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# *In vitro* binding of type 1-fimbriated *Escherichia coli* to uroplakins Ia and Ib: Relation to urinary tract infections

**(epithelial differentiation/urothelium/bladder epithelium/receptor)**

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ABSTRACT **Urinary tract infections, caused mainly by *Escherichia coli*, are among the most common infectious diseases. Most isolates of the uropathogenic *E. coli* can express type 1 and P fimbriae containing adhesins that recognize cell receptors. While P fimbriae recognize kidney glycolipid receptors and are involved in pyelonephritis, the urothelial receptors for type 1 fimbriae were not identified. We show that type 1-fimbriated *E. coli* recognize uroplakins Ia and Ib, two major glycoproteins of urothelial apical plaques. Anchorage of *E. coli* to urothelial surface via type 1 fimbriae–uroplakin I interactions may play a role in its bladder colonization and eventual ascent through the ureters, against urine flow, to invade the kidneys.**

Urinary tract infections are among the most common infectious diseases, accounting for almost 5 million cases annually and causing considerable morbidity and mortality (1). Increasing incidence of antibiotic-resistant *E. coli*, which causes up to 95% of these infections, calls for additional therapeutic considerations. One useful approach entails the inhibition of bacterial attachment to urothelial surface, a crucial initial event involving precise interactions between a group of bacterial adhesive molecules called adhesins and their cognate urinary tract receptors (2–4). Knowledge of the molecular details of the receptor–adhesin interface may provide a basis for rational drug design for preventing and treating urinary tract infections.

To facilitate attachment to eukaryotic receptors, *E. coli* assemble fimbriae capped with adhesin molecules (5–7). Two major classes of fimbriae of uropathogenic *E. coli* have been functionally defined. The P fimbriae are expressed in ≈70% of the pyelonephritis isolates, and they bind to the Gal$\alpha$(1–4)Gal moiety in the glycolipid receptors (8). The type 1 fimbriae are expressed by more than 90% of the uropathogenic *E. coli*, and they can bind, via mannose moieties, to the urothelial surface (9–11). Immunohistochemical staining of voided urothelial cells of urinary infection patients showed adhering *E. coli* with type 1 fimbriae alone (12). Animal studies showed that *E. coli* expressing type 1 fimbriae, but not those harboring mutated ones, can cause urinary tract infections (13, 14). These results clearly establish the functional importance of the mannose-sensitive type 1 fimbriae in urinary tract infections. Virtually nothing is known, however, about the urothelial receptors that presumably bear the mannoses recognized by the type 1 fimbriae. Consequently, the precise role of this kind of fimbriae and their functional relationship with the P fimbriae in various types of urinary tract infections are not well understood (2–7, 15).

Significant progress has recently been made to characterize biochemically the apical surface of mammalian urothelium, which is covered with numerous rigid-appearing, 0.3- to 0.5-$\mu$m plaques. In cross-sections, the luminal leaflet of the plaque membrane is twice as thick as the cytoplasmic leaflet, hence the term asymmetrical unit membrane (AUM) (16). We have recently isolated milligram quantities of bovine urothelial AUMs and showed that they contain four major integral membrane proteins, which we named uroplakin Ia (UPIa; 27 kDa), UPIb (28 kDa), UPII (15 kDa), and UPIII (47 kDa) (17, 18). All of these major AUM proteins have dominant luminal domains with relatively little or, in the cases of uroplakin I proteins and UPII, almost no cytoplasmic domains (19–21). The asymmetrical distribution of the uroplakin domains across the lipid bilayer suggests that the luminal domains may interact to form the 16-nm protein particles protruding luminally and may explain why AUM's luminal leaflet is thicker than its cytoplasmic leaflet (22, 23). Finally, ultrastructural localization confirmed that the uroplakins are associated with the AUM plaques *in situ* (17, 18). Because these plaques occupy 70–80% of the urothelial apical surface and are only interrupted by short interplaque "hinge" areas, these four uroplakins, as the major AUM subunits, are the predominant protein components of the urothelial apical surface. This raises the question as to whether any uroplakins may be the hypothesized urothelial receptors of some uropathogenic *E. coli*.

## MATERIALS AND METHODS

**Preparation of Asymmetric Unit Membranes from Mammalian Urinary Bladders.** The AUMs were isolated from urinary bladders of cattle, human, monkey, and mouse by sucrose gradient centrifugation followed by Sarkosyl and NaOH wash (22). AUMs dissolved in 1% SDS were quantitated using bicinchoninic acid reagent (Pierce).

**Determining Fimbrial Specificity by Agglutination Tests.** Yeast and erythrocyte agglutination tests were performed on glass slides (24, 25). Briefly, 5 $\mu$l of radiolabeled bacteria ($10^{10}$ cells per ml) were mixed with 10 $\mu$l of 1% (wt/vol) *Saccharomyces cerevisiae* suspended in PBS. Human P1 erythrocytes were identified by their agglutinability with anti-P1 antisera (Immucor, Norcross, GA). For hemagglutination, citrated whole blood was washed three times with PBS by centrifugation at 500 × *g*. Ten microliters of washed erythrocytes (4%) were mixed with an equal volume of radiolabeled bacteria ($10^{10}$ cells per ml). After the mixtures were incubated at room temperature for 5 min, the agglutination was read, both macroscopically and microscopically, and graded (−, + to ++++). In some of the experiments, the bacteria were suspended in 2% D-mannose before incubation with yeast or erythrocytes.

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Abbreviations: AUM, asymmetrical unit membrane; UPIa, uroplakin Ia; endo, endoglycosidase.

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***E. coli* Strains Expressing Defined Fimbrial Adhesins.** The fimbrial adhesins of various *E. coli* strains were assessed by their abilities to agglutinate yeast, as well as erythrocytes of various animal species, in the absence and presence of D-mannose (Table 1). J96 is a human pyelonephritis isolate that agglutinated yeast and guinea pig erythrocytes in a mannose-sensitive fashion; this agglutination property suggests that it expresses type 1 fimbriae that harbor FimH adhesins (24). In addition, it agglutinated erythrocytes of human, sheep, and rabbit in a mannose-resistant fashion; this is consistent with the fact that J96 also expresses P fimbriae carrying G-1 and G-3 adhesins (25). SH48 and HU849 are recombinant derivatives of nonfimbriated P678-54 (an *E. coli* K-12 derivative) through transfections using J96 genomic DNAs encoding type 1 or P fimbriae, respectively. These two strains collectively exhibit the chemical, serological, and functional properties of their parent strain, J96 (24). SH48 strongly agglutinated, in a mannose-sensitive manner, yeast and erythrocytes of all species tested; this result confirmed that SH48 expresses exclusively type 1 fimbriae (24, 26). HU849 strongly agglutinated human and rabbit erythrocytes (mannose-resistant), confirming its production of G-1 adhesin (24–26). The recombinant strain $IA_2$ (HB101/pDC1) agglutinated human and sheep erythrocytes, consistent with its expression of P fimbriae carrying G-2 adhesin (25, 27). As expected, the nonfimbriated *E. coli*, P678-54, was nonagglutinating (24). These results, summarized in Table 1, established that all *E. coli* strains expressed, under current culture conditions, the expected fimbrial adhesins (24–27).

The bacteria were grown in Luria–Bertani medium for 16 hr and labeled with $^{35}$[S]methionine [DuPont/NEN; specific activity > 1000 Ci/mmol (1 Ci = 37 GBq)] in a methionine- and glucose-free medium at 37°C for 2 hr. The labeled bacteria were washed three times, resuspended in PBS containing 30% glycerol, and stored at −70°C until use.

***In Vitro* Bacterial Adherence Assay.** Purified AUMs were suspended in PBS and incubated in 96-well polystyrene microtiter plates at room temperature for 30 min, then at 4°C for 16 hr. All subsequent steps were carried out at room temperature. After being washed three times with PBS for 2 hr, the immobilized AUMs were incubated with 2% BSA in PBS for 2 hr, and $^{35}$S-labeled bacteria in 2% BSA and 0.1% $NaN_3$ for 2 hr. The wells were then washed four times with PBS, and the bound bacteria were dissolved in 1% SDS for 30 min and quantitated by scintillation counting. All binding studies were performed in triplicate.

**Bacterial Overlay Assay.** AUM proteins were resolved by SDS/PAGE (17% acrylamide; acrylamide/bisacrylamide = 120:1) and electrophoretically transferred to nitrocellulose. After a brief incubation in 3% BSA in PBS to block the unoccupied sites, the nitrocellulose sheet was incubated with $^{35}$[S]methionine-labeled bacteria in 2% BSA and 0.1% $NaN_3$. After three washings in PBS, the nitrocellulose was air-dried and autoradiographed.

**Enzymatic Deglycosylation.** Purified AUMs were dissolved in 0.1% SDS at room temperature. The solution was adjusted to a final concentration of 1% octyl glucoside, 0.05% $NaN_3$, 5 mM EDTA, 50 mM sodium acetate buffer (pH 5.5), and 33 milliunits/ml endoglycosidase (endo) H. Another fraction was made to contain 40 mM sodium phosphate buffer (pH 7.4) and 14 units/ml *N*-glycosidase F (Boehringer Mannheim). After the mixtures were incubated at 37°C for 16 hr, the proteins were resolved by SDS/PAGE and either stained by silver nitrate or blotted onto nitrocellulose for the bacterial overlay assay.

## RESULTS

**Type 1-Fimbriated *E. coli* Bind to Isolated Urothelial Plaques.** To determine whether the AUM proteins can serve as *E.* coli receptors, we measured the binding of five strains of [$^{35}$S]methionine-labeled, type 1- and P-fimbriated *E. coli* to highly purified bovine AUMs using an *in vitro* adherence assay. Bovine urothelial plaques were isolated by discontinuous sucrose gradient centrifugation plus detergent wash, taking advantage of the remarkable insolubility of AUMs in many detergents, including 2% Sarkosyl (22). These highly purified plaques exhibit, after negative staining, two-dimensional crystalline arrays of 16-nm protein particles (23) and give rise to four major uroplakin bands by SDS/PAGE. In the binding assay, purified AUMs were used to coat the wells of a microtiter plate and incubated with $^{35}$S-labeled *E. coli*, and the radioactivities of the bound bacteria, dissolved in 1% SDS, were quantitated. Of the bacterial strains expressing both type 1 and P (strain J96), type 1 only (SH48), P only (HU849 and $IA_2$), or neither (P678-54 as a control) (24–27), only the first two type 1-fimbriated *E. coli* were able to bind the AUMs (Fig. 1*A*). Although J96 expresses both type 1 and P fimbriae (of the G-1 and G-3 types), its binding to AUMs could be completely blocked by D-mannose (Fig. 1*B*), suggesting that the type 1, but not the P, fimbrial adhesin was responsible for the observed binding.

To test whether the binding between type 1 fimbriae and AUMs was species-specific, we performed the *in vitro* adherence assay using AUMs isolated from bovine, human, monkey, and mouse bladders. The fact that AUMs of all these species showed strong binding suggests that the urothelial plaque receptors are highly conserved (Fig. 2). Moreover, with a constant amount of immobilized AUM, this binding was linearly proportional to the bacterial input and was saturable (Fig. 3*A*); reversing the experiment by immobilizing increasing amounts of AUMs with a constant bacterial input yielded a similar saturating kinetics (Fig. 3*B*). These data clearly indicate that type 1-fimbriated, but not the P-fimbriated, *E. coli* can bind specifically to the AUM plaques that cover the bulk of the urothelial apical surface.

Table 1. *E. coli* strains and their adhesive properties*

| Strains | Yeast† agglutination | Hemagglutination (species) | | | | | Adhesin | | AUM binding |
|---|---|---|---|---|---|---|---|---|---|
| | | Guinea pig | Human‡ | Sheep | Rabbit | Horse | 1 | P | |
| J96 | ++/−§ | ++/− | +/+ | +/+ | ++/+ | ++/− | H¶ | G-1, G-3‖ | ++/− |
| SH48 | ++++/− | ++++/− | +++/− | ++/− | +++/− | +++/− | H | − | +++/− |
| HU849 | −/− | −/− | +++/+++ | −/− | +++/+++ | −/− | − | G-1 | −/− |
| $IA_2$ | −/− | −/− | +++/+++ | ++/++ | −/− | −/− | − | G-2 | −/− |
| P678-54 | −/− | −/− | −/− | −/− | −/− | −/− | − | − | −/− |

*The degree of agglutination and AUM binding was graded from ++++ to − to denote strong to negative reactions, respectively.
†*S. cerevisiae*.
‡Human P1 erythrocytes.
§Values before and after the slash denote the degrees of agglutination in the absence and presence of 2% D-mannose, respectively.
¶FimH adhesin of type 1 fimbriae.
‖G-1, G-2, and G-3 are the three major types of adhesins of P fimbriae.

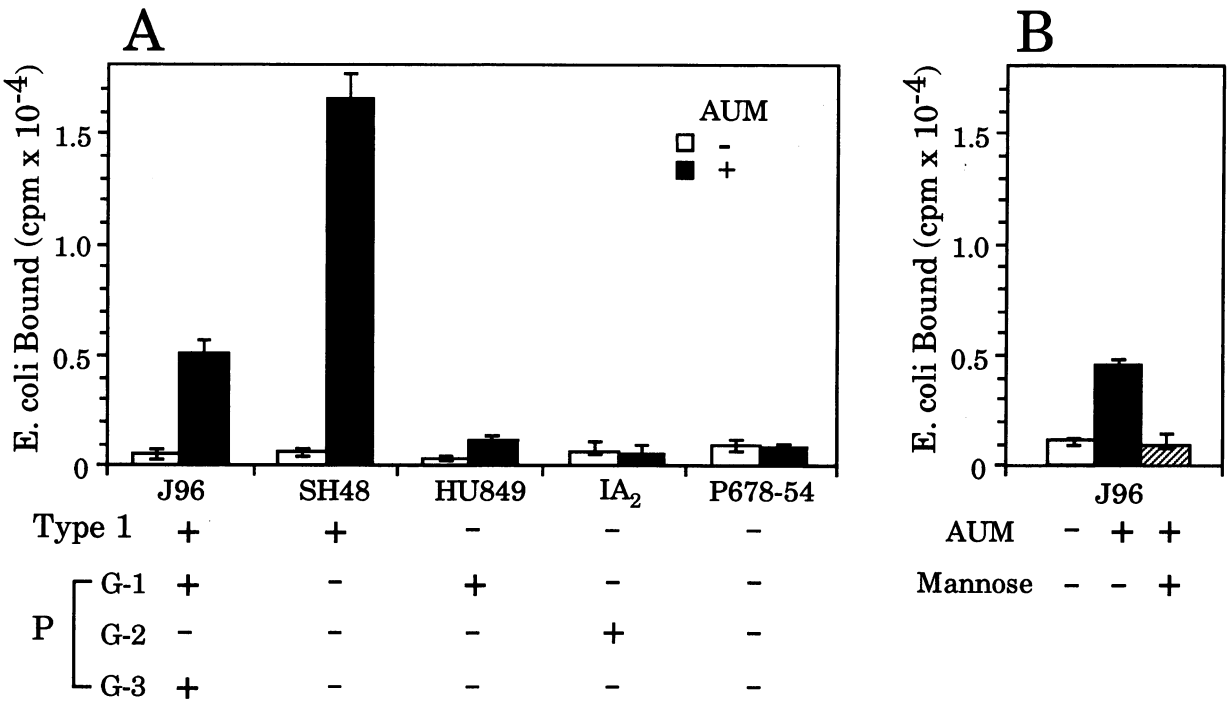


FIG. 1. *In vitro* adherence of type 1-fimbriated *E. coli* to bovine urothelial plaques. (*A*) *In vitro* binding of various *E. coli* strains to bovine urothelial plaques. Purified bovine urothelial plaques, consisting of AUMs that cover 70–80% of urothelial apical surface, were immobilized on microtiter wells (0.2 μg per well); the unoccupied binding sites were blocked with 3% BSA in PBS. BSA was used to coat the control wells. The *E. coli* strains used were J96, which expresses type 1 fimbriae as well as P fimbriae carrying the G-1 and G-3 adhesins; SH48, which expresses type 1 fimbriae only; HU849, which expresses P fimbriae carrying G-1 adhesin; $IA_2$, which expresses P fimbriae carrying G-2 adhesins; and nonfimbriated P678-54 (see Table 1). [$^{35}$S]Methionine-labeled bacteria were added to each well ($2 \times 10^5$ cpm in $10^7$ bacteria suspended in PBS). After incubation (at 25°C for 2 hr), the wells were PBS-washed, and the radioactivities of the bound bacteria, dissolved in 1% SDS, were counted. Each value represents the means of triplicates bracketed by standard deviation (±15%). Note that only the type 1-fimbriated J96 and SH48 strains, but not the P-expressing HU849 and $IA_2$ or the nonfimbriated P678-54, adhere to the AUMs. (*B*) The relative contribution of type 1 and P fimbriae in the binding of J96 to AUMs. $^{35}$S-labeled J96 ($2 \times 10^5$ cpm) bacteria were incubated with immobilized AUMs in the absence or the presence of 2% D-mannose. Note that the binding of J96 to AUMs could be completely blocked by mannose.

To determine whether this *in vitro* binding is physiologically relevant, we tested whether it can be blocked by mannose and its analog, methyl-mannopyranoside, both of which have been shown earlier to inhibit the binding of type 1-fimbriated *E. coli* to urothelium *in vivo* (28, 29). We found that indeed these sugars can abolish the binding of type 1 fimbriae to AUM (Fig. 4), suggesting that the observed *in vitro* interaction is specific and mimics the *in vivo* occurrence.

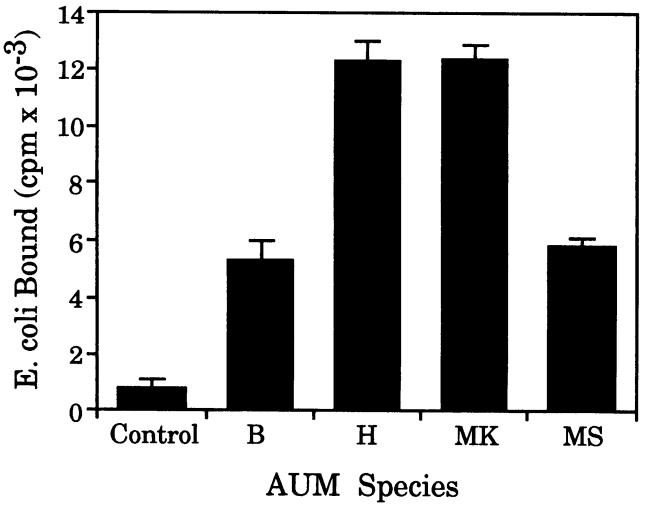


FIG. 2. *In vitro* binding of type 1-fimbriated bacteria to AUMs of various mammalian species. AUMs of cattle (B), human (H), monkey (MK), and mouse (MS) were immobilized on microtiter wells and incubated with radiolabeled, type 1-fimbriated *E. coli* (strain SH48). Note that the bacteria bound to AUMs of all four species.

**UPIa and UPIb as Receptors of Type 1-Fimbriated *E. coli*.** To determine which of the AUM proteins are responsible for this binding, we performed a gel overlay assay. Uroplakins were resolved by SDS/PAGE (Fig. 5 *A* and *B*), transferred to nitrocellulose, and incubated with radiolabeled bacteria. Autoradiography showed that, similar to the results obtained with intact AUMs, only type 1-fimbriated bacteria bound protein bands (Fig. 5*C*), and the binding could be inhibited by mannose but not by galactose (data not shown). The two major bacterial binding proteins were identified as the two closely related uroplakin I proteins, i.e., the 27-kDa UPIa and the 28-kDa UPIb, according to their sizes and immunoreactivities (Fig. 5*B*, lanes 1 and 2). No binding was observed with the mature UPII, which is not glycosylated, nor with UPIII, which is *N*-glycosylated with 20-kDa equivalents of complex type sugars (Fig. 5 *C* and *D*). To determine whether the carbohydrate moieties of uroplakin I proteins were responsible for the binding of the type 1 fimbriae, as one might expect from the inhibitory effects of mannose (Fig. 4), we tested the effects of deglycosylation (Fig. 5 *D* and *E*). Endo H removed ≈3-kDa equivalents of sugars from uroplakin I proteins (Fig. 5*D*, lanes 2 and 5). This abolished the uroplakin I molecules' abilities to bind the bacteria (Fig. 5*E*, lanes 2 and 5). These results provide additional evidence for the specificity of the *in vitro* type 1 fimbriae–AUM interaction. Moreover, the data established that UPIa and UPIb are the main AUM-associated receptors for type 1-fimbriated bacteria and that the high-mannose type sugars of the uroplakin I proteins are responsible for the binding.

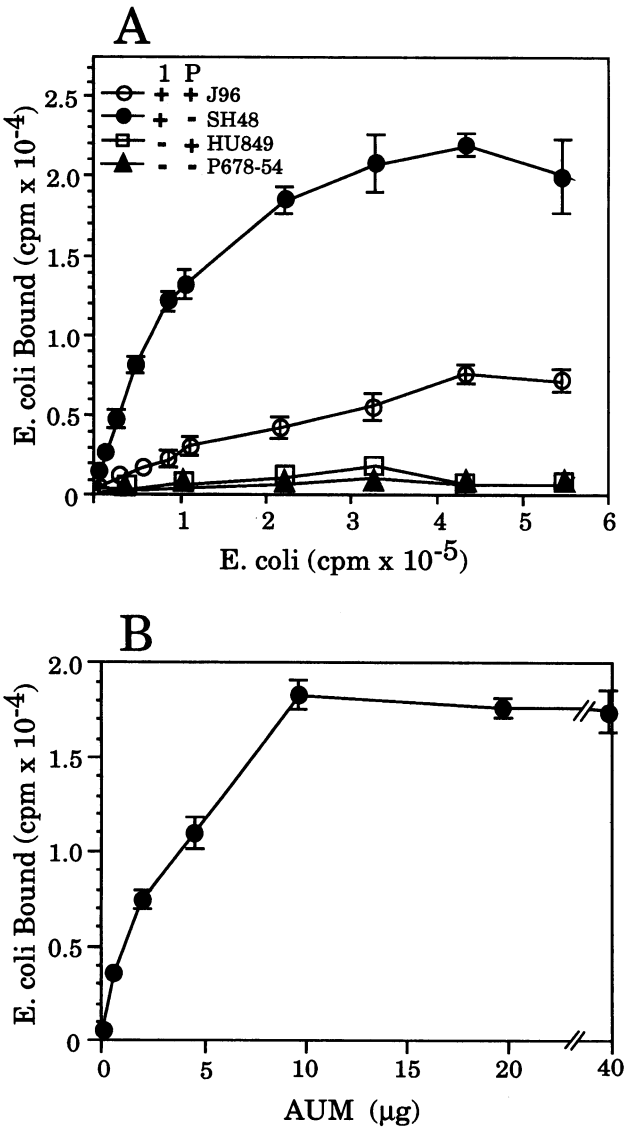


FIG. 3. Saturation kinetics of bacterial binding to bovine urothelial plaques. (*A*) A fixed amount (0.2 μg) of immobilized bovine AUMs were incubated with increasing amounts of radiolabeled *E. coli* (four strains as indicated). (*B*) A fixed number of radiolabeled *E. coli* (strain SH48; $10^7$ bacteria containing $2 \times 10^5$ cpm) were incubated in microtiter wells that had been coated with increasing amounts of AUMs.

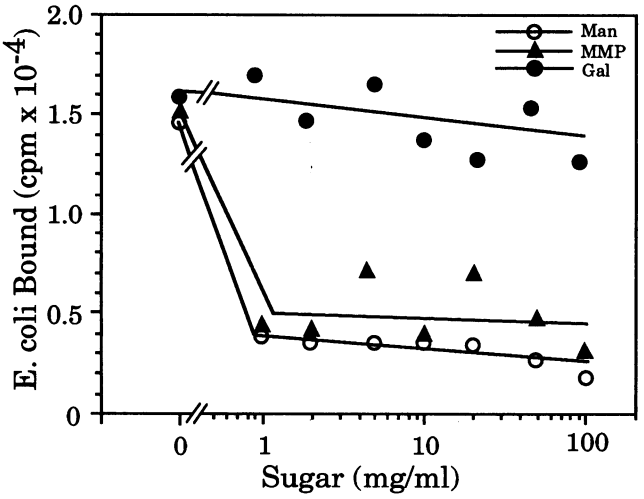


FIG. 4. Effects of sugars on the binding of type 1-fimbriated *E. coli* to bovine urothelial plaques. Radiolabeled *E. coli* (SH48; $2 \times 10^5$ cpm) were preincubated with D-mannose (Man), methyl-α-D-mannopyranoside (MMP), or D-galactose (Gal) before they were incubated with 0.2 μg of immobilized bovine AUMs. Note that the bacterial binding to AUMs was greatly inhibited by D-mannose and its analog methyl-α-D-mannopyranoside, but not by D-galactose.

## DISCUSSION

**Unique Features of the Uroplakin I Receptors.** Although existing data strongly suggest that type 1 fimbriae play an important role in urinary tract infections, their urothelial receptors have been elusive (2–4, 15). The present work provides the first evidence that UPIa and UPIb, two major glycoproteins of AUM plaques covering >70% of the urothelial apical surface, can serve as receptors for type 1-fimbriated *E. coli*. These uroplakin receptors have several interesting properties. First, UPIa and UPIb, two closely related isoforms sharing 39% amino acid sequences, belong to a novel family of integral membrane proteins all having four transmembrane domains (19, 31). Members of this gene family include several important leukocyte differentiation-related surface antigens (CD9, CD37, and CD53), a tumor-associated antigen (CD63), a prostate tumor metastasis suppressor gene (CD82/KAI1), and two Schistosoma antigens (31, 32). Second, they form, together with UPII and UPIII, 16-nm luminal protein particles that are arranged in two-dimensional crystalline arrays (22, 23). Image processing revealed that each 16-nm particle consists of six inner and six outer subdomains interconnected, forming a continuous strand in the shape of a "twisted ribbon" (23). As a part of such a highly organized structure, which can be readily isolated in milligram quantities, uroplakin I proteins are uniquely suitable for detailed structural analysis, both for their protein backbones and their sugar moieties. Third, consistent with the fact that AUM is a hallmark of differentiated urothelial umbrella cells, uroplakins, as the major AUM subunits, are urothelium-specific and differentiation-dependent (17–22, 33). Thus, uroplakins are found so far only in the differentiated urothelial cells. The striking tissue- and differentiation-dependence of uroplakin I proteins suggests that this class of type 1 fimbriae receptor, although important in urinary tract infections, is not involved in the adherence of type 1-fimbriated Enterobacteria in nonurinary organs. Finally, we have shown recently that uroplakin I proteins, like UPII and UPIII, are highly conserved during mammalian evolution (22). This was based on the observation that uroplakins of nine mammalian species, including bovine and human, showed similar sizes, antigenicities, and in some cases, amino acid sequences. Our present finding that type 1-fimbriated *E. coli* can bind with similar facility to AUMs of bovine, human, monkey, and mouse (Fig. 2) extends this conservation to include the terminal mannose moieties recognizable by the adhesin of type 1 fimbriae, i.e., the 30-kDa FimH (5–7). This observation justifies the use of bovine AUMs, which are available in large quantities, as a physiologically relevant and convenient system for studying the molecular details of, and for screening drugs that can interfere with, the interactions between type 1 adhesin and its urothelial receptors.

It is unclear whether the binding of FimH to uroplakin I receptors can trigger intracellular signaling, which has been shown in other adhesin/host cell systems to cause cytoskeletal changes, membrane ruffling, phagocytosis, and the release of inflammatory mediators (3). Because both major hydrophilic loops of amino acids that interconnect transmembrane domains 1 and 2 as well as 3 and 4 of uroplakin I proteins extend luminally, these receptors have practically no cytoplasmic domains (19–21). Although uroplakin I proteins may still be able to transduce signal via its interaction with UPIII, which has a cytoplasmic domain, the fact that uroplakins are parts of

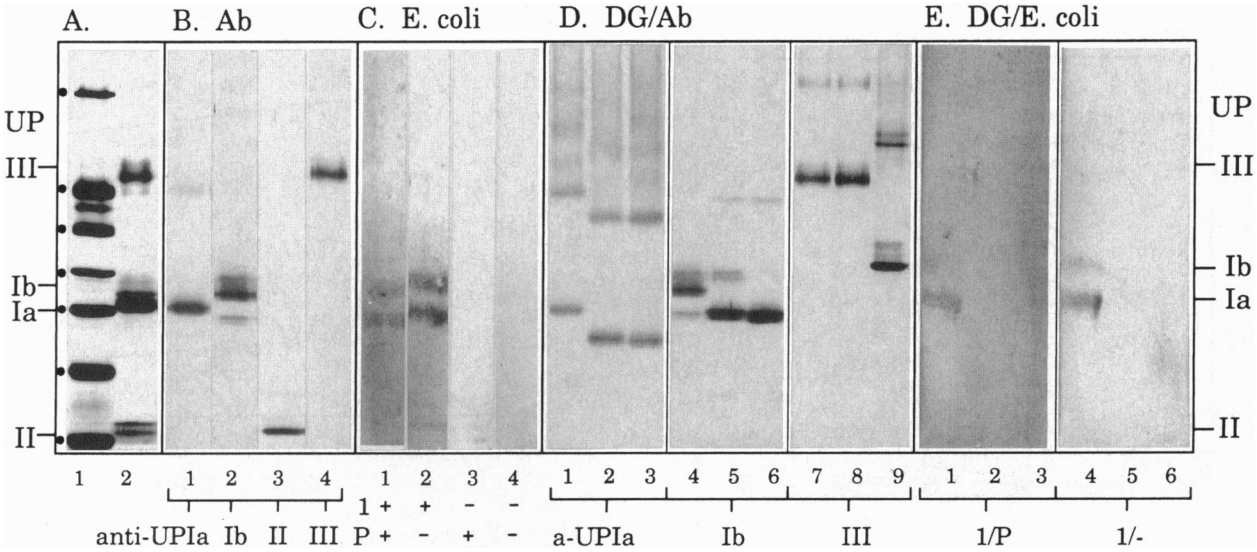


FIG. 5. Binding of type 1-fimbriated *E. coli* to AUM protein subunits. (*A*) Electrophoretic pattern of bovine urothelial plaques. Proteins of bovine AUMs were dissolved in 1% SDS, separated by SDS/PAGE, and visualized by silver nitrate staining (lane 2). Note the separation of three major protein bands, i.e., the 47-kDa UPIII, the 27- to 28-kDa UPIa and UPIb, and the 15-kDa UPII. Lane 1 shows molecular weight markers (dotted; 66, 45, 36, 29, 24, 20, and 14 kDa). (*B*) Identification of uroplakins by immunoblotting. Uroplakins were electrophoretically transferred to nitrocellulose and immunoblotted using antibodies (22) against synthetic peptides corresponding to UPIa (lane 1), UPIb (lane 2), UPII (lane 3), and UPIII (lane 4). (*C*) Binding of *E. coli* to uroplakins. Uroplakins that had been electrophoretically transferred to nitrocellulose were incubated with radiolabeled *E. coli* strains that express both type 1 and P fimbriae (strain J96; lane 1), type 1 only (SH48; lane 2), P only (HU849; lane 3), or none (P678-54; lane 4). Note that the type 1-expressing *E. coli* bind predominantly to UPIa and UPIb. (*D*) Deglycosylation (DG) of uroplakins. Bovine uroplakins were dissolved in 0.1% SDS, treated with a buffer as a control (lanes 1, 4, and 7), or endo H to remove the high-mannose type of sugars (lanes 2, 5, and 8), or endo F (lanes 3, 6, and 9). The uroplakins were then resolved by SDS/PAGE and immunoblotted using antibodies to uroplakins as indicated. Note the removal of ≈3-kDa equivalents of the high-mannose type of sugars from UPIa and UPIb by endo H; and the removal of ≈20-kDa equivalents of sugars, most likely the complex type, from UPIII by endo F. As shown earlier, incubation of SDS-dissolved UPIa (overnight at 37°C) results in its oligomerization (30); very little of these oligomers are present, however, in the AUM fraction used in bacterial binding assays (cf. *B*, lane 1). (*E*) *E. coli* binding to the deglycosylated uroplakins. Uroplakins that had been treated with buffer (controls; lanes 1 and 4), endo H (lanes 2 and 5), or endo F (lanes 3 and 6) were transferred to nitrocellulose and incubated with radiolabeled *E. coli* expressing both type 1 and P fimbriae (1/P; strain J96) or type 1 only (1/−; SH48) as indicated. Note that the binding of type 1-fimbriated bacteria to UPIa and UPIb was abolished by endo H (and endo F) treatment, suggesting the involvement of a high-mannose type of sugars in the binding.

a tightly organized and rigid-looking AUM structure makes this improbable (23). On the other hand, bacterial adherence may induce physiological changes in the microorganism leading to, for example, the release of cytotoxins and proteases that can damage the urothelial defense (3).

**Possible Roles of Type 1 and P Fimbriae in Urinary Tract Infections: Cooperativity and Selection.** Our results showed that the FimH adhesin of type 1 fimbriae, but not the three major G adhesins of the P fimbriae, were able to bind AUMs (Fig. 1; Table 1). This suggests that type 1 and P fimbriae may play different roles in various stages of bacterial infection by recognizing distinct receptors, i.e., the urothelial uroplakin I proteins and kidney glycolipids, respectively. In a relatively early phase of urinary tract infection, *E. coli* has to attach to urothelial surface in the bladder, most likely via type 1 fimbriae–uroplakin I interactions. This allows the bacteria to colonize to maintain a sufficient number of infectious agents possibly causing cystitis. Moreover, since the uroplakin I-containing urothelium covers almost the entire urinary tract (17–20), this provides a mechanism allowing the type 1-fimbriated *E. coli* to ascend through the ureters, against the urine flow, to invade the kidneys. Once reaching the kidney, P fimbriae may then take over as the primary mediator of bacterial attachment, via their binding to the glycolipid receptors (8, 26). This scheme emphasizes the cooperative relationship between the type 1 and P fimbriae in kidney infection (pyelonephritis), and suggests a selection mechanism that explains why a great majority of urinary infection isolates are type 1-fimbriated and why most *E. coli* isolates from pyelonephritis patients are in addition P-fimbriated (2, 4, 10, 11, 34).

**Blocking Bacterial Binding to Urothelial Receptors by Urinary Soluble Proteins and Mucus: A Host Defense Mechanism.** Although type 1 fimbriae are known to be able to recognize several nonurothelial molecules, including a 65-kDa protein of guinea pig erythrocytes, leukocyte adhesion molecules CD11 and CD18, laminin, fibronectin, and uromodulin (35–39), these molecules are not present on urothelial surface and, therefore, clearly cannot be the urothelial receptors of the bacteria. However, uromodulin (also known as the Tamm–Horsfall protein), a kidney-derived, mannosylated protein present in an extraordinarily high concentration in the urine (20 to 30 mg/liter) (40), may play a defensive role. It can saturate all the mannose-binding sites of the type 1 fimbriae, thus potentially blocking bacterial binding to the uroplakin I receptors of the urothelium. Another possible defense mechanism involves the mucus layer that coats the urothelial surface. It has been demonstrated in animal models that type 1-fimbriated *E. coli* cannot bind to normal bladder surface that is covered by an intact mucus layer (41). Damage of the mucus layer allows the bacteria to gain access, however, to the receptors of the underlying urothelium, thus allowing adherence. Defects in these defense mechanisms, which entail the combined effects of the soluble uromodulin and the urothelial mucus, may lead to the adherence of *E. coli* (41), via FimH–uroplakin I receptor interactions, to urothelial surface, thus setting the stage for urinary tract infections. Additional studies are needed to characterize the *in vivo* binding of type 1-fimbriated *E. coli* to the uroplakin I receptors to determine how this interaction is regulated by the soluble urinary proteins and the insoluble urinary mucus components, and to determine whether differences in uroplakin I expression may contribute to different susceptibility of individuals to urinary tract infections.

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