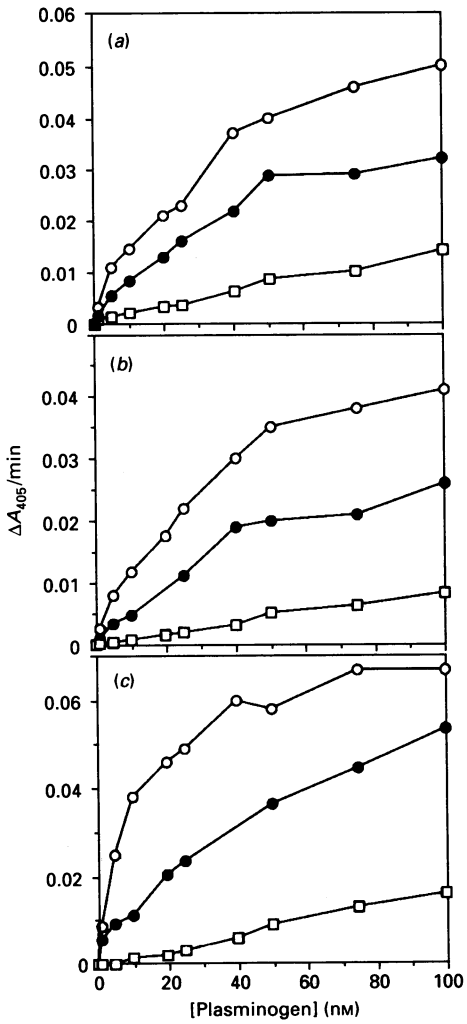

Fig. 5. E.l.i.s.a. of plasminogen binding to immobilized collagen type IV, gelatin type IV and fibrinogen

Plasminogen was added in increasing concentrations to 96-well micro-titre plates coated with (*a*) collagen type IV, (*b*) gelatin type IV or (*c*) fibrinogen. Binding was analysed by e.l.i.s.a. as described in the Materials and methods section. ○, Plasminogen only; ●, plasminogen in the presence of 100 mM-benzylamine; □, plasminogen in the presence of 100 mM-6-AHA.

conditions (results not shown). In order to determine whether this interaction occurred via the lysine-binding sites of plasminogen, blots were incubated with collagen type IV in the presence of either 100 mM-6-AHA (Figs. 3*c* and Fig. 4, lane 3) or 100 mM-benzylamine (Figs. 3*d* and Fig. 4, lane 4). Neither compound significantly decreased the binding of soluble collagen to immobilized plasminogen.

E.l.i.s.a. of plasminogen binding to immobilized collagen type IV, gelatin type IV and fibrinogen

Because blot-binding assays indicated a specific interaction between collagen IV and plasminogen, an e.l.i.s.a. was developed to examine further the interaction between plasminogen and immobilized proteins. Incubation of plasminogen with collagen type IV, gelatin type IV or fibrinogen passively adsorbed on micro-titre wells led to specific saturable binding of the zymogen to the immobilized proteins (Figs. 5*a–c*). No binding of plasminogen to uncoated plates was observed (results not shown). The apparent dissociation constants ($K_{d,app.}$) calculated from the binding data are 6.8 nM, 11.5 nM and 12.7 nM for fibrinogen, collagen type IV and gelatin type IV respectively. Addition of 100 mM-6-AHA significantly inhibited the interaction of plasminogen with all immobilized proteins (Fig. 5), indicating the participation of plasminogen lysine-binding sites in the interaction. However, incubation with 100 mM-benzylamine (Fig. 5) or a 300-fold excess of soluble protein to immobilized protein (results not shown) had only a slight inhibitory effect on plasminogen binding to immobilized proteins.

DISCUSSION

Although the interaction of plasminogen with intact ECMs as well as several isolated matrix protein components has been previously described (Salonen *et al.*, 1985; Knudsen *et al.*, 1986; DePoli *et al.*, 1989; Preissner, 1990), the binding of plasminogen to the major ECM protein, type IV collagen, has remained uncharacterized. We have previously demonstrated that protein components of the ECM are capable of regulating plasminogen activation catalysed by tissue-associated plasminogen activators (Stack *et al.*, 1990). Type IV collagen and gelatin were found to be the most efficient stimulators of plasminogen activation in the absence of fibrin. The present study uses both blot binding and e.l.i.s.a. to demonstrate direct binding of plasminogen to native and denatured type IV collagen.

Our initial studies qualitatively demonstrated the binding of ^{125}I-labelled plasminogen to both the α1(IV) and α2(IV) chains of collagen IV. To determine whether the plasminogen kringle domains were involved in this interaction, the effect of 6-AHA and benzylamine on plasminogen binding to collagen was determined. The first four kringle domains of plasminogen contain binding sites for lysine or lysine analogues (such as 6-AHA) (Vali & Patthy, 1982; Christensen, 1984), whereas kringle 5 interacts strongly with benzylamine and benzamidine (Varadi & Patthy, 1981; Thewes *et al.*, 1990). Both 6-AHA and benzylamine attenuated the plasminogen–collagen interaction, with preferential inhibition of binding to the α2(IV) chain, suggesting that plasminogen binding to the α2(IV) chain is lysine-binding-site-dependent. Binding to the α1(IV) chain was unaffected by 6-AHA and benzylamine and may therefore represent a lysine-binding-site-independent component of binding.

Since inhibition of binding by 6-AHA and benzylamine suggested that plasminogen binding to collagen may involve one or more of the plasminogen kringle domains, experiments were performed to identify the region(s) of the plasminogen molecule responsible for collagen binding. A strong interaction between collagen type IV and the kringles 1–3 and kringle 4 fragments of plasminogen was observed, with minimal binding to mini-plasminogen. However, when plasminogen was the immobilized ligand, addition of up to 100 mM-6-AHA or benzylamine had no effect on the plasminogen–collagen type IV interaction. Similar results were obtained by DePoli *et al.* (1989), who demonstrated that lysine inhibited the interaction between plasminogen and the ECM protein thrombospondin only when the latter was the immobilized ligand and plasminogen was soluble. These authors speculated that immobilization of plasminogen may result in a conformational restriction of the zymogen that would prevent lysine-induced conformational changes that mediate its interaction with thrombospondin. Alternatively, failure of 6-AHA or benzylamine to inhibit binding of collagen type IV to immobilized plasminogen may reflect the lysine-binding-site-independent plasminogen–α1(IV) component of binding observed in Fig. 1.

In addition to blot-binding studies, the interaction of plasminogen with immobilized type IV collagen and gelatin was probed by using e.l.i.s.a. Previous studies on collagen type IV self-assembly with the use of reconstituted collagen films *in vitro* have demonstrated that reconstituted collagen type IV assumes

a structure very similar to intact basement-membrane collagen type IV, indicating that the information for assembly is an integral part of the collagen molecule (Yurchenco & Schnittny, 1990). This suggests that binding of plasminogen to collagen-coated microwells may closely model interactions that occur in the intact basement membrane. Our studies demonstrated that interaction of plasminogen with immobilized collagen and gelatin was strongly inhibited by 6-AHA, but was relatively unaffected by benzylamine. Because both kringles 1–3 and kringle 4 contain binding sites for 6-AHA (Vali & Patthy, 1982; Christensen, 1984), this result supports data obtained in blot-binding assays that indicated strong binding of collagen type IV to both the kringle 1–3 and kringle 4 fragments of plasminogen and suggests that the plasminogen–collagen type IV interaction is primarily lysine-binding-site-dependent. In the light of this result, it is interesting to note that, relative to interstitial collagens (types I and III), basement-membrane collagen (type IV) is particularly lysine-rich (Weiss, 1984). Furthermore, type IV collagen-specific collagenases contain a domain not found in other secreted metalloproteinases that is homologous with the type II structures of the gelatin-binding domains of fibronectin (Collier *et al.*, 1988; Wilhelm *et al.*, 1989). This region of fibronectin has been shown to have structural and functional homology with proteinase kringles (Patthy *et al.*, 1984). In type IV collagenases, the fibronectin-homologue region is believed to be involved in targeting the enzyme to its specific substrate (Collier *et al.*, 1988; Wilhelm *et al.*, 1989, Banyai & Patthy, 1991). On the basis of these observations, it is interesting to speculate that the kringle domains of plasminogen may target the zymogen to the same region of the collagen type IV molecule as is recognized by type IV collagenases. However, the e.l.i.s.a. studies also demonstrated that a fraction of the plasminogen binding to immobilized collagen type IV is not inhibitable by 6-AHA, providing further evidence for the lysine-binding-site-independent interaction demonstrated in Fig. 1. Mini-plasminogen contains a specific benzylamine-binding site on kringle 5 (Varadi & Patthy, 1981; Thewes *et al.*, 1990). Failure of benzylamine to inhibit plasminogen binding significantly in the e.l.i.s.a. is also in agreement with blot-binding data that demonstrated a relatively weak interaction between collagen type IV and mini-plasminogen.

The apparent dissociation constants ($K_{d,app.}$) calculated from the binding data indicate that the strength of plasminogen binding to basement-membrane collagen and gelatin is of the same order of magnitude as the plasminogen–fibrinogen interaction. The $K_{d,app.}$ values calculated for the interaction of plasminogen with type IV collagen and gelatin are also similar to those reported for plasminogen binding to other immobilized matrix protein components. For example, Silverstein *et al.* (1985) reported a K_d of 35 nM for plasminogen binding to thrombospondin whereas a K_d of 91 nM was obtained in studies of plasminogen interaction with fibronectin (Salonen *et al.*, 1985). These data suggest that under conditions that result in exposure of the basement membrane to circulating plasminogen (2 μM in plasma), complete saturation of collagen type IV and gelatin type IV with plasminogen would occur. Collagen-bound plasminogen can be activated by plasma or tissue-associated plasminogen activators, resulting in the formation of plasmin in the basement membrane. Although plasmin itself is a relatively poor collagenase (Liotta *et al.*, 1981), it participates in the activation of pro-matrix metalloproteinases (Nagase *et al.*, 1990) that readily hydrolyse basement-membrane components including type IV collagen. In this respect plasminogen (or plasmin) bound to basement-membrane collagen may be viewed as a reservoir for activation of matrix metalloproteinase zymogens in close proximity to their target substrates.

In summary, we have demonstrated specific binding of plasminogen to type IV collagen and gelatin. The primary interaction involves the lysine-binding sites of plasminogen located within kringles 1–4. A minor lysine-binding-site-independent interaction was also observed. Previous kinetic data indicated that both type IV collagen and gelatin stimulate plasminogen activation catalysed by tissue-associated plasminogen activators at least as effectively as fibrinogen (Stack *et al.*, 1990). Together these data demonstrate that protein components of the extracellular matrix specifically bind plasminogen and regulate its interaction with activator proteins. On the basis of these data, we propose that interaction of plasminogen with ECM proteins such as type IV collagen may represent a fine regulatory mechanism for localized generation of plasmin activity within the basement membrane.

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