Laminin interacts with plasminogen and its tissue-type activator

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1. INTRODUCTION

Laminin (M_r 900000) and fibronectin (M_r 440000) are noncollagenous glycoproteins of the extracellular matrix, both known to promote cell adhesion and to bind to heparin and related sulfated glycosaminoglycans [1,2]. Fibronectin is characterized by its multiple additional interactions such as those with *Staphylococcus aureus* [3], fibrin [4], collagens and gelatin [5] and C-reactive protein [6]. Fibronectin, which unlike laminin also occurs in plasma and other body fluids [2,4], is well known for its high susceptibility to various proteinases [7,8], while laminin has been found to be relatively resistant [7,9,10]. We here report that immobilized laminin binds plasminogen and its tissue-type activator; these interactions may have significance in the proteolytic cleavage of laminin in vivo.

2. MATERIALS AND METHODS

2.1. *Materials*

Laminin was purified from EHS mouse tumor, rich in basement membrane material as in [1,11]. Plasminogen prepared from Cohn supernatant I fraction of human plasma was from Kabi (Stockholm) and contained 20 CU/mg. t-PA purified from culture medium of Bowes human melanoma cells [12] was a gift from Dr Desiré Collen and contained 62.5 μg/ml (2566 PU/ml). u-PA was purified using benzamidine–Sepharose [13] from a commercial preparation (Calbiochem, La Jolla, CA). Human serum albumin was from Kabi. The following rabbit antisera were used: anti-laminin [14], anti-plasminogen (Dako, Copenhagen), anti-t-PA (a gift from Dr D. Collen) and anti-u-PA prepared as in [15] using benzamidine–Sepharose purified u-PA as antigen. Dow-Latex particles (diameter, 1.1 μm) were from Serva (Heidelberg, FRG).

Abbreviations: BSA, bovine serum albumin; PBS, 0.01 M phosphate-buffered 0.15 M saline, pH 7.4; PU, Plough unit(s); SDS–PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type PA; Tween, polyoxyethylene sorbitane monolaureate 20

2.2. *Methods*

Polystyrene microtiter wells (immobilization area 0.65 cm^2; Nunc, Roskilde, Denmark) or tubes (1.73 cm^2; Labsystems, Helsinki) were coated from a solution of 2 μg/ml protein in PBS using an overnight incubation at room temperature. Radioiodination of protein was performed as in [16] using ^{125}I (Amersham) in a modification of the chloramine-T method. The spec. act. was 2.5 μCi/μg. In the binding experiments coated wells (or tubes) received 75 μl (or 200 μl) of the protein solution diluted in PBS-Tween supplemented with 4% (w/v) polyethylene 6000 (Fluka, Buchs, Switzerland) known to speed up solid-phase protein–protein interactions [17]. The incubation was for 120 min at room temperature and was followed by repeated washes with PBS-

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Tween and H_2O. Enzyme immunoassays with predetermined dilutions of the specific rabbit antisera, and alkaline-phosphatase labeled swine anti-rabbit IgG (Orion Diagnostica, Espoo, Finland) were as in [6].

For SDS–PAGE we used polyacrylamide gradient (5–16%) slab gels under nonreducing conditions as in [18] using autoradiography for ^{125}I-labeled samples. Coated latex particles were prepared by incubating 500 μl of 2% (w/v) particles with an equal volume of 100 μg/ml of protein solution in 0.1 M Na–glycine-buffered 0.15 M saline (pH 8.2) for 2 h at 37°C and centrifugation and washing as in [6].

3. RESULTS AND DISCUSSION

Immobilization of laminin onto polystyrene surface was quantitated using ^{125}I-labeled laminin. A large proportion of the laminin added was immobilized, e.g., from 10 μg in 200 μl 86% bound and from 3.2 μg 30%; a coating concentration of 400 ng/200 μl was chosen, from which 270 ng (0.30 pmol, 67%) bound per 1.73 cm^2 surface area in each tube, for all subsequent experiments.

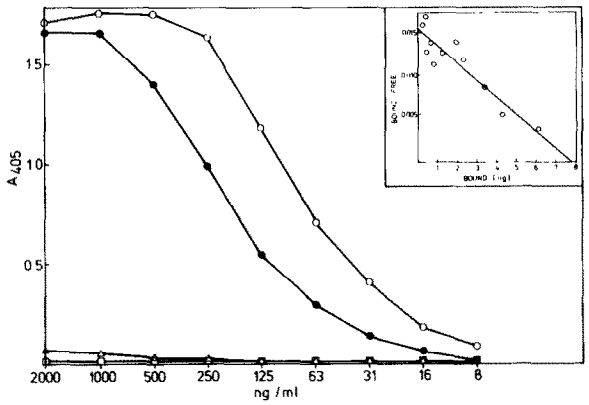

Fig.1. Interaction between immobilized laminin and soluble plasminogen. Polystyrene wells containing 270 ng laminin per 1.73 cm^2 received different concentrations of unlabeled (○) or ^{125}I-labeled (●) plasminogen for 2 h at room temperature. The bound plasminogen was determined using radioactivity for ^{125}I-labeled plasminogen and EIA for both labeled and unlabeled plasminogen. As a control, wells coated with human serum albumin (△) or uncoated wells (□) were used in similar binding experiments. Different amounts of [^{125}I]plasminogen (8–2000 ng/ml) were incubated with a constant amount (270 ng/1.73 cm^2) of laminin coated to the polystyrene surface. The inset shows Scatchard plot of the binding of [^{125}I]plasminogen to solid-phase laminin.

Soluble plasminogen added was found to bind to the immobilized laminin. This binding was dose-dependent and was demonstrated using both [^{125}I]plasminogen, unlabeled plasminogen and enzyme immunoassay (fig.1). The dissociation constant (K_d) (n = 9) for the labeled plasminogen using Scatchard plot analysis (fig.1, inset) was 2.8 × 10^{-8} M. Values calculated from plots of $1/A$ *vs* $1/c$ (A, absorbance value at 405 nm; c, concentration) for labeled and unlabeled plasminogens (see fig.1) were K_d = 6.7 × 10^{-8} M and K_d = 3.0 × 10^{-9} M, respectively. The difference in these values indicates that the radioiodination procedure had affected the binding or antigenic properties of plasminogen. Maximally, each mol of immobilized laminin bound 12.7 mol [^{125}I]plasminogen. What is not shown in fig.1, is that if >500 ng laminin was immobilized per tube, the binding of plasminogen was much lower, suggesting that the higher density of laminin molecules causes steric hindrance.

The interaction between laminin and plasminogen was also demonstrated using Latex agglutination tests as documented in fig.2. The agglutination of plasminogen-coated Latex particles by laminin shows that it has at least 2 binding sites for plasminogen per molecule.

Of the two types of plasminogen activators used, t-PA but not u-PA was bound by immobilized

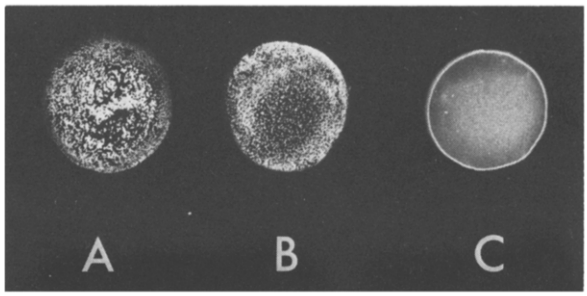

Fig.2. Binding of laminin to plasminogen-coated Latex particles. A volume of 10 μl laminin solution containing 1.25 μg (A) or 300 ng (B) PBS–Tween buffer was incubated with an equal volume of plasminogen-coated Latex particles and photographed within 5 s. As a control, 10 μl laminin (5 μg) was incubated with 10 μl BSA-coated Latex particles and photographed (C). Magnification × 5.

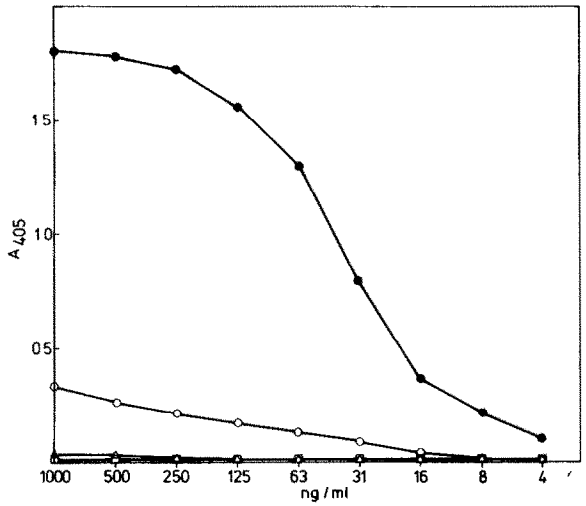

Fig.3. Interaction between immobilized laminin and soluble plasminogen activators. Conditions identical to those in fig.1 were employed to study the binding of t-PA (●) and u-PA (○), which were determined using EIA. As a control, wells coated with albumin (△) or uncoated wells (□) were used.

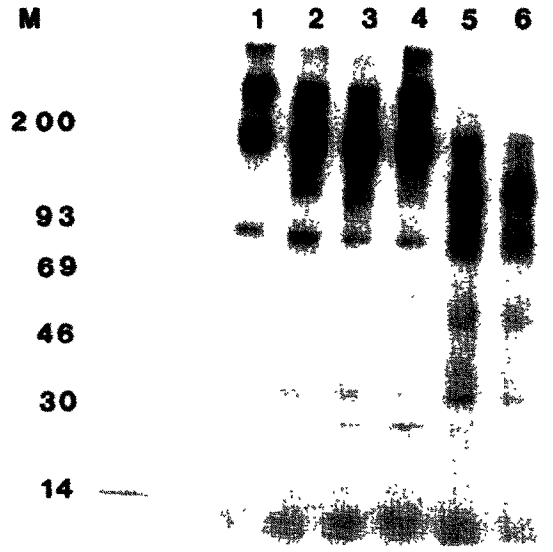

Fig.4. Cleavage of [^{125}I]laminin by plasminogen activated with PA. The indicated mixtures in PBS were incubated in an end-over-end mixer at 37°C for 48 h and then analyzed by SDS–PAGE under reducing conditions followed by autoradiography. Lane 1, [^{125}I]laminin (20 μg); lane 2, [^{125}I]laminin (20 μg) + plasminogen (5 μg); lane 3, [^{125}I]laminin (20 μg) + u-PA (20 mPU); lane 4, [^{125}I]laminin (20 μg) + t-PA (20 mPU); lane 5, [^{125}I]laminin (20 μg) + plasminogen (5 μg) + u-PA (20 mPU); lane 6, [^{125}I]laminin (20 μg) + plasminogen (5 μg) + t-PA (20 mPU). M shows the molecular masses of ^{14}C-labeled marker proteins in kDa.

laminin (fig.3). The binding of both plasminogen and t-PA occurred both in the presence of 0.1% Tween and in the presence and absence of polyethylene glycol. No binding to uncoated or albumin-coated surfaces was found. Fig.4 shows that laminin is cleaved in the presence of plasminogen and either type of activator but not in the presence of plasminogen or the activators alone.

The observed interactions between plasminogen, t-PA and immobilized laminin and the cleavage of laminin by activated plasmin suggest a mechanism for its regulated proteolysis. It should be noted that under the conditions used the cleavage of laminin was relatively slow if compared to that of fibronectin. Nevertheless, it seems that laminin does not inhibit t-PA from activating plasminogen.

Neuroblastoma cells are known to produce both laminin [19] and PA [20], which at least in some cell lines is t-PA (unpublished). Interestingly, cell-associated PA of tumorigenic rat neuroblastoma cells was suppressed by substratum-attached matrix material of nontumorigenic neuroblastoma cells with little or no PA activity [20].

We have no explanation for the differential binding between the two activators t-PA and u-PA to laminin. They are distinct entities with differences in antigenic and molecular properties [12]. A striking difference of possible relevance for our observations is that t-PA but not u-PA binds to fibrin, which greatly enhances its ability to cleave plasminogen [21]. It remains to be determined whether the binding of t-PA to laminin has any relationship to its binding to fibrin.

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