JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5530–5536
0021-9193/87/125530-07$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 169, No. 12

Identification of Two Ancillary Subunits of *Escherichia coli* Type 1 Fimbriae by Using Antibodies against Synthetic Oligopeptides of *fim* Gene Products

SOMAN N. ABRAHAM,[1,2]* JON D. GOGUEN,† DAXI SUN,[1] PER KLEMM,[3] AND EDWIN H. BEACHEY[1,2,4]

Department of Medicine[1] and Department of Microbiology and Immunology,[2] University of Tennessee, Memphis, and Veterans Administration Medical Center,[4] Memphis, Tennessee 38163; and Department of Microbiology, Technical University of Denmark, Lyngby, Denmark[3]

Received 27 April 1987/Accepted 14 September 1987

We have chemically synthesized oligopeptides corresponding to the NH_2-terminal stretch of two gene products, designated FimG and FimH, of the *fim* gene cluster of *Escherichia coli*. These synthetic peptides, designated S-T1FimG(1-16) and S-T1FimH(1-25)C, evoked antibodies in rabbits that reacted with 14- and 29-kilodalton subunits, respectively, of dissociated fimbriae encoded by the recombinant plasmid pSH2 carrying the genetic information for the synthesis and expression of functional type 1 fimbriae. Neither of these fimbrial proteins was detected in dissociated fimbrial preparations from nonadhesive *E. coli* cells carrying the mutant plasmid pUT2002, containing a restriction site-specific deletion of *fimG* and *fimH*. Anti-S-T1FimH(1-25)C inhibited the adherence of type 1 fimbriated *E. coli* to epithelial cells. Immunoelectron microscopy revealed that anti-S-T1FimH(1-25)C, but not anti-S-T1FimG(1-16), bound to intact type 1 fimbriae of *E. coli* at the fimbrial tips and at long intervals along the fimbrial filaments. Anti-S-T1FimG(1-16) appeared to be directed at epitopes not accessible on the intact fimbriae and consequently failed to bind to intact fimbriae or to block fimbrial attachment. Our results suggest that the *fimG* and *fimH* gene products are components of type 1 fimbriae and that FimH may be the tip adhesin mediating the binding of type 1 fimbriated *E. coli* to D-mannose residues on mucosal surfaces.

Type 1 fimbriae are hairlike structures which emanate from the surface of *Escherichia coli* and other members of the family *Enterobacteriaceae* (5, 10, 28). These organelles are characterized by their ability to promote attachment of bacteria to D-mannose-containing receptors on a variety of eucaryotic cells (10, 28). Each fimbrial filament is approximately 1 μm long and 7 nm in diameter and is composed of protein subunits which are held together by strong hydrophobic interactions (5). Harsh treatments such as boiling in acid or exposure to saturated guanidine hydrochloride are required to disrupt the fimbrial filament into its subunits (9, 12). To date, only a single 17-kilodalton (kDa) subunit species has been reported to account for the structure of isolated type 1 fimbriae (9, 12).

While there is general agreement that type 1 fimbriae mediate D-mannose-specific binding, there are differing views on the location of the D-mannose-binding moiety on the fimbrial filament. Brinton suggested that the adhesive moiety resides on the fimbrial tips (5). Other workers have proposed adhesive sites along the length of type 1 fimbriae (28, 30).

Recently, we investigated the genetic determinant of adhesive function of type 1 fimbriae of *E. coli* by restriction site deletion analysis of a recombinant plasmid, pSH2, carrying the genetic information for the synthesis and expression of functional type 1 fimbriae (23). A mutant plasmid, pUT2002, containing a deletion remote from the structural gene (Fig. 1), encoded the 17-kDa subunit protein of type 1 fimbriae but failed to exhibit D-mannose-specific binding, even though the bacteria expressed fimbriae morphologically and antigenically indistinguishable from those produced by the intact recombinant plasmid (23). The isolated mutant fimbriae failed to agglutinate guinea pig erythrocytes, but reacted with a monoclonal antibody specific for quaternary structural determinants of type 1 fimbriae. Moreover, the dissociated 17-kDa fimbrial subunits from this mutant were indistinguishable from subunits of normal fimbriae by their migration in sodium dodecyl sulfate (SDS) gels, by their reactivity with a monoclonal antibody directed against a 17-kDa subunit-specific epitope, and in enzyme-linked immunosorbent assays (ELISAs) with monospecific antisera (23). We have concluded that the adhesive function of type 1 fimbriae is dependent on a factor(s) encoded by a gene other than those required for synthesis, assembly, and expression of the 17-kDa subunit (23). This is consistent with recent findings of Maurer and Orndorff, who showed that fimbriae obtained from a pSH2 deletion mutant mapping in the same region as the deletion in pUT2002 failed to agglutinate guinea pig erythrocytes (21, 22). Similar findings have been reported in pyelonephritogenic *E. coli*: the adhesive function of Pap fimbriae is mediated by three ancillary gene products, PapE, PapF, and PapG (20, 24, 32). That PapE and PapF are components of the Pap fimbriae was demonstrated by the development in animals immunized with purified Pap fimbriae of specific antibodies against both PapE and PapF as well as the major PapA proteins (20).

Several laboratories have provided different designations for the genes and gene products of the type 1 *fim* locus (13, 16, 18, 22, 23). For convenience, we have utilized the designations of Klemm et al. (18). The restriction map and nucleotide sequence of the region in the type 1 *fim* gene cluster corresponding to and contiguous with the deletion on pUT2002 were recently reported by Klemm and Christiansen (17). On the basis of the nucleotide sequence, the

* Corresponding author.
† Present address: Department of Molecular Genetics and Microbiology, University of Massachusetts, Worcester, MA 01605.

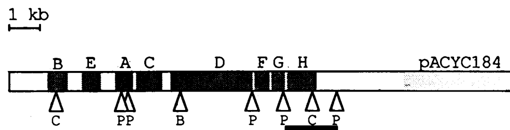

FIG. 1. A partial restriction map of plasmid pSH2, showing the locations of the *fimA* through *fimH* genes. These locations are based on the DNA sequences determined by Klemm et al. (17) from an independently constructed recombinant plasmid carrying the *fim* genes of *E. coli* K-12 strain PC31. All of the restriction endonuclease recognition sites (P, *Pvu*II; C, *Cla*I; B, *Bam*HI) indicated by the vertical arrows are predicted by the sequence data and were found to be present in pSH2. The heavy black bars indicate the *Pvu*II fragment deleted in the mutant plasmid pUT2002 (23).

primary structure of three ancillary fimbrial proteins, FimF, FimG, and FimH, were derived.

Because our mutant plasmid, pUT2002, encoded nonadhesive fimbriae and possessed a deletion of *fimH* and part of *fimG* (see Fig. 1), we investigated the possibility that either or both gene products were structural components of type 1 fimbriae that may serve as the adhesin of organisms bearing these fimbriae. For this purpose, we employed antibodies raised against synthetic peptides copying the amino termini of the mature FimG and FimH proteins. In this paper, we report evidence that both proteins are present in wild-type, type 1 fimbriae but not in the mutant fimbriae encoded by pUT2002. Moreover, FimH appears to be located at the fimbrial tips and at long intervals along the length of the fimbrial filaments, and antibodies to synthetic peptides of FimH, but not of FimG or of FimA, are antiadhesive.

MATERIALS AND METHODS

Synthesis of peptides corresponding to proteins of type 1 fimbriae. Copies of the NH_2 termini of FimG and FimH were synthesized in an automated peptide synthesizer (Beckman Instruments, Fullerton, Calif.) by a solid-phase method (4, 7) and purified as described previously (4, 8, 11). The purity of the peptide product was determined by high-performance liquid chromatography on a reverse-phase column and by quantitative amino acid analysis (8, 11). The peptides synthesized for this study are designated S-T1FimG(1-16) and S-T1FimH(1-25)C, or S-FimG and S-FimH.

Conjugation of synthetic peptides. The synthetic peptides were conjugated to keyhole lympet hemocyanin by using succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (Pierce Chemical Co., Rockford, Ill.) as described (35), with the modifications of Rothbard et al. (27).

Immunization of rabbits. Each of three New Zealand white rabbits was injected subcutaneously with 100 μg of the conjugated peptides, which had been emulsified in complete Freund adjuvant (2, 4, 8). Blood was obtained before the initial injection and at 2-week intervals thereafter. At 4 and 10 weeks, the animals were given booster injections of 100 μg of the conjugated peptide in phosphate-buffered saline.

Assays of antibody reactivity. Detection and titration of antibodies were performed by using an ELISA of the antiserum. The assay was performed as previously described (4, 8) except that purified fimbriae (5 μg/ml) or unconjugated synthetic peptide (10 μg/ml) was adsorbed to ELISA trays as the solid-phase antigen. ELISA inhibition experiments were performed by making serial twofold dilutions of the inhibitor in 0.5 ml of phosphate-buffered saline to which was added a constant dilution of antiserum. Each of the antisera used in this assay was first absorbed with the mutant nonadhesive fimbriae to remove all antibodies that might cross-react with the major fimbrial subunit. The mixture was incubated at 37°C for 30 min and then added to the antigen-coated trays. After this incubation, standard ELISA procedures were carried out.

Bacterial strains and culture conditions. *E. coli* ORN103 [*thr-1 leu-6 thi-1* Δ(*argF-lac*)*U169 xyl-7 ara-13 mtl-2 gal-6 rpsL tonA2 fhuA2 minA minB recA13* Δ(*fimABCDEFGH*)], which lacks all of the *fim* genes (22) (kindly furnished by Paul Orndorff, North Carolina State University, Raleigh), was used as the host strain for plasmid pSH2 and a derivative, pUT2002, which code for type 1 and mutant nonadhesive fimbriae, respectively. Plasmid pSH2 (a kind gift from Shiella Hull, Baylor Medical College, Houston, Tex.) contains a cloned segment of chromosomal DNA with the *fim* locus from a clinical isolate of *E. coli*. The properties of these plasmids have been described in detail elsewhere (15, 23, 26). For best expression of fimbriae, strain ORN103 was grown in Luria broth or in brain heart infusion, both under static conditions for 48 h. To maintain the plasmid in the host, 20 μg of chloramphenicol per ml was incorporated into the medium.

Other type 1 fimbria-expressing strains of *E. coli* employed in this study were CSH50 [*ara* Δ(*lac-pro*)*rpsL thi*], a well-characterized K-12 strain (3, 9, 13), and CI5, a previously described strain isolated from a patient with a urinary tract infection (1, 29).

Isolation and purification of type 1 fimbriae. Type 1 fimbriae were isolated and purified from all the *E. coli* strains by the method of Dodd and Eisenstein (9). The purity of the fimbrial preparations was verified by SDS-polyacrylamide gel electrophoresis (PAGE) and by electron microscopy.

SDS-PAGE. SDS-PAGE was performed in a 1.5-mm-thick 15% or 18% slab gel by the system of Laemmli (19). Samples of fimbriae were dissociated before electrophoresis by heating in acid (9).

Immunoelectroblots of fimbriae. After SDS-PAGE, the bands of fimbrial protein were electrophoretically transferred onto nitrocellulose paper as described previously (3, 29, 31). After transfer, the nitrocellulose paper strips were incubated with appropriate dilutions of antisera (1:50 to 1:200), followed by enzyme-linked goat anti-rabbit immunoglobulin G antibody and finally by the substrate (29).

Immunoelectron microscopy. Samples of type 1 fimbriae were applied to Formvar-coated copper grids and placed on a drop of 0.5% bovine serum albumin for 15 min. The grids were next placed on a drop of antiserum diluted 1:40 for 20 min at ambient temperature, washed several times in deionized water, and incubated for 20 min at ambient temperature in a drop of gold-conjugated protein A (5- or 10-nm-sized gold particles) diluted 1:30. The grids were then washed several times and finally stained with phosphotungstic acid. The samples were examined with a Zeiss electron microscope.

Assays of the inhibition of *E. coli* attachment to immobilized epithelial cells. The ability of antibodies evoked against synthetic fimbrial peptides to block attachment of type 1 fimbriated *E. coli* to human buccal epithelial cells was examined by employing a recently described ELISA technique (25) in which epithelial cells scraped from human oral mucosa are immobilized onto flat-bottomed microtiter plates and the adhesion of biotinylated *E. coli* cells to the epithelial cells is assayed with peroxidase-labeled avidin as described previously (25). This procedure of biotinylation has been shown not to affect the D-mannose-binding property of the organisms (25). The biotinylated bacteria were preincubated

with the appropriate dilution of the antisera for 30 min at ambient temperature, and then this mixture was added to microtiter wells containing the immobilized cells. The microtiter plates were rotated horizontally for 1 h at ambient temperature and then washed four times with phosphate-buffered saline. After the last wash was removed, the plates were fixed by heating at 65°C for 10 min. A 1:5,000 dilution of the avidin-peroxidase conjugate was added to each well and incubated for 30 min at 37°C. The plates were then washed three times with phosphate-buffered saline, and *o*-phenylenediamine solution was added to each well. The color was allowed to develop for 15 min, and the A_{650} was measured. Controls consisted of preimmune sera instead of hyperimmune sera and wells without bacteria to ensure that the primary antibody did not react with the endogenous bacteria or with the host cells. The percentage of inhibition was calculated by comparing the absorbance values obtained with the test sera with the values obtained with the preimmune sera: Percentage of inhibition = [1 − (A_{405} of immune serum/A_{405} of preimmune serum)] × 100.

RESULTS

The synthetic fimbrial peptides were purified by high-performance liquid chromatography, and their sequences were confirmed by automated Edman degradation as ADVTITVAGKVVAKPC for S-T1FimG(1-16) and CKTANGTAIPIGGSANVYVNLAPVVC for S-T1FimH(1-25)C. These sequences were found to be identical to the corresponding amino-terminal regions of FimG and FimH predicted from the nucleotide sequences reported by Klemm and Christiansen (17). The purified synthetic peptides and a synthetic peptide copying the amino-terminal region of FimA (2) were employed in the following studies.

Immunogenicity of synthetic peptides. The synthetic peptides were coupled to keyhole limpet hemocyanin by the bifunctional cross-linking agent as described in Materials and Methods. The conjugated peptides, emulsified in complete Freund adjuvant and injected into rabbits, evoked antibodies against the respective peptides ranging in dilutions from 1:52,200 to 1:102,400 against the respective peptides by ELISA. Anti-S-FimG and anti-S-FimH reacted with isolated type 1 fimbriae at dilutions of 1:1,000 to 1:2,500 by ELISA. These antisera and antisera previously (2) raised against S-T1FimA(23-35)C were used to probe the intact and dissociated type 1 fimbriae.

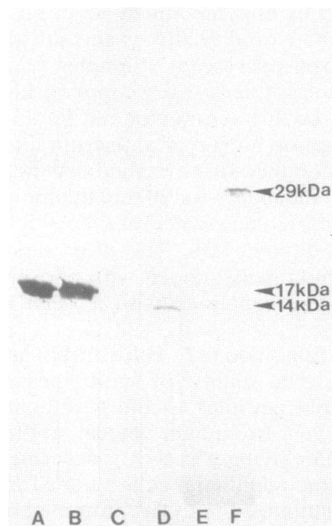

FIG. 2. Western blots of dissociated fimbriae from *E. coli* ORN103(pUT2002) (lanes A, C, and E) and ORN103(pSH2) (lanes B, D, and F). Lanes A and B were stained with amido black. Lanes C and D were reacted with anti-S-FimG, and lanes E and F were reacted with anti-S-FimH.

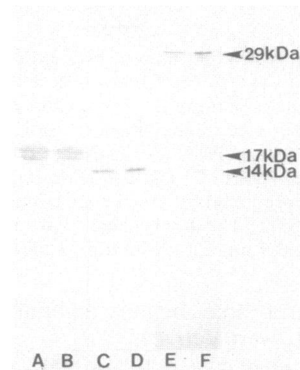

FIG. 3. SDS-PAGE and Western blot analysis of dissociated type 1 fimbriae from *E. coli* CSH50 (lanes A, C, and E) and CI5 (lanes B, D, and F). Lanes A and B were stained with Commassie blue. Lanes B and C were reacted with anti-S-FimG, and lanes E and F were reacted with anti-S-FimH.

Demonstration of FimG and FimH as minor components of type 1 fimbriae. Purified preparations of *E. coli* ORN103 (pSH2) and ORN103(pUT2002) fimbriae were dissociated by boiling at pH 1.5, electrophoresed on a 15% SDS gel, transferred onto nitrocellulose, and reacted with each of the synthetic peptide antisera. Anti-S-FimA reacted strongly with a 17-kDa band in both the adhesive and nonadhesive fimbriae, as previously reported (2). Anti-FimG and anti-FimH reacted with a 14-kDa and a 29-kDa band, respectively, only in the wild-type fimbriae; these bands were conspicuously absent in the mutant pUT2002 fimbriae (Fig. 2). The 14- and 29-kDa bands were not detectable in the wild-type fimbriae by amido black stains of the separated components transferred to nitrocellulose (Fig. 2). These results suggest that FimG and FimH are minor but integral components of adhesive type 1 fimbriae.

Presence of FimG and FimH in other strains of type 1 fimbriated *E. coli*. Isolated type 1 fimbriae prepared from a K-12 strain, CSH50, and a clinical isolate, CI5, were similarly probed with the synthetic peptide antisera. Protein bands identical in molecular weight to FimG and FimH were stained in Western blots (immunoblots) of the separated fimbrial components by anti-S-FimG and anti-S-FimH, respectively (Fig. 3). Interestingly, in previous studies polyclonal and monoclonal antibodies against FimA CSH50 fimbriae reacted with a 17-kDa band of CSH50 fimbriae but not with CI5 fimbriae (3). These results further suggest that FimG and FimH are conserved structural components of *E. coli* type 1 fimbriae.

Reactions of anti-S-FimG and anti-S-FimH with native type 1 fimbriae. The recognition by the antibodies of FimG and FimH in intact fimbriae was demonstrated by ELISA and ELISA inhibition tests. Anti-S-FimG reacted with isolated wild-type 1 fimbriae at a dilution of 1:1,280, and anti-S-FimH

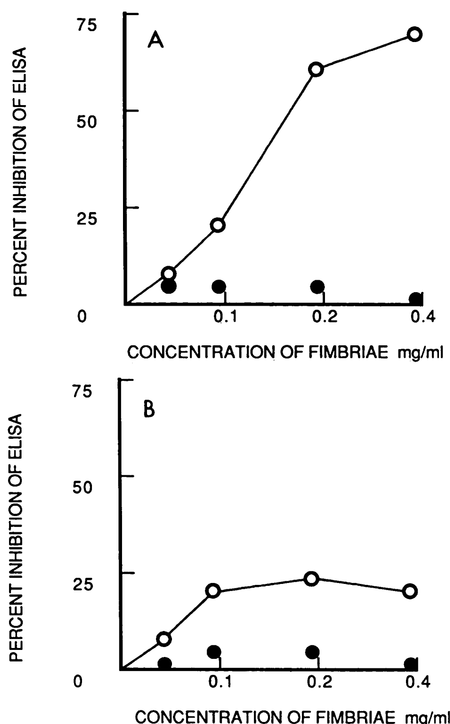

FIG. 4. ELISA inhibition assays of (A) anti-FimH and (B) anti-FimG with isolated type 1 fimbriae using as inhibitor either wild-type (○) or mutant (●) fimbriae.

reacted with such fimbriae at a dilution of 1:2,560. The reactions of anti-S-FimG and anti-S-FimH with the wild-type fimbriae were inhibited by suspended wild-type but not mutant (*fimG* and *fimH*) fimbriae (Fig. 4). The inhibition of anti-S-FimG by the suspended wild-type fimbriae was weaker than that of anti-S-FimH, suggesting that the epitopes against which the antibodies were directed are well exposed both in the immobilized and the suspended state for FimH but only in the immobilized state for FimG.

Immunoelectron microscopy of type 1 fimbriae with anti-S-FimG and anti-S-FimH. To visualize the location of FimG and FimH in the fimbrial filament, we examined the binding of each of the antibodies to type 1 fimbriated *E. coli* by electron microscopy. Binding of the antibodies to the fimbriae was probed with protein A conjugated to gold particles. In organisms treated with anti-S-FimG, no gold particles were detected associated either with the fimbriae or with the outer surfaces of the bacteria (Fig. 5A). In contrast, anti-S-FimH decorated fimbriae mainly at the tips, but also at long intervals along the length of the fimbrial filaments (Fig. 5B and C). Neither anti-S-FimG nor anti-S-FimH reacted with the nonadhesive, mutant pUT2002 fimbriae by immunoelectron microscopy (not shown in figure). These results indicate that FimH is located mainly at the tips of type 1 fimbriae and that the mutant *fimG fimH* fimbriae lack these minor components. The location of FimG could not be determined because the anti-S-FimG appears to be directed at epitopes of FimG that are inaccessible on native type 1 fimbriae.

Antiadhesive properties of anti-S-T1FimH(1-25)C. Because the nonadhesive mutant fimbriae lacked both FimG and FimH it was considered possible that the adhesive property of type 1 fimbriae is dependent on the presence of either one or both of these proteins. If either of these ancillary proteins is involved in mediating adherence or is close to the adhesive moiety, then antibody directed against the protein should block the adhesive function of the fimbriae. Therefore, we attempted to block the attachment of type 1 fimbriated *E. coli* to human buccal epithelial cells by employing each of the antisera as an inhibitor. The binding of type 1 fimbriated *E. coli* to human buccal epithelial cells was inhibited by 12-week rabbit antiserum raised against S-FimH (58% inhibition), but not by similar antisera to S-FimA (7%) or S-FimG (2%) or by a 1:10 dilution of preimmune rabbit sera (0%). Taken together with the results obtained above, these results suggest that FimH, a fimbrial tip protein, serves as the adhesin of *E. coli* type 1 fimbriae. Although FimG also appears to be a minor structural component of type 1 fimbriae, no conclusion can be drawn as to its location or its role in adhesion.

DISCUSSION

In our earlier studies we demonstrated that the genetic determinant for the 17-kDa subunit of type 1 fimbriae of *E. coli* was distinct from the determinant mediating D-mannose binding. Furthermore, we postulated the possibility that a minor fimbrial component(s) may mediate binding. We attempted to find such a component(s) by immunochemical analysis of fimbriae isolated from a wild-type strain, ORN103(pSH2), and its nonadhesive mutant derivative ORN103(pUT2002). In this communication, we demonstrate that type 1 fimbriae of *E. coli* are composed of proteins other than the 17-kDa subunit. We have identified two hitherto undescribed fimbrial subunits of type 1 fimbriae, FimG (14 kDa) and FimH (29 kDa). Either or both may play a role in mediating attachment because both are absent in the nonadhesive mutant fimbriae. We have also demonstrated that antibodies directed at FimH and not those directed at FimA or FimG inhibit the adhesion of type 1 fimbriated *E. coli* to epithelial cells.

Although suggestive, our data do not prove that FimH is the determinant of mannose-specific binding of type 1 fimbriae. Nevertheless, there are some highly suggestive observations from this and other laboratories which support this notion. Recently, we observed that a hyperadhesive mutant derived from the pSH2 clone of *E. coli* expressed large quantities of a 29-kDa protein (S. N. Abraham, J. D. Goguen, and E. H. Beachey, manuscript in preparation). The isolated 29-kDa protein manifested many of the D-mannose-binding properties of isolated type 1 fimbriae. Recently, Maurer and Orndorff (21, 22) described a gene, *pilE*, on the pSH2 plasmid which appears to map in the same region as the pUT2002 deletion in the *fim* gene cluster. These workers reported that inactivation of *pilE*, either by restriction site mutagenesis or Tn*5* insertion, resulted in loss of receptor binding capacity (21, 22). The *pilE* gene product was revealed to be a 31-kDa protein (22) which is comparable to FimH.

Brinton (5) proposed that the adhesive moiety on type 1 fimbriae is situated at the fimbrial tips because isolated fimbriae were observed to attach to polystyrene latex spheres only by their tips. However, others found the concept of a single specific binding site located at the tip of the fimbriae untenable, because this would make isolated fimbriae monovalent. It would then be difficult to explain the hemagglutinating capacity of isolated fimbriae (30). A lateral site for the adhesive moiety was proposed by Sweeny and Freer to explain both the hemagglutinating property of isolated type 1 fimbriae and the observation that fragmentation of isolated fimbriae did not decrease their ability to bind

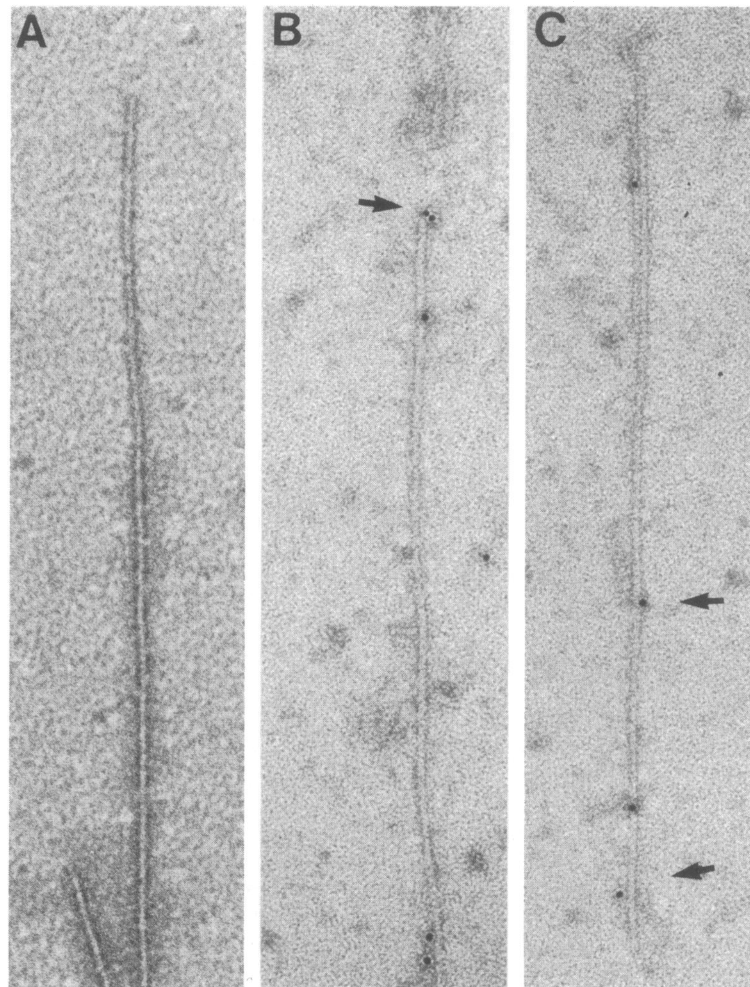

FIG. 5. Electron micrographs of immunogold labeling of isolated fimbriae from *E. coli* ORN103(pSH2) reacted with anti-S-FimG (A) and anti-S-FimH (B and C). Note gold particles are not associated with fimbriae in panel A. In contrast, gold particles are seen at the fimbrial tips in panel B and along the length of the fimbrial filament in panel C. Magnification, ×138,500.

to eucaryotic cells (30). Interestingly, our immunoelectron microscopic findings suggest that FimH resides both at the tips and at long intervals along the length of the fimbriae. If FimH is indeed the adhesive moiety of type 1 fimbriae, then it would appear that our findings are consistent with both concepts.

Antisera directed at FimG did not block attachment of type 1 fimbriae. It is also clear, however, that we could not visualize its binding to the intact fimbriae. The apparent ability of anti-S-FimG to recognize FimG in dissociated or immobilized fimbriae but not in the assembled, suspended, or intact fimbriae is consistent with the notion that the amino terminus of FimG is inaccessible to antibodies in the intact fimbriae. Thus, it is conceivable that FimG may yet have a role in determining the adhesive property of type 1 fimbriae. Indeed, recent complementation studies of Klemm and Christiansen (17) suggest that FimH in concert with FimG or FimF confers adhesive properties to type 1 fimbriae; FimH alone is insufficient. Thus, FimG may be involved in the structural assembly of FimH or as a coadhesin. Similar studies of Pap fimbriae of *E. coli* have suggested the role of more than one fimbrial protein in mediating attachment (20, 24).

It is now widely accepted that foretelling antigenic sites from the primary structure of a protein based solely on Chou and Fassman (6) and Hopp and Wood (14) predictions is far from accurate (33). Our reason for employing the amino-terminal region as an immunogen is based on recent reports that the immunodominant region of fimbrial subunits resides in the amino terminus. For example, Worobec et al. have shown that the immunodominant region of strain EDP208 fimbriae resides specifically within the amino-terminal dodecapeptide (34). Furthermore, antibodies raised against these amino-terminal synthetic peptides reacted avidly with the native EDP208 fimbriae. We have previously demonstrated the immunogenicity of the amino-terminal region of FimA (2). The results of our current study demonstrate that the selected amino-terminal regions of FimG and FimH were highly immunogenic and that the resulting antibodies were able to detect the native protein in the fimbrial preparation by Western blots. Whether or not other sequences of FimG will evoke antibodies that recognize the subunit in native fimbriae requires further study.

It is of interest that proteins corresponding immunologically and structurally to FimG and FimH on the pSH2 fimbriae are present on type 1 fimbriae of a K-12 and a

clinical strain of *E. coli*. This suggests that the basic structural composition of the type 1 fimbrial filament is the same in at least two markedly different strains of *E. coli*. Whether or not these structures are antigenically conserved in type 1 fimbriae of other members of the *Enterobacteriaceae* requires further study.

Few studies on the structure of type 1 fimbrial filaments have been undertaken since the X-ray fiber diffraction studies of Brinton (5), which indicated that type 1 fimbriae are comprised of 17-kDa subunits arranged in a right-handed helix with 3 1/8 subunits per turn. Our findings that type 1 fimbriae contain at least two additional subunits, one of which is located at the tips and interspersed at long intervals among the major subunits in the fimbrial filament, suggests the need to reassess the current concept of the three-dimensional structure of type 1 fimbriae. A comparison of the quaternary structural arrangements of subunits in adhesive and nonadhesive fimbriae may shed further light on fimbrial structure and assembly. Continued efforts to identify the adhesive moiety and the functions of the other ancillary subunits of type 1 fimbriae should also allow the development of approaches to prevent fimbrial attachment and subsequent bacterial colonization.

ACKNOWLEDGMENTS

The peptides used in this study were synthesized in the Protein Chemistry Laboratory of the Veterans Administration Medical Center under the supervision of Jerome M. Seyer, whose assistance is deeply appreciated. We also thank Edna Chiang for expert technical assistance, Loretta Hatmaker for excellent electron micrographs, and Johnnie Smith for efficient secretarial assistance.

This study was supported by research funds from the U. S. Veterans Administration and by Public Health Service grants AI-23821, AI-13550, and AI-10085 from the National Institutes of Health.

LITERATURE CITED

1. **Abraham, S. N., J. P. Babu, C. S. Giampapa, D. L. Hasty, W. A. Simpson, and E. H. Beachey.** 1985. Protection against *Escherichia coli*-induced urinary tract infections with hybridoma antibodies directed against type 1 fimbriae or complementary D-mannose receptors. Infect. Immun. **48:**625–628.
2. **Abraham, S. N., and E. H. Beachey.** 1987. Assembly of a chemically synthesized peptide of *Escherichia coli* type 1 fimbriae into fimbria-like antigenic structures. J. Bacteriol. **169:** 2460–2465.
3. **Abraham, S. N., D. L. Hasty, W. A. Simpson, and E. H. Beachey.** 1983. Anti-adhesive properties of a quaternary structure-specific hybridoma antibody against type 1 fimbriae of *Escherichia coli*. J. Exp. Med. **158:**1128–1144.
4. **Beachey, E. H., and J. M. Seyer.** 1986. Protective and nonprotective epitopes of chemically synthesized peptides of type 6 streptococcal M protein. J. Immunol. **136:**2287–2292.
5. **Brinton, C. C.** 1965. The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. Trans. N.Y. Acad. Sci. **27:**1003–1165.
6. **Chou, Y., and D. Fassman.** 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. **47:**45–75.
7. **Dale, J. B., and E. H. Beachey.** 1986. Localization of protective epitopes of the aminoterminus of type 5 streptococcal M protein. J. Exp. Med. **163:**1176–1191.
8. **Dale, J. B., J. M. Seyer, and E. H. Beachey.** 1983. Type-specific immunogenicity of a chemically synthesized peptide fragment of type 5 streptococcal M protein. J. Exp. Med. **158:**1727–1730.
9. **Dodd, D. C., and B. I. Eisenstein.** 1982. Antigenic quantitation of type 1 fimbriae on the surface of *Escherichia coli* cells by enzyme-linked immunosorbent inhibition assay. Infect. Immun. **38:**764–773.
10. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of *Enterobacteriaceae*, p. 185–217. *In* E. H. Beachey (ed.), Bacterial adherence, receptors and recognition, series B, vol. 6. Chapman and Hall, London.
11. **Edman, P., and G. Begg.** 1967. Automated equipment for sequence determination. Eur. J. Biochem. **1:**80–91.
12. **Eshdat, Y., F. J. Silverblatt, and N. Sharon.** 1981. Dissociation and reassembly of *Escherichia coli* type 1 pili. J. Bacteriol. **148:**308–314.
13. **Frietag, C. S., and B. I. Eisenstein.** 1983. Genetic mapping and transcriptional orientation of the *fimD* gene. J. Bacteriol. **156:**1052–1058.
14. **Hopp, T. P., and K. R. Wood.** 1981. Prediction of protein antigenic determinants from amino acid sequence. Proc. Natl. Acad. Sci. USA **78:**3824–3828.
15. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli*. Infect. Immun. **33:**933–938.
16. **Klemm, P.** 1984. The *fimA* gene encoding the type 1 fimbrial subunit of *Escherichia coli*. Eur. J. Biochem. **143:**395–399.
17. **Klemm, P., and G. Christensen.** 1987. Three *fim* genes required for the regulation of length and mediation of adhesion of *Escherichia coli* type 1 fimbriae. Mol. Gen. Genet. **208:**439–445.
18. **Klemm, P., B. J. Jorgensen, I. van Die, H. de Ree, and H. Bergmans.** 1985. The *fim* genes responsible for synthesis of type 1 fimbriae in *Escherichia coli*, cloning and genetic organization. Mol. Gen. Genet. **199:**410–414.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **117:**680–685.
20. **Lindberg, F., B. Lund, and S. Normark.** 1986. Gene products specifying adhesion of uropathogenic *Escherichia coli* are minor components of pili. Proc. Natl. Acad. Sci. USA **83:**1891–1895.
21. **Maurer, L., and P. E. Orndorff.** 1985. A new locus, *pilE* required for the binding of type 1 piliated *Escherichia coli* to erythrocytes. FEMS Microbiol. Lett. **30:**59–66.
22. **Maurer, L., and P. Orndorff.** 1987. Identification and characterization of genes determining receptor binding and pilus length of *Escherichia coli* type 1 pili. J. Bacteriol. **169:**642–645.
23. **Minion, F. C., S. N. Abraham, E. H. Beachey, and J. D. Goguen.** 1986. The genetic determinant of adhesive function in type 1 fimbriae of *Escherichia coli* is distinct from the gene encoding the fimbrial subunit. J. Bacteriol. **165:**1033–1036.
24. **Norgren, M., S. Normark, D. Lark, P. O'Hanley, G. Schoolnik, S. Falkow, C. Svanborg-Eden, M. Baga, and B. E. Uhlin.** 1984. Mutations in *E. coli* cistrons affecting adhesion to human cells do not abolish Pap pili fiber formation. EMBO J. **3:**1159–1165.
25. **Ofek, I., H. S. Courtney, D. M. Schifferli, and E. H. Beachey.** 1986. Enzyme-linked immunosorbent assay for adherence of bacteria to animal cells. J. Clin. Microbiol. **24:**512–516.
26. **Orndorff, P. E., and S. Falkow.** 1984. Identification and characterization of a gene product that regulates type 1 piliation in *Escherichia coli*. J. Bacteriol. **160:**61–66.
27. **Rothbard, J. B., R. Fernandez, and G. K. Schoolnick.** 1984. Strain specific and common epitopes of gonococcal pili. J. Exp. Med. **160:**208–213.
28. **Salit, I. E., and E. C. Gotschlich.** 1977. Type 1 *Escherichia coli* fimbriae: characterization of binding to monkey kidney cells. J. Exp. Med. **146:**1182–1192.
29. **Schifferli, D. M., S. N. Abraham, and E. H. Beachey.** 1986. Synergistic effects of Trimethoprim and Sulfamethoxazole on synthesis, expression and haemagglutinating activity of type 1 fimbriae of *Escherichia coli*. J. Infect. Dis. **154:**490–496.
30. **Sweeney, G., and J. H. Freer.** 1979. Localization of binding sites on common type 1 fimbriae of *Escherichia coli*. J. Gen. Microbiol. **112:**321–328.
31. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and applications. Proc. Natl. Acad. Sci. USA **76:**4350–4352.
32. **Uhlin, B. E., M. Norgren, M. Baga, and S. Normark.** 1985. Adhesion to human cells by *Escherichia coli* lacking the major

subunit of a digalactoside-specific pilus adhesin. Proc. Natl. Acad. Sci. USA **82:**1800–1804.

33. **Watts, T. H., P. A. Sastri, R. S. Hodges, and W. Paranchych.** 1983. Mapping of the antigenic determinants of *Pseudomonas aeruginosa* PAK polar pili. Infect. Immun. **42:**113–121.
34. **Worobec, E. A., W. Paranchych, J. M. Parker, A. K. Taneja, and R. S. Hodges.** 1985. Antigen-antibody interaction: the immunodominant region of EDP208 pili. J. Biol. Chem. **260:** 938–945.
35. **Yoshitake, S., Y. Yamada, E. Ishikawa, and R. Masseyeff.** 1979. Conjugation of glucose oxidase from *Aspergillus niger* and rabbit antibodies using N-hydroxysuccimide ester of N-(4-carboxycyclohexylmethyl) malemide. Eur. J. Biochem. **101:** 395–398.