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Isolation and Separation of Physicochemically Distinct Fimbrial Types Expressed on a Single Culture of *Escherichia coli* O7:K1:H6

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The fimbrial (pili) profile of a single strain of *Escherichia coli* O7:K1:H6 (WF96) was evaluated. Fimbriae were isolated by sucrose density gradient ultracentrifugation, purified from flagellae by the use of 0.4% sodium dodecyl sulfate (SDS), and separated into distinct fimbrial types. Analysis of the purified WF96 fimbriae by SDS-polyacrylamide gel electrophoresis revealed two polypeptide bands with molecular weights of 16,000 and 21,000. Treatment of the fimbrial mixture with saturated guanidine hydrochloride resulted in the appearance of a third band with a molecular weight of 19,500. The relative susceptibilities of the WF96 fimbrial types to disrupting chemicals (octyl-glucoside, urea, SDS, and guanidine hydrochloride) were assessed by exposure of the fimbrial mixture to each agent, separation of the depolymerized fimbriae from intact fimbriae by gel filtration on Sepharose CL-4B, and identification of the disaggregated fimbrial types by SDS-polyacrylamide gel electrophoresis of column fractions. The physicochemical heterogeneity of the three fimbrial types coexpressed on WF96 was exploited to develop a method for separation of individual fimbriae.

Fimbriae (also called pili) are expressed on the surface of many gram-negative bacteria as filamentous polymers of highly hydrophobic protein subunits (3, 20). These bacterial appendages may, under certain circumstances, play an important role in the host-parasite interaction by mediating the attachment of bacteria to mammalian cells via specific eucaryotic receptors (2, 7, 17, 20, 22). Adherence of fimbriae to specific receptors is also the basis for the agglutination of erythrocytes (RBCs) by fimbriated bacteria. The agglutination of guinea pig RBCs by some strains of *Escherichia coli* can be blocked with D-mannose or its analogs (6, 20). Fimbriae from such strains are called mannose-sensitive hemagglutinins, or type 1 fimbriae. These mannose-sensitive adhesins are found on 50 to 70% of all *E. coli* isolates and are particularly prevalent among normal flora isolates of *E. coli* (21). By contrast, most strains of *E. coli* that are associated with intestinal or extraintestinal disease in humans agglutinate guinea pig RBCs even in the presence of mannose (21).

The finding that pathogenic strains of *E. coli* exhibited both mannose-sensitive agglutination of guinea pig RBCs and mannose-resistant agglutination of other kinds of RBCs (5, 9) first suggested that a single culture of *E. coli* can produce more than one kind of fimbria. Subsequently, Jann et al. (10) showed that fimbrial preparations from certain pathogenic strains of *E. coli* formed multiple bands when subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). However, as they pointed out (10), these multiple SDS-PAGE bands could represent either single subunits from different types of fimbriae or different subunits from a single kind. Similar multiple bands have been described by Korhonen et al. for P-antigen-recognizing fimbriae from *E. coli* (13). More recently, Klemm et al. (11) used both crossed immunoelectrophoresis and N-terminal amino acid analysis to unequivocally demonstrate expression of multiple fimbrial types by a single strain of *E. coli*.

Analysis of the importance of a particular fimbrial type in the adhesion of a multiply fimbriated *E. coli* strain to mammalian cells necessitates that each fimbrial type be isolated in pure form. Rhen et al. (19) were the first to accomplish such a separation. They used sequential immunoprecipitation to isolate three fimbrial types expressed by *E. coli* strain KS71. The limitation of such a procedure is that only serologically distinct fimbrial types can be isolated.

The purpose of this study was to isolate, purify, and characterize the fimbriae expressed by a single strain of *E. coli* O7:K1:H6. It will be shown that this *E. coli* strain coexpresses three distinct kinds of fimbriae and that these fimbriae are soluble to varying degrees in chemical-disrupting agents. We also describe a method for separation and purification of each of the fimbrial types based on these physicochemical differences among the fimbriae.

MATERIALS AND METHODS

Bacteria. *E. coli* strain WF96, serotype O7:K1:H6, was obtained from St. Mary's Hospital, London, England. This strain belongs to hemagglutination type VII-C as performed by the hemagglutination assay described by Evans et al. (9). *E. coli* strain D509 of serogroup O86 was originally obtained from J. P. Duguid, Dundee University, Dundee, Scotland. The D509 isolate exhibits only mannose-sensitive hemagglutination of guinea pig RBCs and hence expresses type 1 fimbriae. Because the effect of various disruptive chemicals on type 1 fimbriae is well documented (8, 16, 20), the fimbrial preparation from strain D509 was used throughout this study as a standard against which similarly treated WF96 fimbriae were compared.

Bacteria were grown in Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md.) for 48 h at 37°C without agitation.

Purification of fimbriae. Fimbriae were isolated from *E. coli* WF96 and D509 by a modification of the procedure described by Korhonen et al. (12). Briefly, bacteria were

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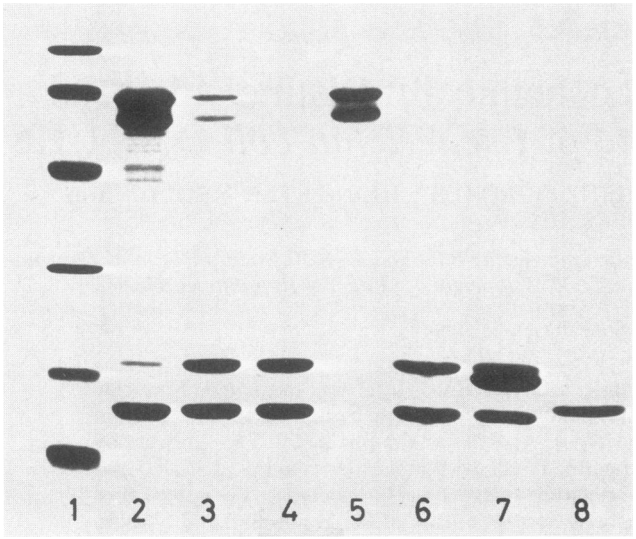

FIG. 1. SDS-PAGE of purification of fimbriae from *E. coli* WF96. Lane 1, Molecular weight markers purchased as a mixture from Pharmacia (low-molecular-weight proteins kit; contains phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin [see text for molecular weights]). Lane 2, 20% ammonium sulfate precipitate from WF96 fimbriae after blending in an Omnimixer. Lane 3, Fimbriae (and flagellae)-containing fractions from sucrose gradient ultracentrifugation. Lane 4, Pooled fractions (10 to 15) from the void volume of the Sepharose CL-4B chromatography shown in Fig. 2. Lane 5, Low-molecular-weight fractions (22 to 26) of the same column. Lane 6, Purified fimbriae from WF96 boiled in sample buffer (5 min, 2% SDS). Lane 7, The sample from lane 6 after pretreatment with 8 M guanidine hydrochloride. Lane 8, Type 1 fimbriae from strain D509 run on this gel for comparison.

collected by centrifugation (6,000 × *g*, 4°C, 30 min), suspended in 10 mM Tris-hydrochloride (pH 7.2), blended in a Sorvall Omnimixer (top speed, 2 min, 4°C), and pelleted by centrifugation (8,000 × *g*, 30 min). The fimbriae (and flagellae for some strains) were precipitated with 20% ammonium sulfate, collected by centrifugation at 15,000 × *g* for 30 min, suspended in 10 mM Tris-hydrochloride (pH 7.2), and layered on top of a 35-ml linear sucrose gradient (15 to 50% [wt/wt] sucrose in 10 mM Tris-hydrochloride). This gradient was subjected to ultracentrifugation (Kontron TGA 55; Kontron Analytical, Munich, Federal Republic of Germany) at 90,000 × *g* in a swinging rotor for 14 h and 4°C. The optical density of each 1-ml fraction was determined by refractometry. Fimbriae banded at a density of 1.117 to 1.137 g/cm^{-3}, as shown by electron microscopy. Fimbria-containing fractions were combined and dialyzed for 24 h against distilled water to remove sucrose.

When the heavily flagellated WF96 strain was used for fimbrial purification, the procedure described resulted in significant contamination with flagellae. Therefore, fimbriae were incubated for 1 h at 37°C with 0.4% SDS to selectively deaggregate contaminating flagellae. The dissociated flagellae were separated from intact fimbriae by gel filtration on Sepharose CL-4B (Pharmacia, Uppsala, Sweden) equilibrated in 5 mM Tris-hydrochloride buffer (pH 7.2) that contained 0.4% SDS–0.1% EDTA. Only fimbriae eluted in the void volume (measured with blue dextran 2000; Pharmacia); flagellae depolymerized under these conditions (as assessed by electron microscopy) and eluted as low-molecular-weight components. After extensive dialysis to remove detergent, this fimbrial preparation was used for separation of the distinct fimbrial types.

Separation of distinct fimbrial types. The purified fimbrial preparation was incubated (2 h, 37°C) in 10 mM Tris-hydrochloride buffer (pH 7.2) containing 30 mM octyl-glucoside (Sigma Chemical Co., Munich, Federal Republic of Germany). This suspension was passed over a Sepharose CL-4B column and eluted with 10 mM Tris-hydrochloride buffer (pH 7.2) containing 0.1% EDTA to prevent reaggregation of fimbrial subunits. Native fimbriae were presumed to be present only in the void-volume fractions, and this assumption was confirmed by electron microscopy. Disaggregated fimbriae were obtained as low-molecular-weight components. The column was calibrated with commercially available proteins of known molecular weights. For further separation of fimbriae from the multifimbriated WF96 strain, fractions from the void volume were combined, dialyzed against distilled water, and treated with 2% SDS (5 min, 100°C) to isolate the detergent-resistant fimbrial type. The suspension was rechromatographed under the conditions described above. Fimbriae resistant to depolymerization in 2% SDS were recovered from the void volume, whereas subunits were obtained in the low-molecular-weight fractionation range of this column.

Protein estimation. For protein determination, intact fimbriae were acidified with 0.015 N HCl solution, heated to 100°C for 5 min, and neutralized with NaOH. The protein content was estimated according to Markwell et al. (15), with bovine serum albumin (Serva, Heidelberg, Federal Republic of Germany) as the standard.

Electrophoresis. SDS-PAGE was performed according to Lugtenberg et al. (14) with the modification that the running gel contained 11% polyacrylamide. All samples were boiled for 5 min in 2% SDS–1% β-mercaptoethanol at pH 6.8 before application to the gel. Type 1 fimbriae (about 0.5 mg) were incubated (1 h, 37°C) in 2 ml of 8 M guanidine hydrochloride solution (8). After dialysis against distilled water containing 10 mM EDTA (1) for 24 h at 4°C, samples were lyophilized and boiled for 5 min in the sample buffer described above. The following proteins from Pharmacia (electrophoresis calibration kit) were used as molecular weight standards: phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soy bean trypsin inhibitor (20,100), and α-lactalbumin (14,400). Gels were stained with Coomassie brilliant blue R250.

Electron microscopy. For negative staining, samples were applied to Formvar-coated specimen grids and stained with 2% sodium phosphotungstatic acid at pH 6.8.

RESULTS

Analysis of efficacy of the fimbrial purification scheme. With sucrose gradient ultracentrifugation most of the contaminating proteins (Fig. 1, lane 2) present after the ammonium sulfate precipitation step were removed (Fig. 1, lane 3). The two proteins near the 69,000-molecular-weight marker represent flagellae and were removed by suspending the material in 0.4% SDS at 37°C for 1 h followed by chromatography on Sepharose CL-4B. The resulting gel filtration profile is shown in Fig. 2. One major peak which emerged in the void volume and a minor peak which eluted later were observed. Two peptides with molecular weights of 64,000 and 67,000 were seen on SDS-PAGE of peak 2 fractions (Fig. 1, lane 5). Analysis of the void-volume material revealed two peptide bands with molecular weights of 16,000 and 21,000 (Fig. 1, lane 4). This material was further examined by electron microscopy. Figure 3B shows that it consisted of intact

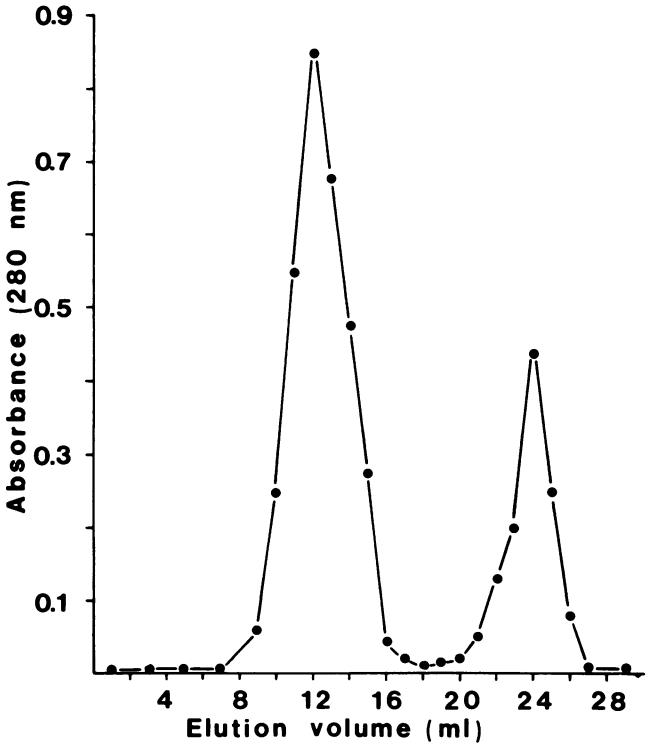

FIG. 2. Separation of fimbriae from contaminating flagellae by gel filtration on Sepharose CL-4B in the presence of 0.4% SDS.

fimbriae that retained their morphology in the presence of 0.4% SDS, whereas flagellae that were present in the samples from sucrose gradient fractions (Fig. 3A) were completely disaggregated under these conditions.

Characterization of purified fimbriae. When nondenatured purified fimbriae from *E. coli* WF96 were subjected to SDS-PAGE, two polypeptide chains with apparent molecular weights of 16,000 and 21,000 were evident (Fig. 1, lanes 4 and 6). In addition, a band was observed in the stacking gel

TABLE 1. Dissociation of native *E. coli* fimbriae into subunits after exposure to detergents or denaturing agents

Treatment	Mol wt ($\times 10^3$)[a] of dissociated fimbrial subunits isolated from *E. coli* strain:	
	WF96	D509
30 mM octyl-glucoside (37°C, 2 h)	16	
2% SDS (100°C, 5 min)	16, 21	
8 M guanidine hydrochloride (37°C, 1 h)	16, 19.5, 21	17
6 M urea	16, 21	

[a] Estimated by SDS-PAGE after exposure of native fimbriae to physicochemical agents, chromatography of treated material on Sepharose CL-4B in the presence of 10 mM Tris buffer–0.1% EDTA, and elution of low-molecular-weight fractions.

(not shown) which represented WF96 fimbrial material too large to enter the gel, i.e., intact fimbriae. A similar phenomenon has been reported for type 1 fimbriae (16), and, as previously noted (8), these fimbriae must be exposed to guanidine hydrochloride to be dissociated into subunits. Therefore, the WF96 fimbrial preparation was similarly treated. All guanidine hydrochloride-exposed WF96 fimbrial proteins appeared to enter the gel, resulting in a new broad band to 19,500 (19.5K) (Fig. 1, lane 7) in addition to the bands observed in SDS-exposed samples. These highly detergent-resistant fimbriae resemble type 1 fimbriae in their stability against SDS. However, the molecular weight of 19,500 for the fimbrial subunit is about 2,500 higher than that shown for type 1 fimbriae of *E. coli* D509 run on the same gel for comparison (Fig. 1, lane 8).

Susceptibility of different fimbrial types to depolymerization by disruptive agents. As a first step in the development of a scheme to separate the different WF96 fimbriae, the stability of fimbriae within the purified mixture was assessed after incubation with octyl-glucoside, SDS, urea, and guanidine hydrochloride. Treated samples were subjected to Sepharose CL-4B column chromatography to exploit the differences in size between intact fimbriae and depolymerized fimbriae, and fractions eluted from the column were then

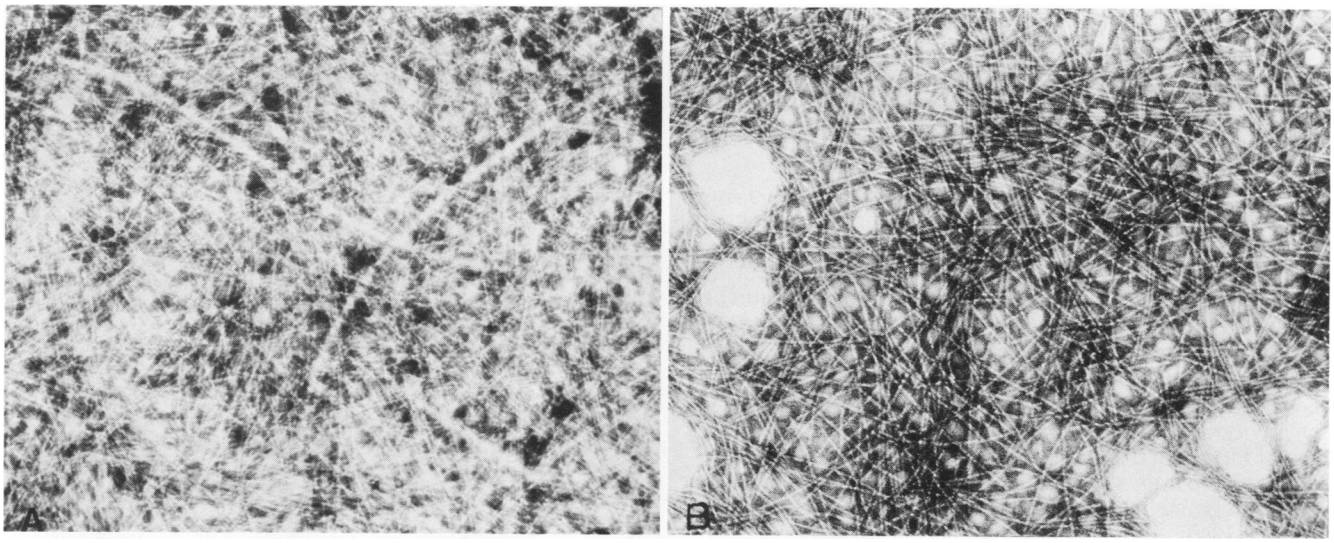

FIG. 3. Electron micrograph of fimbriae (×52,640) before (A; note contaminating flagellae) and after (B) column chromatography in the presence of 0.4% SDS.

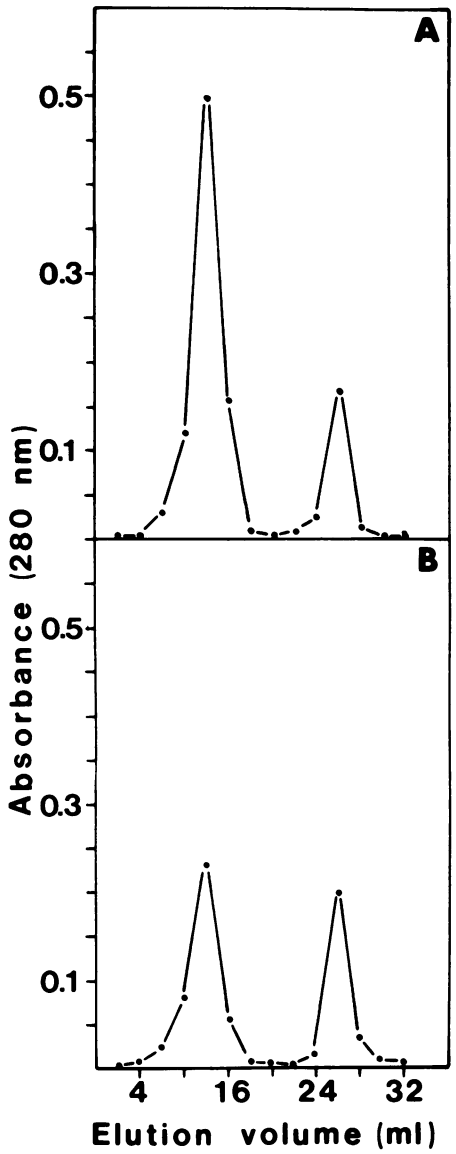

FIG. 4. Separation of fimbrial subunits of strain WF96 after sequential dissociation of fimbriae into subunits. (A) Elution profile of fimbriae after incubation in 30 mM octyl-glucoside–10 mM Tris-hydrochloride, pH 7.2 (2 h, 37°C), and chromatography on a Sepharose CL-4B column (1.5 by 25 cm, 6 ml/h) that was eluted with 10 mM Tris-hydrochloride–0.1% EDTA, pH 7.2. (B) Elution profile from the void-volume peak from column A that was dialyzed, heated in 2% SDS (5 min), and rechromatographed on a Sepharose CL-4B column under the elution conditions described for (A).

examined by SDS-PAGE. The depolymerization effects of a variety of chemicals on the WF96 fimbrial mixture are summarized in Table 1. Type 1 fimbriae from *E. coli* D509 that were similarly treated are included for comparison.

Isolation of different fimbrial subunits by selective physicochemical treatments. From the information on the differential sensitivity of various WF96 fimbrial types to detergents and denaturing agents, a sequential isolating protocol was designed to separate the 16K, 19.5K, and 21K subunits. The purified mixture of intact fimbriae was first treated with 30 mM octyl-glucoside followed by chromatography on Sepharose CL-4B with Tris-EDTA buffer to prevent reaggregation of fimbrial subunits (Fig. 4A). Only one homogeneous peptide of a molecular weight of 16,000 was seen on SDS-PAGE of peak 2 fractions (Fig. 5, lane 1). The void-volume material from this first chromatography step was dialyzed and exposed to 2% SDS (100°C, 5 min) and then subjected to a second Sepharose CL-4B chromatographic step (Fig. 4B). This procedure disaggregated the 21K subunit (Fig. 4, lane 3). Fimbriae resistant to depolymerization with 2% SDS, i.e., material in the void volume of the second Sepharose CL-4B column, were only dissociated by exposure to 8 M guanidine hydrochloride. SDS-PAGE of this sample revealed one band with a molecular weight of 19,500 (Fig. 5, lane 5). If only isolated type 1-like fimbriae are required, the sequence of steps performed can be altered so that the fimbrial mixture is first exposed to 2% SDS. This procedure disaggregates part of the fimbriae from WF96 into their 16K and 21K subunits that are eluted at low-molecular-weight fractions (Fig. 5, lane 6), whereas the material from the void volume yields a single homogeneous peptide on SDS-PAGE identical to that shown in Fig. 5 (lane 5). The purified 16K and 21K subunits were dialyzed in the presence of $MgCl_2$. The efficacy of reassembly was measured by gel chromatography. About 50% of the protein applied to the column eluted with the void volume. When this material was examined by electron microscopy, it proved to consist of rodlike structures. However, the size of the reaggregates was less than one-third of that of the detached intact fimbriae.

DISCUSSION

In this communication, a description of the separation and characterization of three fimbrial types coexpressed on the

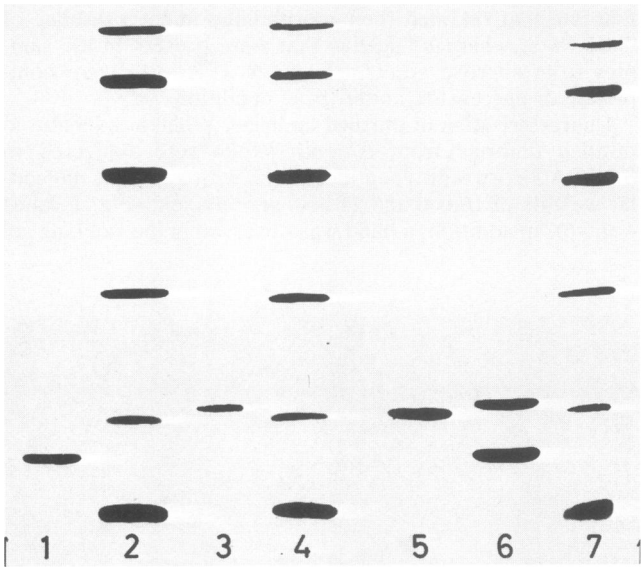

FIG. 5. SDS-PAGE of the separated fimbrial subunits of strain WF96. Lane 1, Low-molecular-weight fractions (25 to 27) from the Sepharose CL-4B column shown in Fig. 4A. Lane 2, Molecular weight markers (see Fig. 1) for the sample in lane 1. Lane 3, Low-molecular-weight fractions (25 to 28) from the Sepharose CL-4B column shown in Fig. 4B. Lane 4, Low-molecular-weight markers for the sample in lane 3. Lane 5, Void-volume fractions (10 to 14) from the Sepharose CL-4B column shown in Fig. 4B. This material was resistant to depolymerization by SDS and was pretreated with saturated guanidine hydrochloride before electrophoresis. Lane 6, 16K and 21K fimbrial subunits that were separated from type 1-like fimbriae by a one-step separation procedure (for details, see text). Lane 7, Molecular weight markers for the samples in lanes 5 and 6.

same strain is presented. Proof is also given for the theory proposed by Dodd and Eisenstein (4) that physicochemical differences exist among *E. coli* fimbriae, and it is further shown that such differences are evident even among fimbriae produced by the same bacterial culture. In addition, a technique is detailed for the isolation of individual fimbriae based on the differential depolymerization of fimbriae in various disrupting agents.

Studies on the physicochemical properties of *E. coli* fimbriae have primarily focused on type 1 fimbriae. This type of fimbria has been shown to resist disruption by such chemicals as urea (12, 20), SDS (16), and 6 M guanidine hydrochloride (8). In this study, the stability of type 1 fimbriae in 6 M urea and in ionic detergents was confirmed. Type 1 fimbriae were also shown to be insoluble in the nonionic detergent octyl-glucoside, a reagent used to completely dissociate the fimbriae of *Pseudomonas aeruginosa* (23). When the fimbriae of WF96 were exposed to the same chemical, only one of the fimbriae proved to be extremely stable and difficult to disassemble into its 19.5K subunits. The stability of this fimbrial type in various disrupting agents enabled us to readily purify it from the other fimbrial types coexpressed by WF96. Similarly, Klemm et al. (11) demonstrated differences in the stabilities of fimbrial types 1A, 1B, 1C, and F7 to depolymerization by 6 M guanidine hydrochloride. The instability of the other two WF96 fimbrial types when exposed to certain disrupting chemicals (Table 1) made us aware that the agents commonly used to separate fimbriae from flagellae probably disaggregate some of the fimbrial types and hence the fimbriae so purified are not completely representative of those present in the bacterial culture. We therefore recommend treatment of fimbriae-flagellae mixtures with 0.4% SDS at 37°C to selectively disaggregate flagellae. The method of isolation of fimbriae from multipiliated *E. coli* has been applied successfully to other *E. coli* strains of different serotypes (Karch et al., manuscript in preparation).

The separation of fimbriae from a mixture of fimbrial types by physicochemical means, as described in this report, has several advantages over immunological methods (19). First, purification of a single fimbrial type by immunoprecipitation requires monospecific antisera. Because of the confusion and complexity in serological classification of fimbriae (18) and the difficulty heretofore encountered in purification, only a few such antisera are available in reference laboratories. Thus, a fimbrial purification scheme based on serological distinctions among the diverse fimbriae of *E. coli* will probably only become practical with the availability of a panel of monoclonal antibodies that recognize specific fimbrial epitopes.

One of the potential limitations of a physicochemical approach to the separation of multiple fimbrial types is that the behavior of dissociated subunits that are permitted to reaggregate may not mimic native fimbriae. For example, Watts et al. (23) found that fimbriae isolated from *P. aeruginosa* PAK by solubilization in octyl-glucoside that were then allowed to reassemble failed to bind bacteriophage PO4 as did intact fimbriae. On the other hand, Abraham et al. (1) showed that reassembled type 1 fimbriae reacted with monoclonal antibodies specific for the fimbrial quaternary structure.

Purification of the different fimbrial types makes it possible to study more intensively the biological function of the different types. Furthermore, how these fimbriae are expressed on the individual bacterial cells, whether a multifimbriated culture of bacteria represents individual cells that produce all fimbrial types, and whether the various kinds of fimbriae are selectively expressed by different bacterial subpopulations can be investigated.

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LITERATURE CITED

1. **Abraham, S. N., D. L. Hasty, W. A. Simpson, and E. H. Beachey.** 1983. Antiadhesive properties of a quaternary structure-specific hybridoma antibody against type 1 fimbriae of *Escherichia coli*. J. Exp. Med. **158**:1114–1128.
2. **Beachey, E. H.** 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**:325–345.
3. **Brinton, 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**:1003–1054.
4. **Dodd, D. C., and B. I. Eisenstein.** 1982. Antigenic quantitation of type 1 fimbriae on the surface of *Escherichia coli* cells by an enzyme-linked immunosorbent inhibition assay. Infect. Immun. **38**:764–773.
5. **Duguid, J. P., S. Clegg, and M. I. Wilson.** 1979. The fimbrial and non fimbrial haemagglutinins of *Escherichia coli*. J. Med. Microbiol. **12**:213–227.
6. **Duguid, J. P., and R. R. Gillies.** 1957. Fimbriae and adhesive properties in dysentery bacilli. J. Pathol. Bacteriol. **74**:397–411.
7. **Duguid, J. P., I. W. Smith, G. Dempster, and P. N. Edmunds.** 1955. Non flagellar filamentous appendages ("fimbriae") and haemagglutinating activity in Bacterium coli. J. Pathol. Bacteriol. **70**:335.
8. **Eshdat, Y., F. J. Silverblatt, and N. Sharon.** 1981. Dissociation and reassembly of *Escherichia coli* type 1 pili. J. Bacteriol. **148**:308–314.
9. **Evans, D. J., Jr., D. G. Evans, L. S. Young, and J. Pitt.** 1980. Hemagglutination typing of *Escherichia coli*: definition of seven hemagglutination types. J. Clin. Microbiol. **12**:235–242.
10. **Jann, K., B. Jann, and G. Schmidt.** 1981. SDS polyacrylamide gel electrophoresis and serological analysis of pili from *Escherichia coli* of different pathogenic origin. FEMS Microbiol. Lett. **11**:21–25.
11. **Klemm, P., I. Ørskov, and F. Ørskov.** 1982. F7 and type 1-like fimbriae from three *Escherichia coli* strains isolated from urinary tract infections: protein, chemical, and immunological aspects. Infect. Immun. **36**:462–468.
12. **Korhonen, T. K., E. Nurmiaho, H. Ranta, and C. Svanborg-Eden.** 1980. New method for isolation of immunologically pure pili from *Escherichia coli*. Infect. Immun. **27**:569–575.
13. **Korhonen, T. K., V. Räisänen, H. Saxén, H. Hultberg, and S. B. Svenson.** 1982. P-antigen-recognizing fimbriae from human uropathogenic *Escherichia coli* strains. Infect. Immun. **37**:286–291.
14. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the major outer membrane proteins of *Escherichia coli* K12 into four bands. FEBS Lett. **58**:254–259.
15. **Markwell, M. A. K., S. M. Haar, L. L. Bieker, and W. E. Tolbert.** 1978. A modification of the Lowry procedure to simplify protein determinations in membrane and lipoprotein samples. Anal. Biochem. **87**:206–210.
16. **McMichael, J. C., and J. T. Ou.** 1979. Structure of common pili from *Escherichia coli*. J. Bacteriol. **138**:969–975.
17. **Ofek, I., and E. H. Beachey.** 1978. Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect. Immun. **22**:247–254.
18. **Ørskov, I., and F. Ørskov.** 1983. Serology of *Escherichia coli* fimbriae. Progr. Allergy **33**:80–105.
19. **Rhen, M., E. Wahlström, and T. K. Korhonen.** 1983. P-fimbriae

of *Escherichia coli*: fractionation by immune precipitation. FEMS Microbiol. Lett. **18**:227–232.
20. **Salit, I. E., and E. C. Gotschlich.** 1977. Hemagglutination by purified type 1 *Escherichia coli* pili. J. Exp. Med. **146**:1169–1181.
21. **Salit, I. E., J. Vavougios, and T. Hofmann.** 1983. Isolation and characterization of *Escherichia coli* pili from diverse clinical sources. Infect. Immun. **42**:755–762.
22. **Svanborg-Eden, C., and H. A. Hansson.** 1978. *Escherichia coli* pili as possible mediators of attachment to human urinary tract epithelial cells. Infect. Immun. **21**:229–237.
23. **Watts, T. H., D. G. Scraba, and W. Paranchych.** 1982. Formation of 9-nm filaments from pili monomers obtained by octylglucoside dissociation of *Pseudomonas aeruginosa* pili. J. Bacteriol. **151**:1508–1513.