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A novel lectin-independent interaction of P fimbriae of *Escherichia coli* with immobilized fibronectin

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Binding of P fimbriae of uropathogenic *Escherichia coli* to purified human plasma fibronectin and human placental type IV collagen was studied. In an enzyme immunoassay, purified P fimbriae bound strongly to immobilized intact fibronectin and to the aminoterminal 30-kDa fragment and the 120–140-kDa carboxyterminal fragments of fibronectin. Binding to the gelatin-binding 40-kDa fragment of fibronectin was considerably weaker. No binding to immobilized type IV collagen was seen. The interaction between P fimbriae and immobilized fibronectin was not inhibited by α-D-Gal-(1-4)-β-D-Gal-1-*O*-Me, a receptor analog of P fimbriae. Moreover, a mutated P fimbria lacking the lectin activity behaved similarly in the adherence assays. Recombinant strains expressing the corresponding cloned fimbriae genes bound to immobilized fibronectin, but no binding to soluble ^{125}I-labelled fibronectin was found. The results suggest that P fimbriae interact with immobilized fibronectin and that the binding mechanism does not involve the lectin activity of the fimbriae.

Fibronectin; P fimbria; Bacterial adhesion

1. INTRODUCTION

P fimbriae are a major virulence factor of *Escherichia coli* strains associated with human childhood pyelonephritis [1]. They bind to tubular epithelia and vascular endothelium in the kidney and to epithelia of the lower urinary tract of humans [2]. P fimbriae are thought to increase the virulence of *E. coli* by mediating bacterial adherence to epithelial glycoconjugates, thus giving resistance to mechanical clearance by the flow of urine. The receptor structure recognized by P-fimbriated *E. coli* is the disaccharide moiety α-D-Gal-(1-4)-β-D-Gal of the P-blood-group antigens [3].

Fibronectin (FN) is a 450-kDa dimeric glycoprotein found in a soluble form in body fluids, such as blood plasma, and as an insoluble protein in the extracellular matrix and in association with basement membranes [4–6]. One of the main biological functions of FN is to promote attachment and spreading of eukaryotic cells on different substrates. FN is also important in wound healing and involved in clot stabilization. A characteristic of FN is its ability to interact with a wide variety of mammalian molecules and cells [4–6].

The FN molecule is composed of two nearly similar polypeptides joined by two disulfide bridges at the carboxytermini. Over 90% of the molecule consists of repeats of three types of fairly homologous amino acid sequences. On the basis of binding properties, the two chains of FN can be divided into functional domains. So far, many specific domains have been identified, including those binding to fibrin, heparin, collagen, gelatin, DNA and several bacterial species [5]. A specific cell-attachment domain containing the sequence Arg-Gly-Asp-Ser in FN is recognized by fibroblasts, platelets and other eukaryotic cells [5,6].

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Kuusela [7] described the binding of ^{125}I-labelled, soluble FN to *Staphylococcus aureus*. Thereafter, many Gram-positive bacteria, including group A, C and G streptococci [8–10] and strains of staphylococci [11] were shown to interact with soluble FN. Moreover, recently certain human and animal isolates of *E. coli* [12–14] and strains of *Salmonella* [15] have also been found to bind FN. The FN-binding protein of *S. aureus* has recently been identified and its gene cloned [16].

Recent genetic work has shown that the P-fimbrial filament of *E. coli* is composed of a major structural subunit, fimbrillin, and of minor components that are responsible for the lectin activity of the filament [17]. These lectin-like molecules probably are situated at the tip of the filament [18], and it is thought that the function of fimbrillins is to expose the lectin molecules of the fimbriae beyond the lipopolysaccharide and capsular layers of the bacterial cell [19]. In this communication we show that P fimbriae of *E. coli*, both on bacterial cells and as purified proteins, interact with immobilized FN and its fragments. The binding is independent of the lectin activity of the P fimbriae. This work was presented in part at the 88th ASM meeting in Miami, USA, 8–13 May 1988.

2. MATERIALS AND METHODS

2.1. *Bacteria*

E. coli HB101 and the recombinant strains carrying cloned genes for the wild-type P ($F7_1$) fimbriae [HB101(pPIL110-75)] or for the lectin-deficient P fimbriae [HB101(pPIL110-7501)] have been described before [20,21]. The strain IH11128, possessing the O75X adhesin, has previously been characterized for its adhesins and binding properties [22,23]. The bacteria were grown overnight at 37°C on Luria agar plates supplemented with ampicillin for the recombinant strains. For adhesion tests, the bacteria were collected, washed with phosphate-buffered saline (PBS) and finally suspended in PBS containing 0.1% (w/v) bovine serum albumin (BSA; Sigma, St. Louis, MO, USA) (PBS-BSA 0.1%). For purification of fimbriae, the recombinant strains were grown for 48 h at 37°C in Luria broth supplemented with ampicillin.

2.2. *Purified proteins and the antisera*

The purified O75X adhesin and the antiserum against it have been described earlier [22,23]. P fimbriae were purified from strains HB101(pPIL110-75) and HB101(pPIL110-7501) using deoxycholate and concentrated urea [24]. Antiserum against the $F7_1$ fimbriae has been described [25]. Human plasma FN was purified by the method of Engvall and Ruoslahti [26] using the modification of Vuento and Vaheri [27]. The purified 30-kDa, 40-kDa and 120–140-kDa fragments of FN were as in previous work [28,29]. Fetuin and human placental type IV collagen were from Sigma.

2.3. *Binding tests with purified fimbriae*

The binding of the purified P fimbriae and the O75X adhesin (50 μg/ml) to polystyrene microtiter plates coated with FN, type IV collagen or fetuin (25 μg/ml) was tested by enzyme-linked immunosorbent technology as detailed elsewhere [22,23]. Absorbance values in independent duplicates were in general within 10% of the mean. In inhibition experiments, D-glucose (Merck, Darmstadt, FRG) and α-D-Gal-(1-4)-β-D-Gal-1-*O*-Me (Biocarb Chemicals, Lund, Sweden) were tested at 25 mM final concentration, and tetrapeptides Arg-Gly-Asp-Ser or Val-Ala-Ala-Phe (Sigma) at 5 mM final concentration.

2.4. *Adhesion assay with intact bacteria*

Glass slides with ground-edged areas (Chance Propper Ltd, Warley, England) were coated for 16 h at 22°C with the glycoproteins to achieve a final coating concentration of 5 pmol/area. For fetuin, a coating concentration of 50 μg/ml was used. Data for quantitative coating with the glycoproteins was available from previous work [23]. The slides were washed three times for 5 min in PBS-BSA (0.1%) and consequently saturated with PBS-BSA (2%) for 2 h at 22°C. Then the slides were overlaid with bacteria (3×10^{10} cells/ml in 0.1% PBS-BSA; 50 μl/ground-edged area) for 5 h at 22°C. In inhibition tests, α-D-Gal-(1-4)-β-D-Gal-1-*O*-Me or D-glucose (both at a final concentration of 25 mM) were incubated together with the bacterial suspensions. After incubation with bacteria the slides were washed three times with 0.1% PBS-BSA and dried at 22°C. Finally, the adhered bacteria were visualized by staining with methylene blue (Merck) and quantitated by counting the number of bacteria in 20 randomly chosen microscopic fields. The area of one field was 4.8×10^3 μm^2.

2.5. *Binding of radiolabelled glycoproteins to bacteria*

FN and type IV collagen were labelled with carrier-free $Na^{125}I$ (Amersham, Buckinghamshire, England and New England Nuclear, Boston, MA, USA) using the Iodogen [30] (Pierce, Rockford, USA) and the chloramine T methods [31]. The specific activity obtained ranged from 2.5 to 3.6 μCi/μg of protein. The binding assay was performed as detailed in [23]. Briefly, 5×10^7 bacterial cells were mixed with 5 ng of labelled protein in 300 μl of 1% PBS-BSA for 1.5 h at 22°C. After two washed with 1% PBS-BSA, the bound radioactivity was quantitated in a 1270 Rackgamma II counter (LKB, Bromma, Sweden).

3. RESULTS AND DISCUSSION

Systematic studies on the interaction between various purified fimbriae and extracellular matrix components have revealed that the O75X adhesin of *E. coli*, which binds preferentially to basement membranes, strongly interacts with human type IV collagen [23]. Similar type of experiments also showed the interaction of P fimbriae with immobilized FN.

Purified P fimbriae bound efficiently to polystyrene microtiter wells coated with human plasma FN (fig.1A). Absorbance values in control wells, which were processed identically but without the fimbriae, were at the highest 1% of the positive test values (not shown). No significant binding of P fimbriae to immobilized type IV collagen or to the control protein fetuin was seen (fig.1A). The binding to FN was not inhibited by 25 mM α-D-Gal-(1-4)-β-D-Gal-1-*O*-Me (fig.1A and B), a receptor-analog for P fimbriae [3]. This concentration is enough to inhibit binding of purified P fimbriae to human tissues [2]. The mutant fimbriae from strain HB101(pPIL110-7501), lacking the lectin activity, bound as efficiently and selectively to immobilized FN as did the 110-75 P fimbriae (fig.1C). As a further control for the specificity of the binding, the purified O75X adhesin was included in the test. As found in earlier work [23], the O75X adhesin showed a strong binding to type IV collagen but only a weak binding, or none at all, to immobilized FN and fetuin (fig.1D).

The intact FN molecule can be proteolytically cleaved into defined fragments. The 30-kDa aminoterminal fragment contains the binding domains for fibrin, heparin, *S. aureus* and streptococci, and the 40-kDa fragment next to the aminoterminus interacts with collagen and gelatin. The 120- to 140-kDa carboxyterminal fragment contains the cell-attachment domain and another possible binding site for staphylococci and streptococci [5]. To localize the binding sites for P fimbriae within the FN molecule, these fragments were tested for the interaction. The P fimbriae from strain HB101(pPIL110-75) bound efficiently to the 30-kDa aminoterminal and the 120–140-kDa carboxyterminal fragments of FN (fig.2). Only a weak interaction with the gelatin-binding 40-kDa fragment was seen (fig.2).

The adherence of the 110-75 fimbriae to solid-phase intact FN or to the 30-kDa and 120–140-kDa fragments of FN was not inhibited by the tetrapeptides Arg-Gly-Asp-Ser (the cell-attachment sequence of FN [6]) or Val-Ala-Ala-Phe (a test control), both tested at 5 mM (not shown). This concentration of the Arg-Gly-Asp-Ser peptide is enough to inhibit adhesion of normal rat kidney cells to immobilized intact FN [6].

To confirm the binding results obtained with purified fimbriae, the adhesion of intact bacterial cells to glycoproteins immobilized on glass slides was determined. The recombinant strain HB101(pPIL110-75), carrying wild-type P fimbriae genes, adhered about ten times more effi-

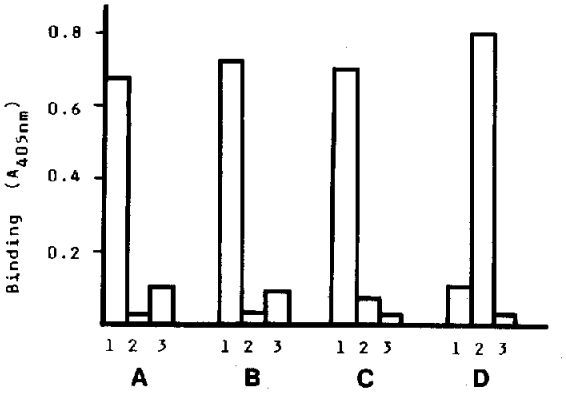

Fig.1. Binding of purified P fimbriae to microwells coated with FN (bar 1), type IV collagen (bar 2) and fetuin (bar 3). (A) Binding of the 110-75 fimbriae in the presence of 25 mM D-glucose and (B) in the presence of 25 mM α-D-Gal-(1-4)-β-D-Gal-1-*O*-Me. (C) Binding of the lectin-deficient 110-7501 fimbriae. For comparison, binding of the type-IV-collagen-binding O75X adhesin is also shown (D).

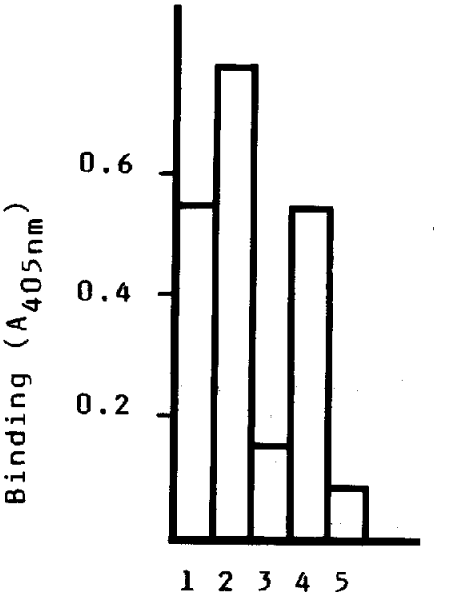

Fig.2. Binding of the purified 110-75 fimbriae to microwells coated with FN or its fragments. (Symbols) Bar 1, FN; bar 2, the aminoterminal 30-kDa fragment; bar 3, the gelatin-binding 40-kDa fragment; bar 4, the 120–140-kDa carboxyterminal fragment; bar 5, fetuin.

ciently to slides coated with FN than to those coated with type IV collagen or fetuin (fig.3A). The adhesion was not inhibited by 25 mM α-D-Gal-(1-4)-β-D-Gal-1-*O*-Me (fig.3B). The recombinant strain HB101(pPIL110-7501), expressing the mutated P fimbriae genes, showed the same binding pattern (fig.3C). The control strain *E. coli* HB101, lacking fimbriae, did not adhere to FN (fig.3D). For comparison, the adhesion of the O75X-positive strain IH11128 is also shown (fig.3E). As expected from earlier work with purified O75X adhesin [23], the strain bound efficiently to type IV collagen, more weakly to FN and not at all to fetuin.

We next tested binding of ^{125}I-labelled FN and type IV collagen to the recombinant bacteria. No significant binding of ^{125}I-labelled FN was seen with either HB101, HB101(pPIL110-75) or HB101(pPIL110-7501) cells (not shown). The iodination methods used had no effect on the result. However, in similar experiments, the O75X-positive strain IH11128 bound ^{125}I-labelled type IV collagen efficiently but failed to bind soluble FN [23].

Our results show that P fimbriae of uropathogenic *E. coli* interact with immobilized FN. Specificity of the interaction is indicated by lack of P-fimbrial binding to immobilized type IV collagen or fetuin (fig.1), and by the finding that recombinant bacteria expressing the corresponding fimbriae showed similar adhesion patterns (fig.3). Adhesion of the fimbriated *E. coli* strains to immobilized FN was weak but repeatable and constantly higher than that of the recipient strain HB101. Moreover, the O75X adhesin of *E. coli* which is known to interact with type IV collagen [23], did not show binding to FN (figs 1 and 3), indicating target specificity in our test systems. The findings that the interaction of the wild-type P fimbriae with FN was not inhibited by α-D-Gal-(1-4)-β-D-Gal-1-*O*-Me (figs 1A,B and 3A,B), and that the interaction was seen also with the 110-7501 fimbriae lacking the lectin activity (figs 1C and 3C), strongly suggest that this novel interaction is independent of the lectin activity of the P-fimbrial filament. At present, it is impossible to say whether the P-fimbrial filament is recognized by FN, which is known to possess several binding properties, or whether the fimbrillin subunits have binding properties of their own, i.e. independently of the α-D-Gal-(1-4)-β-D-Gal binding via the lectin-like minor components of the filament.

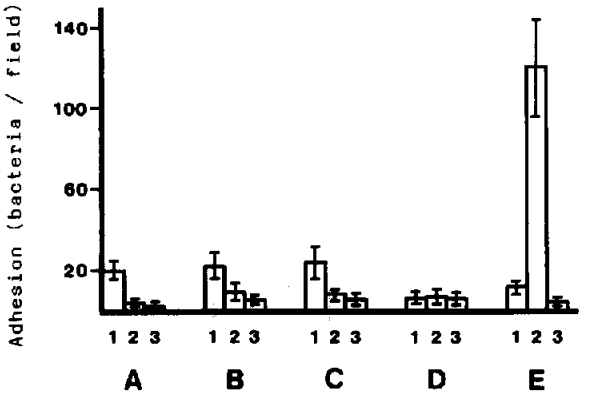

Fig.3. Adhesion of recombinant bacteria to FN (bar 1), type IV collagen (bar 2) and fetuin (bar 3). (A) Adhesion of strain HB101(p110-75) in the presence of 25 mM D-glucose and (B) in the presence of 25 mM α-D-Gal-(1-4)-β-D-Gal-1-*O*-Me. (C) Adhesion of the strain HB101(p110-7501) expressing mutated P fimbriae genes. (D) Adhesion of the strain HB101 lacking fimbriae. (E) Adhesion of the O75X-positive strain IH11128. Bacteria in twenty randomly chosen fields of 5×10^3 μm^2 were counted in each case, standard deviations are also shown.

Interestingly, the interaction between P fimbriae and FN appears to involve mainly the aminoterminal 30-kDa and the carboxyterminal 120–140 kDa fragments (fig.2) that interact also with staphylococci and streptococci [5]. Whether these bacteria use the same or different binding site(s) inside each domain remains still to be resolved. The interaction does not seem to involve the cell-binding domain of FN, as the tetrapeptide Arg-Gly-Asp-Ser was not inhibitory.

Although P fimbriae interacted readily with the immobilized form of FN (figs 1–3), no binding of soluble, ^{125}I-labelled FN to bacteria expressing the same P fimbriae was seen. In this respect, the interaction described here differs from binding of FN to *S. aureus* and group G streptococci, which can be detected with the soluble and insoluble forms of FN [7–11]. Moreover, binding of ^{125}I-labelled, soluble FN to *E. coli* cells [12–14] and to fimbriae of *Salmonella enteritidis* [32] have been reported. This suggests that *E. coli* has several mechanisms for interaction with FN, one active only with the insoluble form and the other with both soluble and insoluble forms of FN.

Interaction of P fimbriae with FN has parallel phenomena involving only the insoluble form of FN. *S. sanguis* adheres to FN immobilized on gelatin-coated plastic but does not bind soluble FN [33]. Similarly, plasminogen [34] and hyaluronate [35] bind to immobilized FN but not to soluble FN. Interestingly, group A streptococci show a reversed binding property: they bind soluble FN but adhere poorly to FN-coated glass surfaces [36]. These differences are best explained by conformational differences between the soluble and the insoluble forms of FN. Soluble FN exists in a globular configuration with the aminotermini of the two chains folded inwards. Upon immobilization, the chains unfold and the molecule has a linear configuration [37].

Our results have an important conclusion for the pathogenetic role of P fimbriae in human upper urinary tract infections. So far, it has been thought that the function of P fimbriae is to mediate bacterial attachment to the α-D-Gal-(1-4)-β-D-Gal-containing glycoconjugates of human epithelial and endothelial cells [2,3] and that the fimbrillins are needed to carry the lectin molecules beyond lipopolysaccharide and capsular layers of the bacterial cell wall [19]. Our results suggest of a further function for the P fimbriae: they interact with a component of the extracellular matrix in a manner that is independent of α-D-Gal-(1-4)-β-D-Gal binding. Such interaction can be useful for the bacteria at later stages of the infection, i.e. after epithelial trauma and exposure of connective tissue when the ascent to kidneys has taken place and the lectin activity of the fimbriae is perhaps not useful anymore.

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