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Binding of plasminogen to *Escherichia coli* adhesion proteins

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Immobilization of plasminogen via its lysine-binding sites is regarded as a prerequisite for its activation and function in fibrinolysis and pericellular proteolysis. In the present study, the interaction of plasminogen with fimbriae found on *Escherichia coli* strains causing invasive human infections was studied. Plasminogen displayed concentration-dependent and saturable binding to immobilized type 1 fimbriae and, several fold lower binding to P and S fimbriae. The binding to fimbriae was effectively inhibited by ε-aminocaproic acid indicating that it was mediated by the lysine-binding sites of plasminogen. Binding studies with mutated fimbriae and inhibition tests indicated that the interaction was not dependent on the lectin subunit of the fimbriae. These results indicate the existence of a novel type of host-microbe interaction which may be important in the invasion by bacteria of host tissues.

Plasminogen; Bacterial adhesin; (*E. coli*)

1. INTRODUCTION

The serine protease plasmin plays a central role in various physiological and pathological processes involving degradation of extracellular matrix proteins [1]. In addition to intravascular fibrinolysis [2], plasmin has been proposed to digest pericellular proteins in inflammatory response [3–5], tumor cell invasion [6], ovulation and implantation [7], and neuronal development [8]. Activation of plasminogen by tissue plasminogen activator is kinetically unfavorable in the fluid phase but is remarkably promoted by immobilization of plasminogen via its lysine-binding sites [9–11]. Immobilization also protects the activated plasmin from α_2-antiplasmin, which in the fluid phase rapidly inactivates plasmin [11,12]. Furthermore, the presence of specific receptors for plasminogen on endothelial cells [11] and inflammatory cells [4,5] is thought to be an important mechanism for generating localized proteolytic activity on the cell surface.

Similarly to invasive animal cells, pathogenic bacteria must evidently also break down extracellular matrix components in order to invade host tissues through basement membranes. The ability of streptokinase to activate plasminogen [13] and the presence of a plasmid-encoded plasminogen activator in *Yersinia pestis* [14] suggest that bacteria might utilize plasmin for this purpose. To investigate further this possibility, we have studied here the interaction of plasminogen with fimbriae found on *Escherichia coli* strains causing invasive infections. Fimbriae are protein filaments typically consisting of a major structural subunit and a few minor proteins including a lectin subunit which mediates bacterial attachment to host cells [15,16]. Recently, *E. coli* P fimbriae were found to bind to fibronectin via a mechanism that does not involve the lectin subunit [17], suggesting that bacterial fimbriae are multifunctional proteins with lectin-dependent and -independent functions. We report here that plasminogen binds via its lysine-binding sites to type 1 fimbriae that commonly occur in *E. coli* strains, including those

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Abbreviations: IgG, immunoglobulin G; EACA, ε-aminocaproic acid (6-aminohexanoic acid); PBS, phosphate-buffered saline; BSA, bovine serum albumin

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causing invasive human infections [15]. Specific binding of plasminogen was also observed to P fimbriae which are closely associated with pyelonephritogenic strains and S fimbriae that occur in strains causing septic neonatal infections [18,19].

2. MATERIALS AND METHODS

2.1. *Materials*

Human Glu-plasminogen and plasmin were from KabiVitrum (Stockholm). Rabbit polyclonal plasminogen-specific IgG was obtained from Dakopatts (Clostrup, Denmark). Labeling of IgG with europium chelate (isothiocyanatophenyldiethylenetriaminepentaacetic acid complexed with europium; Wallac, Turku, Finland) was carried out essentially as described [20]. ϵ-Aminocaproic acid (EACA) and α-methyl mannoside were obtained from Sigma (St. Louis, MO). Laminin was from Bethesda Research Labs (Gaithersburg, MD).

2.2. *Bacterial fimbriae*

Fimbriae were purified by using deoxycholate and concentrated urea [21] from *E. coli* strains that carry recombinant plasmids encoding type 1 fimbriae (EH827) [22], P fimbriae (pPIL110-75), mutated P fimbriae lacking lectin activity (pPIL110-7501) [23], and S fimbriae (HB101(pANN801-4)) [24]. Yeast cell agglutination tests with type-1-fimbriated bacteria were carried out as in [25].

2.3. *Binding assay*

The proteins studied were allowed to attach to polystyrene microtitration strip wells (Titertek, Labsystems, Helsinki, Finland) in 100 μl PBS (10 μg/ml) overnight at 4°C. The wells were washed twice with washing solution [0.15 M NaCl containing 0.05% (v/v) Tween 20] and incubated with PBS (10 mM phosphate buffer, pH 7.4, 150 mM NaCl) containing 1 mg/ml BSA for 3 h at room temperature. The wells were washed twice, plasminogen was added in 100 μl PBS containing 0.05% Tween 20, and incubation performed for 2 h at room temperature with continuous shaking. The wells were washed three times, 100 μl europium-labeled plasminogen-specific IgG was added (about 50 ng IgG/well) in 50 mM Tris buffer, pH 7.8, containing 150 mM NaCl, 5 mg/ml BSA, 1 mg/ml bovine globulin, and 5 mM NaN_3, followed by incubation for 1 h at room temperature with continuous shaking. The wells were washed four times, 200 μl Enhancement solution (Wallac) [20] was added and fluorescence was measured after 20 min in an Arcus Fluorometer (Wallac). The coefficient of variation for duplicate wells was 4.8% on average. Antibody controls, i.e. wells without any plasminogen added but otherwise processed as described above, gave insignificant fluorescence values (less than 2000 cps) with all immobilized proteins.

3. RESULTS

Plasminogen bound in a concentration-dependent manner to all three immobilized fimbriae studied (fig.1). Binding to type 1 fimbriae was about 4–6-times higher than that to S or P fimbriae. Under the test conditions used, plasminogen bound to immobilized laminin, a formerly characterized target of plasminogen [26], to the same extent as to type 1 fimbriae whereas no binding to BSA (fig.1) and transferrin (not shown) was observed. The binding to type 1 fimbriae became saturated at plasminogen concentration of 20–30 μg/ml indicating an apparent K_d value of 0.1–0.2 μM. Binding to P and S fimbriae was less clearly saturable (fig.1).

Binding of plasminogen to the bacterial fimbriae was effectively inhibited by the lysine analog EACA (fig.2). The binding to type 1 fimbriae was most completely inhibited, i.e. by over 90% in the presence of 20 mM EACA. At the same inhibitor concentration, the binding to P and S fimbriae was decreased by about 70 and 50%, respectively.

To assess the involvement of the formerly characterized lectin-activity of the fimbriae in the interaction with plasminogen, binding of plasminogen to mutated P fimbriae lacking the lectin subunit was compared to binding to wild-type P fimbriae. No difference was observed (fig.3). Furthermore, binding of plasminogen to type 1 fimbriae was not affected in the presence of 50 mM methyl α-mannoside (not shown), a sugar

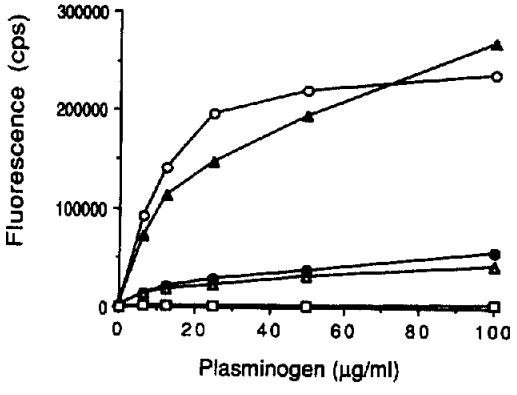

Fig.1. Interaction of plasminogen with immobilized *E. coli* fimbriae. Plasminogen was incubated for 2 h at room temperature in microtiter wells coated with type 1 fimbriae (○), P fimbriae (●), S fimbriae (△), laminin (▲) or BSA (□). After washing, the wells were incubated with a europium-labeled plasminogen-specific antibody and measured for time-resolved fluorescence. Each point represents the mean of duplicate wells.

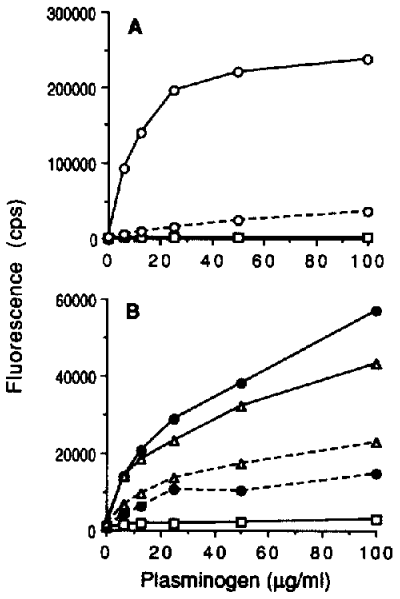

Fig.2. Effect of EACA on plasminogen binding to immobilized fimbriae. Plasminogen was incubated with (---) and without (——) 20 mM EACA in microtiter wells coated with type 1 fimbriae (○), P fimbriae (●), S fimbriae (△) or BSA (□). Bound plasminogen was determined by a europium-labeled antibody. Note the different scale of the *y*-axis in A and B.

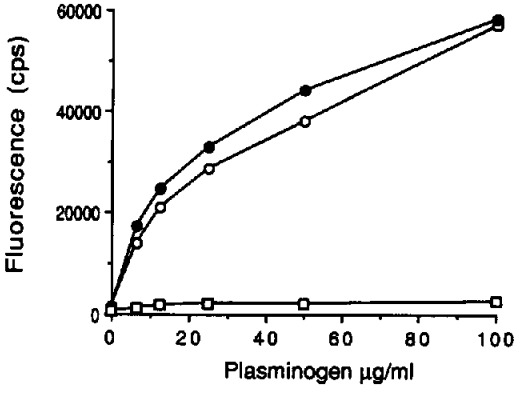

Fig.3. Interaction of plasminogen with intact and mutated P fimbriae. Plasminogen was incubated in microtiter wells coated with P fimbriae (●), mutated P fimbriae lacking the lectin subunit (○) or BSA (□). Bound plasminogen was determined by a europium-labeled antibody.

that totally inhibits the binding of type 1 fimbriae to eukaryotic cells and glycoproteins [15], and yeast cell agglutination by type 1 fimbriae was not inhibited by plasminogen at 100 µg/ml (not shown). On the other hand, treatment of type-1-fimbriated bacteria with activated plasmin (100 µg/ml for 2 h at 37°C) did not influence their ability to agglutinate yeast cells (not shown).

4. DISCUSSION

The present study provides the first evidence on the interaction of plasminogen with surface components of pathogenic bacteria. Our results demonstrate that plasminogen binds to the fimbrial types most commonly found in *E. coli* strains causing pyelonephritis, urosepsis, and neonatal meningitis. The inhibition with EACA [27] indicated that the binding to type 1 fimbriae was almost totally mediated by the lysine-binding sites of plasminogen. The binding of plasminogen to S and P fimbriae was also predominantly lysine binding site-mediated, as indicated by the 50–70% inhibition caused by EACA.

Immobilization of plasminogen via the lysine-binding sites critically enhances its activation by tissue plasminogen activator and protects plasmin from inactivation by the primary plasmin inhibitor, α_2-antiplasmin [9–12]. Binding of plasminogen to bacterial fimbriae may thus generate localized plasmin activity on the bacterial surface. Since plasmin effectively digests different extracellular matrix proteins [28,29] and activates collagenase [30], bacterium-bound plasmin may provide an effective means for bacteria to invade host tissues. On the other hand, the present finding that plasmin treatment of type 1 fimbriae did not influence their ability to agglutinate yeast cells suggests that fimbriae themselves are not digested by plasmin. This is in agreement with earlier observations on the resistance of fimbrial filaments to proteases including trypsin [31].

Several lines of evidence suggest that plasminogen binding is not related to the formerly characterized cell-binding activities of the bacterial fimbriae that are specific for carbohydrate structures [15]. First, mutated P fimbriae lacking the lectin subunit bound plasminogen to an extent similar to that of wild-type P fimbriae. Second, the receptor analog saccharide of type 1 fimbriae, methyl α-mannoside, did not inhibit their plasminogen-binding activity, and third, plasminogen did not inhibit the binding of type 1 fimbriae to yeast cells. Plasminogen binding thus represents a novel property in bacterial fimbriae

and is a further indication of their multifunctional nature. Our results suggest that the pathogenic function of fimbriae is not limited to bacterial adhesion to mucosal surfaces of the host but that fimbriae may also play a part in septicemia. This proposal is also supported by recent evidence indicating that S fimbriae bind to vascular endothelium and choroid plexus [32].

Plasminogen binding to *E. coli* fimbriae indicates the existence of a novel type of host-microbe interaction in bacterial infections. Type 1 fimbriae are commonly found and have a similar structure in most enterobacterial genera [15]; it can therefore be expected that the binding activity described here occurs in bacteria other than *E. coli*.

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