

Spatial Regulation and Activity Modulation of Plasmin by High Affinity Binding to the G domain of the α_3 Subunit of Laminin-5*

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Cells in complex tissues contact extracellular matrix that interacts with integrin receptors to influence gene expression, proliferation, apoptosis, adhesion, and motility. During development, tissue remodeling, and tumorigenesis, matrix components are modified by enzymatic digestion with subsequent effects on integrin binding and signaling. We are interested in understanding the mechanisms by which broad spectrum proteinases such as plasmin are targeted to their extracellular matrix protein substrates. We have utilized plasmin-mediated cleavage of the epithelial basement membrane glycoprotein laminin-5 as a model to evaluate molecular events that direct plasmin activity to specific structural domains. We report that plasminogen and tissue plasminogen activator (tPA) exhibit high affinity, specific binding to the G₁ subdomain of the N terminus of the laminin-5 α_3 subunit, with equilibrium dissociation constants of 50 nM for plasminogen and 80 nM for tPA. No high affinity binding to the G₂, G₃, and G₄ subdomains was observed. As a result of binding to the G₁ subdomain, the catalytic efficiency of tPA-catalyzed plasminogen activation is enhanced 32-fold, leading to increased matrix-associated plasmin that is positioned favorably for cleavage within the G₄ subdomain as we have reported previously (Goldfinger, L. E., Stack, M. S., and Jones, J. C. R. (1998) *J. Cell Biol.* 141, 255–265). Thus, physical constraints dictated by interaction of proteinase and matrix macromolecule control not only enzymatic activity but may regulate substrate targeting of proteinases.

Cells in epithelial tissues are in contact with an array of extracellular matrix (ECM)¹ molecules. Through interaction with cell surface receptors, ECM proteins have a profound influence on gene expression as well as proliferation, adhesion, and motility (see for example, Refs. 1–4). During development,

tissue remodeling, and tumorigenesis, protein components of the ECM are often modified by enzymatic digestion (4–9). Proteolyzed ECM components may bind different cell surface receptors than their intact counterparts and thereby trigger alternative signaling events (10). We have utilized plasmin-mediated cleavage of the epithelial basement membrane glycoprotein laminin-5 as a model to evaluate molecular events that direct plasmin activity to specific structural domains.

Laminin-5 secreted by epithelial cells is a heterotrimeric protein with an $\alpha_3\beta_3\gamma_2$ subunit composition and promotes epithelial cell migration (11, 12). However, we have previously demonstrated that proteolytic processing of the 190-kDa α_3 subunit within the C-terminal globular (G) domain renders laminin-5 competent to induce formation of hemidesmosomes, cell matrix anchorage structures formed in part via binding of laminin-5 to the $\alpha_6\beta_4$ integrin (Fig. 1) (13, 14). As a consequence of hemidesmosome formation, cellular motility is significantly reduced. In cultured cells, this functional and structural modification of the laminin-5 α_3 subunit occurs as a result of limited plasmin proteolysis within the G₄ subdomain and requires tissue plasminogen activator (tPA)-catalyzed plasminogen (Pg) activation (Fig. 1) (14).

Both Pg and tPA co-localize with laminin-5 in the ECM of epithelial cells and bind to intact laminin-5 *in vitro* (14). Further, previous studies with the laminin-1 isoform demonstrated that Pg and tPA binding is localized to the G domain at the C terminus of the laminin α_1 subunit (15). Because these data suggest that the G domain of laminin isoforms may function in proteinase targeting, we have evaluated the interaction of Pg and tPA with the G domain of the α_3 subunit of laminin-5. Our results demonstrate high affinity binding of both Pg and tPA to the G₁ subdomain of the laminin-5 α_3 subunit. As a functional consequence of G₁ binding, the catalytic efficiency of Pg activation is enhanced, resulting in increased plasmin activity. These data suggest that binding of Pg and tPA to the α_3 G₁ subdomain may function in part to focus cleavage site specificity by positioning Pg and tPA in close proximity to the processing site within the G₄ subdomain (14).

MATERIALS AND METHODS

Chemicals, Proteins, and Cloning of α_3 Laminin G Domain Fragments—Synthetic D-Val-Leu-Lys-p-nitroanilide (VLK-pNA) was purchased from Helena Laboratories (Beaumont, TX). The lysine analog ϵ -amino caproic acid (EACA) was purchased from Sigma. Two-chain recombinant tPA was the generous gift of Dr. Henry Berger (Wellcome Research Laboratories, Research Triangle Park, NC). Pg was purified from human plasma by affinity chromatography on L-lysine-Sepharose. Purified Pg and tPA were biotinylated by co-incubation with 1 mg/ml N-hydroxy succinimide biotin at room temperature (Sigma), followed by dialysis against PBS for 12 h. Biotinylation of the proteins was confirmed by processing derivatized polypeptides for SDS-PAGE, followed by transfer to nitrocellulose and Western blotting using a streptavidin-HRP probe (Life Technologies, Inc., Rockville, MD; see below).

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¹ The abbreviations used are: ECM, extracellular matrix; tPA, tissue plasminogen activator; Pg, plasminogen; VLK-pNA, D-Val-Leu-Lys-p-nitroanilide; EACA, ϵ -amino caproic acid; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; HRP, horseradish peroxidase; BSA, bovine serum albumin.

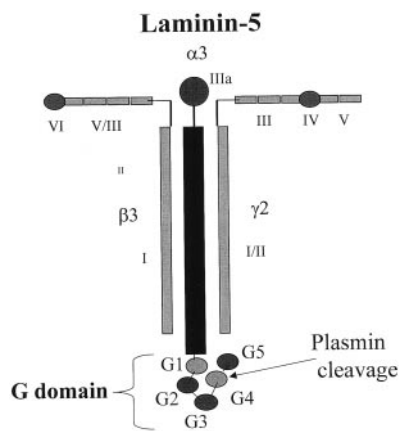


FIG. 1. Schematic diagram of a laminin-5 heterotrimer. Laminin-5 is a cross-shaped structure composed of α_3 , β_3 , and γ_2 subunits (11, 13). The C-terminal portion of the α_3 subunit forms a compact globular domain, called the G domain, which is divided into subdomains G₁–G₅ (16). The putative plasmin cleavage site in the G₄ subdomain, identified by Goldfinger *et al.* (14), is indicated by an arrow.

Laminin-5 was a generous gift of Desmos Inc. (San Diego, CA). For generation of recombinant human laminin α_3 subunit G domain and the G₁, G₂, G₃, and G₄ subdomain proteins, cDNA fragments of 3000, 633, 507, 657, and 553 base pairs, encoding residues 2229–5229, 2374–2907, 2910–3417, 3414–4051, and 4045–4598, respectively, were subcloned by reverse transcription-polymerase chain reaction from mRNA prepared from MCF-10A cells (16). Each of these cDNAs was inserted into either a pET32 or a pBAD-TOPO cloning vector upstream of a sequence encoding six His residues (Novagen, Inc., Madison, WI and Invitrogen, Carlsbad, CA). DNA was transfected into BL21(DE3) or LMG bacteria, and cells containing vector with insert were selected with ampicillin antibiotic. Expression of recombinant protein was induced with either isopropylthio- β -D-galactoside or arabinose. To confirm expression of the appropriate sized recombinant protein, cells were suspended in gel sample buffer. Protein samples were subjected to SDS-PAGE and processed for Western blotting by standard procedures (see below). Recombinant proteins were identified using a His-HRP probe (Pierce, Rockford, IL). To prepare protein from the bacteria, cells were extracted in a buffer containing 40 mM imidazole, 4 M NaCl, and 160 mM Tris-HCl, pH 7.9. The His-tagged proteins were then purified from the cell extracts over a Ni²⁺-Sepharose column according to the instructions of the manufacturer (Novagen). Protein concentrations were determined by standard Bradford assay (Bio-Rad, Hercules, CA) (17).

SDS-PAGE, Gel Staining, Western Blots, and Ligand Blots—Purified laminin-5 and recombinant G domain and subdomain proteins were solubilized in 8 M urea, 1% SDS sample buffer in 10 mM Tris-HCl, pH 6.8, with 15% β -mercaptoethanol. Samples were processed for SDS-PAGE on 6–12% acrylamide gels (18). For silver staining, gels were fixed in 40% methanol and 10% acetic acid followed by secondary fix in a solution of 10% ethanol, 5% acetic acid. Proteins were oxidized and silver stained using reagents from Bio-Rad, according to the instructions of the manufacturer. In addition, separated proteins were transferred to nitrocellulose for Western blotting by standard procedures (19).

For ligand blotting, protein was spotted onto nitrocellulose, and the spot was then allowed to dry. The nitrocellulose was treated with a blocking buffer (0.1% Tween, 0.9% NaCl, 1 mM MgCl₂, 1 mM dithiothreitol, 0.2% fish gelatin, 0.2% phenylmethylsulfonyl fluoride, in 20 mM Tris, pH 7.4) for 2 h and then incubated overnight at 4 °C with biotinylated Pg diluted in the same buffer. After extensive washing the nitrocellulose was incubated in a streptavidin-HRP conjugate, bound proteins were detected with ECL chemiluminescent substrate (Amersham Pharmacia Biotech, Piscataway, NJ), and images were captured on X-Omat Imaging Film (Eastman Kodak Co., Rochester, NY).

Binding Assay by Surface Plasmon Resonance Biosensor—Protein-protein interactions and equilibrium binding constants were studied by surface plasmon resonance using an IAsys evanescent wave biosensor (Affinity Sensor, Paramus, NJ). We used either biotin-coated cuvettes or aminosaline cuvettes, provided by the manufacturer. Streptavidin (100 μ g/ml; Sigma) in PBS-0.05%Tween (PBST) was added to the biotin-coated cuvette, and subsequently, either biotinylated Pg (20 μ g/ml) or biotinylated tPA (200 μ g/ml) in PBST was

immobilized on the cuvette surface by incubation for approximately 20 min at room temperature. In the case of aminosaline cuvettes, the cuvette was first washed with 10 mM HCl for 5 min. After extensive washing with PBS, laminin-5, recombinant G domain, and subdomains were suspended in PBS at a concentration of 200 μ g/ml, added to the cuvette, and incubated for 30 min at room temperature. The cuvettes were washed with PBS to remove unbound protein. In all cases, refraction of the biosensor laser was used as a measure of the affinity of binding of soluble polypeptides incubated at increasing concentrations in the cuvette coated with the immobilized protein under test. All binding experiments were evaluated by nonlinear regression analysis. All curves fit the equation for a rectangular hyperbola with a correlation coefficient of 0.97 or greater.

Pg Activation Rate—Purified laminin-5, recombinant G domain, and G subdomain proteins were coated at 5 μ g/ml in 20 mM HEPES pH 7.4 buffer onto the surfaces of wells of a 96-well plate (Sarsdedt, Arlington Heights, IL) at 4 °C for 14 h. Wells were blocked with 1% BSA for 1 h at 37 °C and then washed with 20 mM HEPES. The effect of laminin-5 and recombinant fragments of laminin-5 on the rate of Pg activation was determined by a coupled reaction in which the amidolytic activity of generated plasmin was monitored colorimetrically. Pg (0–0.3 μ M) was incubated in coated wells for 15 min at 37 °C, followed by addition of 0.3 mM of the plasmin substrate VLK-pNA at 37 °C in a total volume of 175 μ l. Activation of Pg was initiated by the addition of 0.55 nM tPA (20 IU/ml). Hydrolysis of VLK-pNA by the resulting plasmin was recorded as a change in absorbance at 405 nm over time using a Molecular Devices Thermomax microtiter plate reader (Sunnyvale, CA). Kinetic constants were extrapolated from the data by nonlinear regression analysis using Sigmaplot (SPSS Inc., Richmond, CA).

RESULTS

High Affinity Interaction of Pg and tPA with Laminin-5—Pg and tPA are co-localized with laminin-5 in the extracellular matrix of many epithelial cells, and the presence of both zymogen and activator are necessary for limited plasmin proteolysis of laminin-5 and subsequent hemidesmosome assembly (14). Moreover, both purified Pg and tPA have been shown to interact with laminin-5 in a ligand blot assay (14). Because plasmin is a broad spectrum proteinase, these results imply that targeting of cleavage to a distinct domain of the laminin-5 substrate may be regulated by specific protein-protein interactions that position plasmin in close proximity to its cleavage site. To test this hypothesis, the affinity of Pg and tPA binding to laminin-5 was quantified by surface plasmon resonance in an evanescent wave biosensor. Purified laminin-5 was added stepwise in increasing concentrations (0–150 nM) to immobilized Pg or tPA, and the degree of refraction of a light beam crossing the surface of the immobilized protein was used to measure the affinity of protein-protein interaction (20, 21). Laminin-5 binds to immobilized Pg and tPA in a concentration-dependent, saturable fashion with dissociation constants (K_d) of 40 and 35 nM, respectively (Fig. 2, A and B). In control experiments, laminin-5 shows no obvious specific binding to biotinylated control proteins, including biotinylated BSA (Fig. 2C) and biotinylated lactoperoxidase (not shown). To confirm the bindings are at equilibrium, in control experiments, Pg and tPA were coated at approximately half-saturation, and binding of laminin-5 was evaluated as described above. Although the plateau of laminin-5 binding was reduced, the overall K_d for binding was unchanged (not shown). Additional controls demonstrated that the binding of laminin-5 with Pg was rapid and reversible (Fig. 3). To mimic more accurately binding interactions likely to occur in the environment *in vivo*, laminin-5 was passively absorbed onto aminosaline cuvettes and tPA or Pg binding was evaluated as described above. These data confirmed concentration-dependent, saturable binding between Pg or tPA and laminin-5 with dissociation constants (K_d) of 25 and 40 nM, respectively (Fig. 2, D and E). Soluble uPA failed to bind immobilized laminin-5, confirming the specificity of tPA and Pg binding to laminin-5 (Fig. 2F).

Binding Site Localization of Pg and tPA on the α_3 Subunit

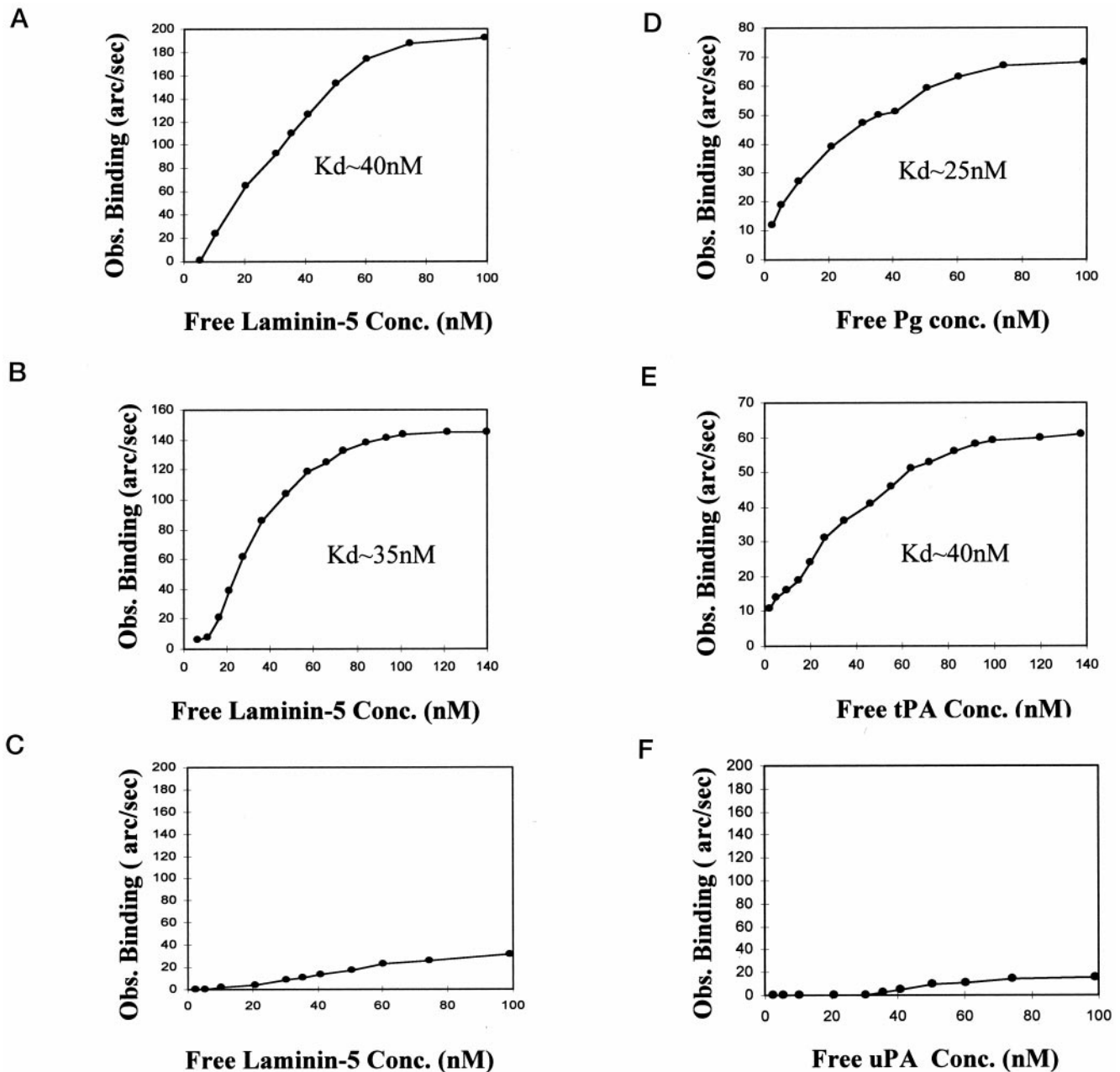


FIG. 2. Pg and tPA bind to laminin-5. Biotinylated Pg (A), tPA (B), or BSA (C) was immobilized on the surface of streptavidin-coated cuvettes in saturating amounts. Soluble laminin-5 was added at concentrations (Conc.) in the range of 0–150 nM. In the reverse experiments (D–F), binding of Pg (D), tPA (E), or uPA (F) to immobilized laminin-5 was evaluated. In this and all subsequent protein binding assays, the level of binding was measured by surface plasmon resonance in an IAsys evanescent wave biosensor. K_d values were calculated by determining the concentration of protein required for half-maximal binding at equilibrium and are indicated in the figure.

of Laminin-5—To investigate whether Pg and tPA binding sites are localized proximal to the plasmin cleavage site in the G domain of the α_3 subunit of the laminin-5 molecule (Fig. 1), binding to recombinant G domain and subdomain polypeptides was also evaluated. Sequences corresponding to the entire laminin-5 G domain or the individual G_1 , G_2 , G_3 , and G_4 subdomains were generated from MCF-10A cell mRNA by reverse transcription-polymerase chain reaction and then cloned into bacteria expression vectors. The latter were transfected into bacterial cells and recombinant proteins with a C-terminal His₆ tag were purified from cell lysates over a Ni²⁺-Sepharose column. Protein recovery was typically approximately 1 mg of purified protein/liter of induced bacterial culture. Purity of recombinant proteins was

confirmed by silver staining following separation by SDS-PAGE (Fig. 4). These same polypeptides are recognized by a His-HRP probe (results not shown). Surface plasmon resonance binding experiments were then performed with the recombinant α_3 subdomain polypeptides. Similar to intact laminin-5, both Pg and tPA bound to immobilized α_3 laminin G domain in a concentration-dependent and saturable manner ($K_d = 12$ and 10 nM, respectively; Fig. 5). In the reverse experiment, recombinant G domain bound to immobilized Pg or tPA with similar affinities ($K_d = 2$ and 4 nM, respectively) (not shown). Similar K_d values were obtained when Pg or tPA were immobilized at half-saturation concentration (not shown).

Because these results demonstrated high affinity of Pg and

tPA binding to the α_3 G domain, interaction specificity was further delineated using recombinant G₁, G₂, G₃, and G₄ subdomain polypeptides. Pg and tPA bind to immobilized G₁ with a K_d of 50 and 80 nM, respectively (Fig. 6, A and B). Conversely, when tPA and Pg are immobilized at saturated or half-saturated level, the respective K_d (25 and 40 nM for Pg and tPA, respectively) for the binding of purified G₁ to these proteins are comparable (not shown). Pg and tPA showed little binding to immobilized G₄ (Fig. 6, C and D). In additional experiments, no binding of Pg or tPA to purified recombinant G₂ or G₃ subdomain proteins was observed (not shown).

Pg has been shown to bind to matrix molecules via interaction with lysine residues (22). To determine whether Pg interacts with the G₁ subdomain in a lysine-dependent manner, G₁ subdomain protein and, as a control, G₄ subdomain protein were spotted onto nitrocellulose at 1 μ g/ml and incubated with purified biotinylated Pg at 10 μ g/ml. Pg bound G₁ but not G₄ protein, consistent with results presented above. The lysine analog, EACA, inhibited Pg binding to the G₁ protein (Fig. 7A). These results were confirmed using surface plasmon resonance, where binding interactions between laminin-5, G domain, or G₁ subdomain with Pg were effectively blocked by EACA (Fig. 7B).

Pg Activation in the Presence of Recombinant G Domain Proteins—Specific localization of Pg and tPA to the G₁ subdomain of laminin-5 may direct subsequent proteolysis within the G₄ subdomain (14). In addition to substrate targeting, protein-

ase binding to immobilized matrix may also influence zymogen activation kinetics (23). This is supported by the observation that both Pg and tPA bind to the same α_3 G subdomain. To evaluate the effect of laminin-5 binding on Pg activation kinetics, tPA catalyzed Pg activation was evaluated in the presence of immobilized G domain or subdomain polypeptides. Michaelis-Menten curves for the activation reactions are shown in Fig. 8, and kinetic constants are summarized in Table I. Whereas activation on G₂, G₃, or G₄ subdomain coated surfaces was similar to that observed in solution, activation on G₁ was significantly enhanced (by a 32 folds increase in K_{cat}/K_m). These data suggest that colocalization of Pg and tPA to laminin-5 containing matrix surfaces via interaction with the α_3 G₁ subdomain may result in increased concentration of plasmin properly positioned laminin-5 processing.

DISCUSSION

Laminin G domains contain binding sites for cells and other ECM molecules and thus contribute significantly to laminin function. The G domains of both the α_1 and α_2 laminin subunits have high affinity binding sites for a variety of extracellular matrix molecules such as perlecan, heparin, and sulfatides, as well as for cell surface receptors including α -dystroglycan (24–26). Fibulin-1 and -2 and nidogen-2 also bind to the laminin α_2 G domain but with somewhat lower affinity (26). In the case of

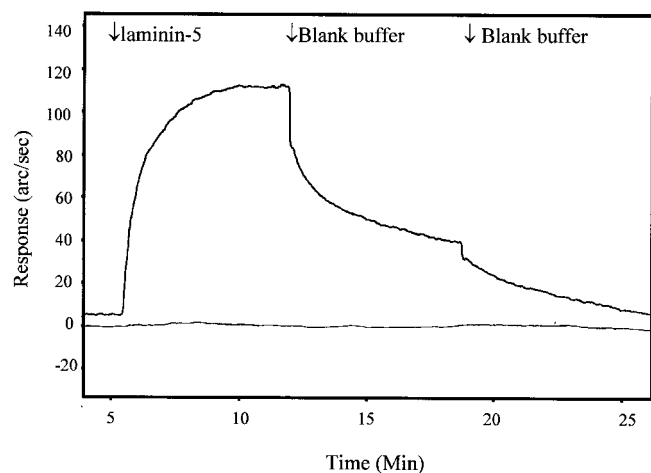


FIG. 3. **Kinetics and reversibility of laminin-5 and Pg interaction.** Binding of laminin-5 to immobilized Pg was initiated by the addition of 400 nM laminin-5 into the cuvettes, and the binding was monitored using surface plasmon resonance. Dissociation was induced by replacing laminin-5 solution with blank buffer.

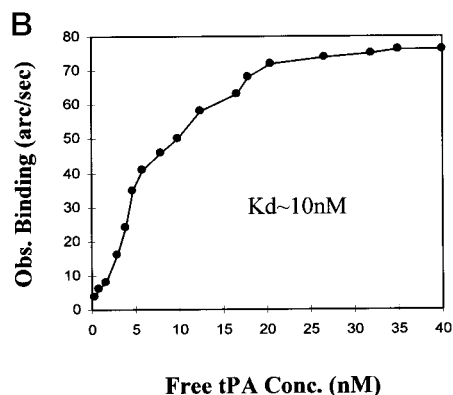
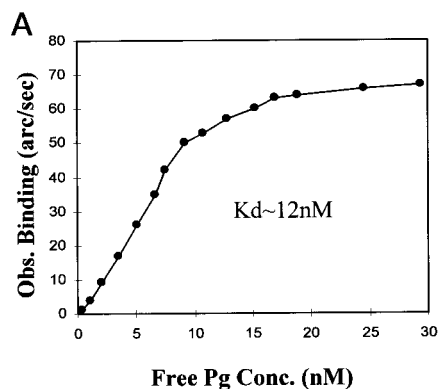


FIG. 5. **Pg and tPA bind to the G domain of the α_3 subunit of laminin-5.** Pg or tPA (0–50 nM) was added to immobilized recombinant α_3 G domain protein and binding evaluated by surface plasmon resonance as described under “Materials and Methods.”

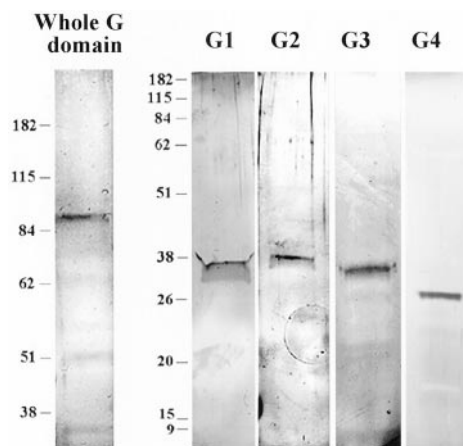


FIG. 4. **Purity of recombinant G domain proteins.** Recombinant His-tagged human laminin α_3 subunit G domain and the G₁, G₂, G₃, and G₄ subdomain proteins were purified over a Ni²⁺-Sepharose column following expression in bacteria. Samples of the pure protein fractions were solubilized in gel sample buffer and subjected to SDS-PAGE. Gels were subsequently fixed and stained with silver reagent. Recombinant whole G domain and G₁, G₂, G₃, and G₄ subdomain proteins migrate at 95, 35, 38, 30, and 28 kDa, respectively. Molecular markers are indicated to the left.

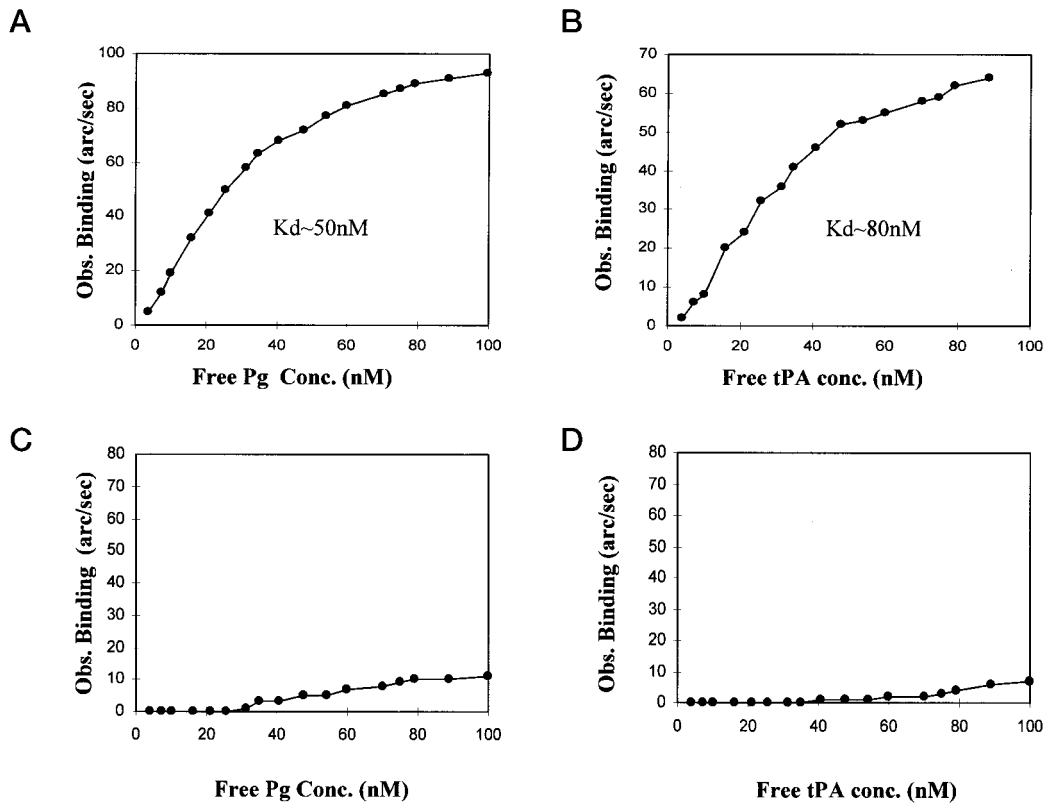


FIG. 6. Pg and tPA bind to the G₁ subdomain of the α_3 subunit of laminin-5. Pg or tPA (0–100 nM) was added to immobilized recombinant α_3 G₁ (A and B) or G₄ (C and D) subdomain protein and binding evaluated by surface plasmon resonance as described under “Materials and Methods.”

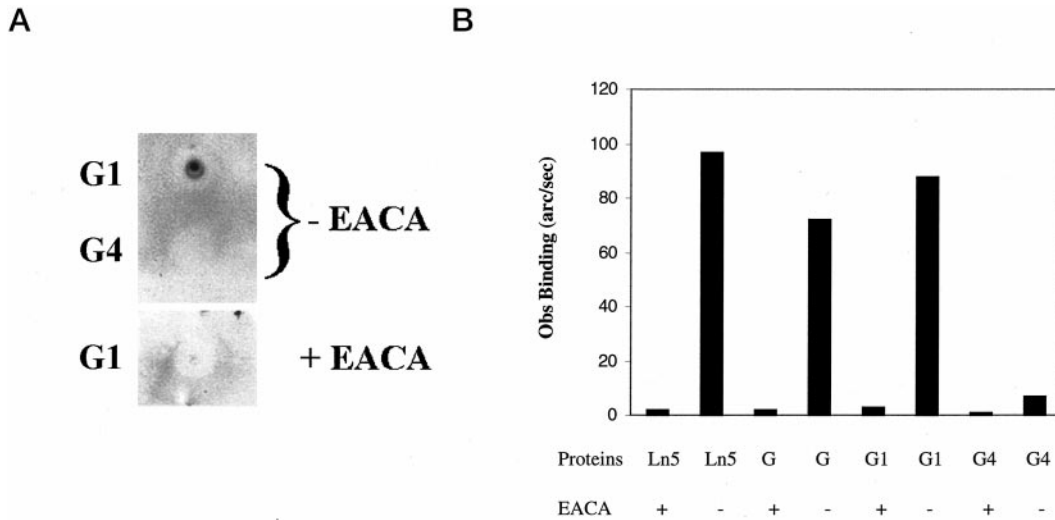


FIG. 7. Pg binding to the G₁ subdomain of the laminin-5 α_3 subunit is lysine-dependent. A, recombinant G₁ or G₄ subdomain (1 μ g/ml) protein was spotted onto nitrocellulose and incubated with biotinylated Pg in the presence or absence of the lysine analog ϵ -amino caproic acid as indicated. Bound Pg was detected with streptavidin-HRP. B, effect of EACA on binding of Pg to immobilized laminin-5 and its α_3 subdomains. Binding of Pg (400 nM) to immobilized laminin-5, recombinant α_3 G, G₁, or G₄ polypeptides in the presence or absence of EACA (50 mM, as indicated) was evaluated as described by surface plasmon resonance as described under “Materials and Methods.”

laminin-5, the $\alpha_3\beta_1$ integrin binding site has been reported to lie within the G₂ subdomain, whereas laminin-5 apparently interacts with heparin and other macromolecules via its G₄ and G₅ subdomains (27). In this study we have demonstrated that both Pg and tPA bind specifically and with high affinity to the G₁ subdomain, the first proximal region of the laminin-5 α_3 chain G domain. The functional significance of these interactions is apparent, because we have previously shown that plas-

min cleavage of laminin-5 has a profound impact on epithelial cell behavior (14).

The G₁ subdomain of the laminin α_3 subunit shows little homology with the corresponding domains of the α_1 and α_2 laminin subunits (16, 28, 29), and in previous studies no binding of Pg or tPA to the α_1 G₁ subdomain was observed (15). Indeed, Pg and tPA appear to bind the laminin α_1 G₄ domain, whereas we detect no such binding to the analogous domain of

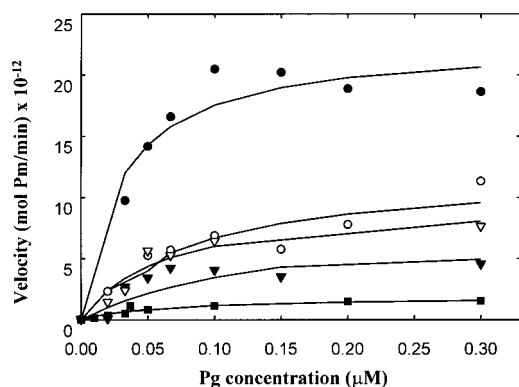


FIG. 8. Effect of laminin-5 α_3 G subdomain fragments on tPA-catalyzed Pg activation kinetics. Micro-titer wells coated with recombinant G₁, G₂, G₃, or G₄ subdomain proteins were preincubated with Pg (0–0.3 μ M) 15 min at 37 °C followed by addition of VLK-pNA (0.3 mM) and tPA (0.55 nM). The resulting hydrolysis of VLK-pNA by plasmin was monitored at 405 nm. Control reactions were performed on wells coated with albumin to correct for nonspecific effects of protein-protein interactions. Activation velocity (mol plasmin generated/min) on surfaces coated with recombinant subdomain proteins or albumin are indicated as follows: G₁ (●), G₂ (○), G₃ (▼), G₄ (▽), and albumin (■). Lines drawn represent predicted fit of the data points to a rectangular hyperbola. Kinetic constants were calculated by nonlinear regression analysis and are summarized in Table I.

TABLE I
Kinetic parameters of Pg activation by tPA in the presence of recombinant laminin-5 subdomain fragments

Kinetic constants were determined from the initial velocity data shown in Fig. 8 using nonlinear regression analysis. Fold change in catalytic efficiency (K_{cat}/K_m) is expressed relative to control reactions on albumin-coated wells.

α_3 subdomain fragment	K_m μ M	K_{cat} s^{-1}	K_{cat}/K_m μ M s^{-1}	Fold change in K_{cat}/K_m
G1	0.029	3.93	135.5	32.3
G2	0.082	2.11	25.7	6.1
G3	0.241	2.04	8.5	2.0
G4	0.134	2.72	20.3	4.8
BSA	0.09	0.38	4.2	1

the α_3 laminin subunit (15). The G₁ subdomain of the laminin α_3 subunit shows 58% similarity with that of the laminin α_4 chain and 53% similarity to that of the human laminin α_5 chain (16, 30–32), raising the possibility that the α subunit G₁ domains of other laminin isoforms may also bind Pg and tPA. Further, the G₄ subdomain of the human α_3 chain, which contains the plasmin cleavage site we have identified previously, has 53% similarity to that of the human α_4 laminin chain, suggesting that the α_4 chain may also be subject to plasmin proteolytic processing (14, 16, 30).

Our data support the idea that Pg binds the laminin α_3 subunit G₁ subdomain in a lysine-dependent manner. Lysine binding site-dependent association of Pg with fibrin or other matrix molecules induces a dramatic conformational change in Pg, resulting in a 15 Å increase in its Stoke's radius. As a result of this conformational change, the Arg⁵⁶¹-Val⁵⁶² peptide bond is more readily accessible to tPA, thus enhancing the catalytic efficiency of the activation (23, 33, 34). In the current study, a significant increase in k_{cat}/K_m is observed in following Pg and tPA interaction with either intact G domain or G₁ subdomain. Taken together, our data suggest a model for the regulation of plasmin cleavage of laminin-5. High affinity association of Pg and tPA with the G₁ subdomain of intact laminin-5 enhances the catalytic efficiency of tPA-catalyzed Pg activation, resulting in generation of plasmin. Because recent data suggest a close

association of the G₁ and G₄ subdomains in intact laminin, the newly generated plasmin is thus properly positioned for proteolytic cleavage within the G₄ subdomain (Fig. 1) (14, 35). Interestingly, there is a precedent for this because it has been shown previously that in endothelial cells, Pg and tPA bind the annexin II tetramer, a calcium- and phospholipid-binding protein, with high affinity (36, 37). Moreover, the formation of the Pg, tPA, and annexin II complex facilitates tPA-dependent conversion of Pg to plasmin (38–40).

Unprocessed laminin-5 in the extracellular matrix promotes migration of epithelial cells, whereas plasmin-cleaved laminin-5 blocks cell migration by inducing formation of hemidesmosomes, which anchor the cells to the cleaved laminin-5 substrate (14). Therefore, the ability of cells to efficiently generate plasmin and to specifically localize the enzyme to a distinct subdomain of the 460-kDa macromolecular laminin-5 substrate may represent key regulatory steps that control the divergent cell processes of migration and adhesion (14).

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