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Antigenic Quantitation of Type 1 Fimbriae on the Surface of *Escherichia coli* Cells by an Enzyme-linked Immunosorbent Inhibition Assay

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Type 1 fimbriae from two strains of *Escherichia coli*, K-12-derived CSH50 and a clinical isolate VL-2, were purified by a simplified procedure, which should be applicable to a variety of bacterial strains. After mechanical removal from the cells, the fimbriae were sedimented in the ultracentrifuge and resuspended in 5 M urea to disaggregate cell membranes and flagella, leaving the urea-resistant fimbriae intact. After several hours at 37°C, this crude fimbrial suspension was diluted to 1 M urea, and the intact fimbriae were sedimented through a 1 M urea–1 M sucrose cushion. The pellet was found to be pure fimbriae by sodium docecyl sulfate-polyacrylamide gel electrophoresis, with apparent subunit molecular weights of 17,000 for the fimbriae from K-12 strain CSH50 and 19,000 for those from the clinical isolate VL-2. High-titer rabbit antiserum raised against CSH50 fimbriae was specific for fimbriae by indirect ferritin labeling and immunoprecipitation and was used to develop an enzyme-linked immunosorbent assay. Competitive inhibition of antifimbrial antiserum in the enzyme-linked immunosorbent assay by a known amount of either purified fimbriae or fimbriae-bearing bacteria permitted precise quantitation of fimbrial antigen in cultures of strain CSH50, thereby providing a simple means of determining the effects of environmental conditions on the synthesis of type 1 fimbriae.

Type 1 fimbriae (or pili) are proteinaceous appendages 7 nm in width projecting from the surface of many gram-negative bacteria (5). They consist of aggregated subunits of a single polypeptide of 17,000 (17K) daltons; their high proportion of hydrophobic residues (2, 26) stabilizes the subunit association and prevents disaggregation by sodium dodecyl sulfate (SDS) (19) or urea (26), but not by hot acid (2, 19) or saturated guanidine (2, 11). Subunits released by saturated guanidine have recently been shown to reassemble in the presence of Mg^{2+} (11).

Escherichia coli cells exhibit mannose-sensitive adherence to a variety of cell types including *Candida* yeast cells, guinea pig erythrocytes (5, 26), and human oral and intestinal epithelial cells (4, 24). Adherence has been shown to be mediated by fimbriae; isolated fimbriae exhibit mannose sensitive adherence to monkey kidney cells (26), and bacteria in the nonfimbriate phase or defimbriated cells lack the ability to adhere (2). Adherence is required for colonization of mucosal surfaces, and colonization has been shown to correlate with the degree of fimbriation (22). Fimbriae that mediate adherence are designated type 1.

The isolation of antigenically pure fimbriae is important for classification of fimbrial serotypes from clinical isolates and in the study of their role in adhesion and pathogenesis. A number of purification procedures have been published, reflecting the fact that fimbriae from different strains possess different physiochemical properties. The methods of Salit and Gotschlich (26), Brinton (2), or Silverblatt (27), with repeated precipitations with either ammonium sulfate or magnesium chloride, give quantitative yields only with fimbriae that aggregate readily. With our strains of *E. coli*, these methods resulted in significant contamination with flagella and poor yields of fimbriae. The more generally applicable procedure of Korhonen et al. (16) takes advantage of the stability of fimbriae in concentrations of urea that disaggregate flagella but requires many complex manipulations and a great deal of laboratory time. We, therefore, modified and greatly simplified the procedure of Korhonen et al., taking advantage of the unique stability of fimbrial organelles in urea solutions.

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Using these antigenically pure fimbriae, we were able to develop a highly specific immunological assay for the precise quantitation of type 1 fimbrial antigen on *E. coli* surfaces. We believe that this assay is a marked improvement over presently popular assays that utilize the electron microscope (21) or that depend on yeast cell or red cell agglutination (22, 26). Although a somewhat similar immunological assay was developed by Buchanan (3) to analyze fimbriae on gonococcal cell surfaces, our method avoids the additional step of first quantitating solid-phase fimbriae by radiometric means as well as certain assumptions used in the Buchanan method.

MATERIALS AND METHODS

Strains and culture conditions. Strain VL-2 is a streptomycin-resistant mutant derived from the clinical isolate VL-1 of *E. coli* (9). Strain CSH50 is a Cold Spring Harbor K-12-derived strain with the genotype F^- *ara* Δ(*lac-pro*) *rpsL thi* (20). VL-361 is a nonfimbriate strain obtained from CSH50 after insertion of bacteriophage Mu *d*(Ap^R*lac*) into a gene necessary for the production of fimbriae (7). For the isolation of fimbriae, bacterial cells were inoculated 1:50 into brain heart infusion broth (BHI; BBL Microbiology Systems, Cockeysville, Md.) from an overnight culture statically grown in nutrient broth and incubated statically at 37°C for 48 h. For immunoprecipitation, cells grown overnight in minimal medium A (20) supplemented with 0.5% glucose were inoculated 1:10 into the same broth and grown with shaking (250 rpm) at 37°C in a New Brunswick G-24 environmental shaker. The cell number was determined from a standard curve of viable counts versus optical density (OD) at 550 nm (OD_{550}) measured in a Coleman Junior II spectrophotometer (Coleman Systems, Inc., Irvine, Calif.).

Chemicals and buffers. All reagents for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were electrophoresis grade from Bio-Rad Laboratories (Richmond, Calif.). Buffers used were Tris buffer (5 mM Tris, 0.02% NaN_3, pH 7.4), urea buffer (10 mM Tris, 5 M urea, pH 7.0), PBS (0.02 M phosphate, 0.15 M NaCl, pH 7.4), and PBS-T (PBS containing 0.05% Tween 20). Polyethylene glycol (PEG), molecular weight 6,000, was obtained from Sigma Chemical Co. (St. Louis, Mo.).

SDS-PAGE. SDS-PAGE was performed in 1.5-mm slab gels (gel concentration, 10%) by the system of Laemmli (17). Fimbriae are resistant to disaggregation by SDS (19) and require treatment with acid or guanidine in order for subunits to enter the gel. Samples were routinely acidified with HCl (pH < 2), heated to 100°C for 5 min, cooled, neutralized, mixed 1:1 with sample buffer (0.125 M Tris, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.002% bromophenol blue [pH 6.8]), heated to 100°C for 2 min, and applied to the gel. Molecular weight standards were from Sigma.

Protein estimation. Protein was estimated by the method of Peterson (25), with bovine serum albumin as the standard.

Electron microscopy. The samples were applied to copper grids coated with Formvar and carbon. Samples were negatively stained with phosphotungstic acid and examined with an EM6B electron microscope (AEI Scientific Apparatus, Inc., Elmsford, N.Y.) as previously described (23). For immunoferritin labeling (28), bacteria-coated grids were placed sequentially on drops of the following series of solutions: PBS, PBS, 1:128 dilutions of either preimmune or 2-week immune serum, PBS, PBS, 1:250 ferritin-labeled goat antirabbit antiserum (Cappel Laboratories, Cochranville, Pa.), deionized water, deionized water, phosphotungstic acid. Grids were left on PBS, water, and phosphotungstic acid drops for 30 s each and on antibody solutions for 10 min and were blotted dry between each drop.

Preparation of antisera. Immunization was by the bentonite method (12). Briefly, 500 μg of fimbriae isolated from CSH50 was mixed with bentonite particles in carbonate buffer at pH 9.6. The fimbriae-coated particles were removed by centrifugation and resuspended in 1 ml of sterile PBS, which was injected intramuscularly into an adult New Zealand white rabbit.

Agglutination titer. A 10-μl amount of a 0.41 OD_{550} suspension of strain CSH50 or VL-361 in PBS was mixed with 10 μl of various dilutions of serum on a microscope slide. The titer was the highest dilution giving visible agglutination after 1 min.

Immunoprecipitation. The method of Ito et al. (15) was used as follows. Bacteria in 1 ml of minimal medium A were grown with shaking at 37°C to an OD_{550} of 0.3 and then labeled for 5 min with 5 μCi (40 Ci/mmol) of [^{3}H]leucine (ICN, Irvine, Calif.). Cells were sedimented and resuspended in 0.1 ml of 1% SDS–50 mM Tris-hydrochloride (pH 8)–1 mM EDTA. After boiling for 5 min, 2 ml of Triton buffer (2% Triton X-100, 50 mM Tris-hydrochloride [pH 8], 0.15 M NaCl, 0.1 mM EDTA) was added, and the cells were removed by centrifugation. A 5-μl amount of antiserum was added to the SDS-solubilized material and incubated overnight at 4°C. A 0.2-ml portion of a 20% suspension of Formalin-fixed *Staphylococcus aureus* (IgGsorb; The Enzyme Center, Boston, Mass.) was added and allowed to incubate for 2 h at 40°C. Bacteria were washed three times with Triton buffer and resuspended in 75 μl of water. SDS-PAGE was performed as described above. Fluorography was performed using En^{3}Hance (New England Nuclear Corp., Boston, Mass.) exactly as suggested by the manufacturer. Film was exposed for 20 h at –80°C.

ELISA. Enzyme-linked immunosorbent assay (ELISA) (10) inhibition determinations were as follows. A 0.15-ml aliquot of a suspension of purified fimbriae at a concentration of 5 μg/ml in 1 M $NaHCO_3$–0.5% PEG (pH 9.6) was allowed to adsorb to each well of a microtiter plate (Dynatech Laboratories, Inc., Alexandria, Va.) overnight at 30°C. After the removal of the coating solution and the washing of the wells three times with PBS-T, serial twofold dilutions of bacteria or fimbriae were prepared in PBS-T so that each well contained 50 μl of either diluted cells or fimbriae. Then 0.1 ml of antiserum diluted 1:2,000 in PBS-T was added and allowed to incubate for 2 h at room temperature. After the plates were washed three times with PBS-T, 150 μl of a 1:1,500 dilution of goat anti-rabbit immunoglobulin G (IgG) conjugated to horseradish peroxidase (Cappel Laboratories) was added and allowed to incubate for 3 h. The plates were again washed three times with PBS-T, and 150 μl of substrate buffer (5-aminosalicylic acid, 0.005% H_2O_2,

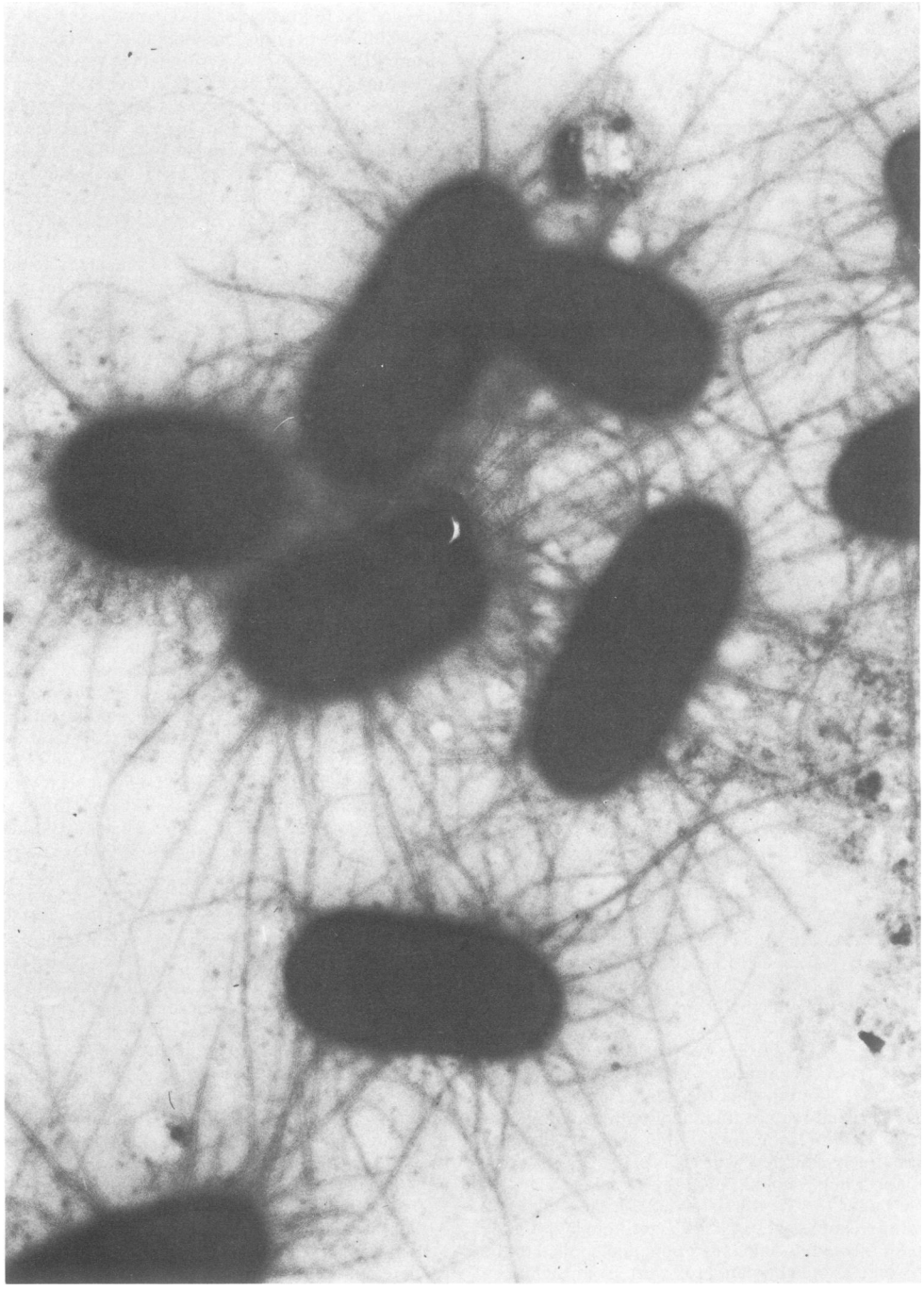

FIG. 1. Electron micrograph of strain CSH50 after growth for 48 h in static BHI. ×15,000.

pH 6.0) was added. The plates were read at 450 nm after 15 min by an MR580 plate reader (Dynatech). Samples were prepared in duplicate or triplicate. The instrument was blanked against wells that received no antifimbrial serum. Wells in which no cells or fimbriae were added were controls. Percent inhibition was calculated as $(OD_{control} - OD_{competitor})/OD_{control} \times 100$.

Fifty percent inhibition was calculated from lines produced by linear regression of 1/cell number versus 1/percent inhibition. Only points with greater than 40% inhibition (usually about five) were used. Values of the linear regression curves generally exceeded 0.99.

Amino acid sequence analysis. For sequence analysis, the fimbriae were first disaggregated in saturated guanidine hydrochloride as described by Eshdat et al. (11). Sequence analyses were performed with an Automatic Protein Sequenator (model 890C, Beckman Instruments, Inc., Fullerton, Calif.) according to the principles of Edman and Begg (6). The peptide-DMAA program (071472 of Beckman Instruments) was used, and the pth amino acids were identified by high-pressure liquid chromatography (Waters Institute) (29) or hydrolysis with 55% HI to their parent amino acids (30).

RESULTS

Purification of fimbriae. Bacteria were grown statically in 8 liters of BHI for 48 h at 37°C. Figure 1 shows typical fimbriate cells of the K-12-derived strain CSH50. The bacteria were collected by centrifuging for 10 min at 10,000 × *g* and 4°C in a J2-21 centrifuge (Beckman Instruments, Inc., Palo Alto, Calif.). All subsequent steps were performed at 4°C unless stated otherwise. The bacterial pellet (35 g, wet weight) was washed in 200 ml of 0.5% NaCl and resuspended in 100 ml of Tris buffer. Fimbriae were removed by blending at the highest speed in an Osterizer blender five times for 2 min each time, with cooling periods in between. The cells were pelleted and washed with 60 ml of Tris buffer, and the supernatants were combined. Only a few protein bands were visible by SDS-PAGE analysis of this fraction for CSH50, with fimbriae as the major component (data not shown).

Figure 2 displays the SDS-PAGE analysis of samples from strain VL-2 and the sequential purification steps showing the marked contamination with other proteins after the blending step (lane a). Centrifugation at 27,000 × *g* for 30 min resulted in the cosedimentation of a large amount of the contaminating flagella and membrane vesicles with only a small amount of fimbriae, as seen by electron microscopy (not shown). By SDS-PAGE analysis (Fig. 2, lane b), this pellet consisted of several protein bands, one most likely being flagella near the 66K molecular weight marker. The partially cleared supernatant was then subjected to ultracentrifugation in a 55.2 Ti rotor (227,000 × *g* for 2 h),

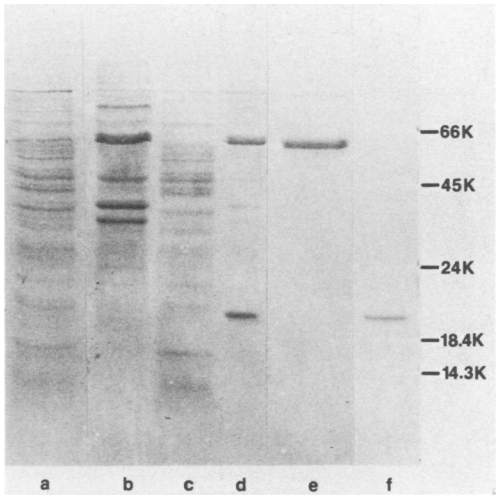

FIG. 2. SDS-PAGE analysis of various stages of fimbria purification from strain VL-2. Lane a, the blended supernatant after removal of cells, 50 μg; lane b, the pellet obtained when the blended supernatant was centrifuged for 30 min at 27,000 × *g*, 45 μg; lane c, supernatant of the centrifugation for 2 h at 227,000 × *g*, 40 μg; lane d, pellet of the centrifugation for 2 h at 227,000 × *g*, 25 μg; lane e, supernatant of the centrifugation for 15 h at 200,000 × *g*, 25 μg; lane f, pellet of purified fimbriae obtained from the centrifugation at 200,000 × *g*, 15 μg. Reference proteins indicated on the right were bovine serum albumin (66K), ovalbumin (45K), trypsinogen (24K), β-lactoglobulin (18.4K), and lysozyme (14.3K).

which completely cleared the suspension of fimbriae (Fig. 2, lanes c and d). The clear gelatinous pellets of semipure fimbriae were easily resuspended in 8 ml of 5 M urea buffer, mixed for several hours at 37°C, diluted to 40 ml with Tris buffer (1 M urea, final concentration), and then layered in four 10-ml aliquots over 10 ml of a 1 M urea–1 M sucrose–5 mM Tris cushion. Pellets of pure fimbriae were obtained by centrifuging these samples for 16 h at 200,000 × *g* in a 55.2 Ti rotor. The supernatant contained no fimbriae but contained a band that migrated at the same apparent molecular weight as a preparation which appeared to be flagella by electron microscopy (Fig. 2, lane e). The heavily fimbriated strain CSH50 gave yields of 30 to 40 mg of pure fimbriae per 35 g (wet weight) of bacteria, while the lightly fimbriated strain, VL-2, gave 3 to 6 mg.

Characterization of fimbriae. Preparations from both strains were morphologically similar. An electron micrograph of CSH50 fimbriae is shown in Fig. 3. An SDS-PAGE lane loaded with 80 μg of CSH50 fimbriae (Fig. 4, lane A) showed no contaminating protein. A similar result was obtained with VL-2 (not shown). The

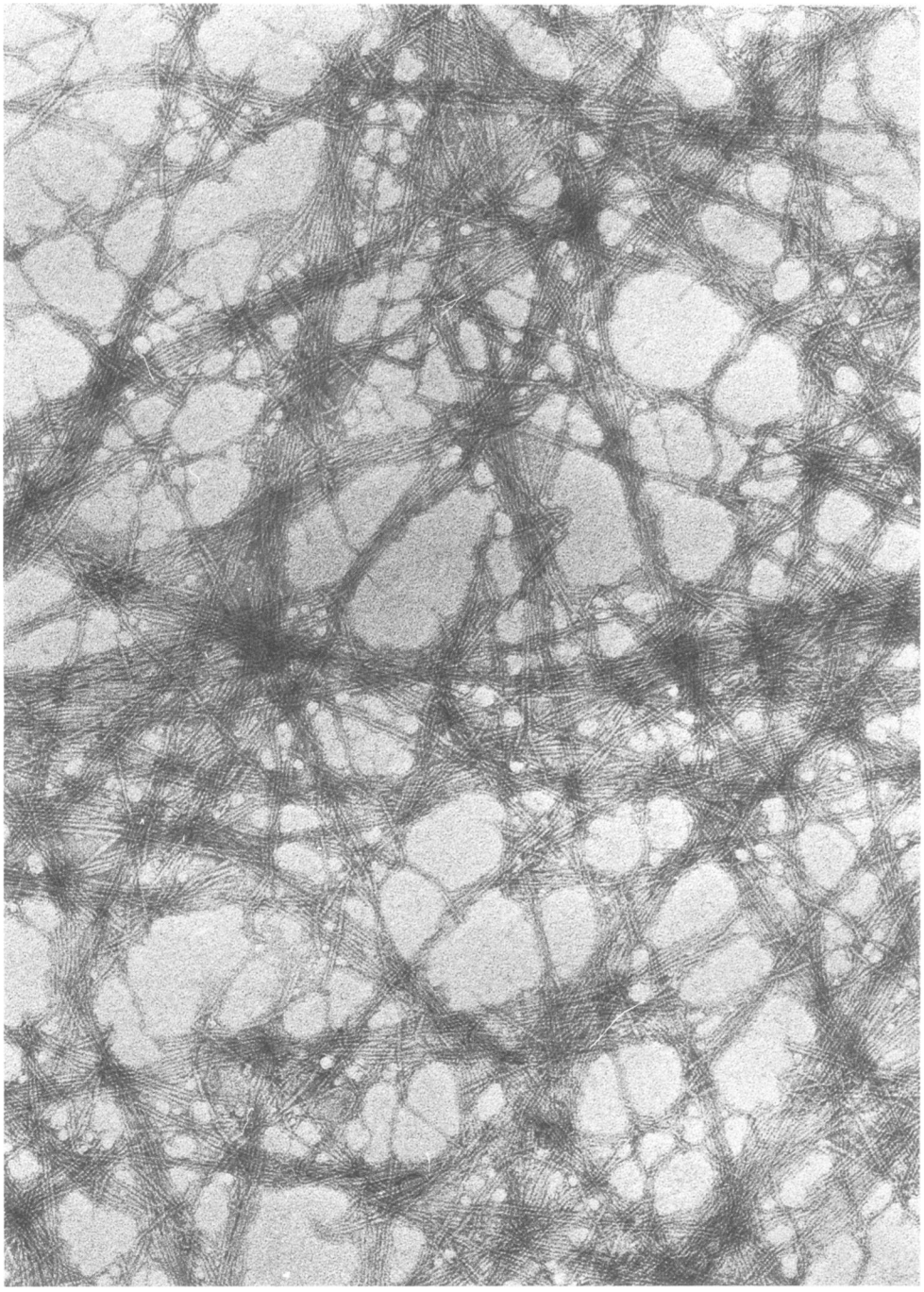

FIG. 3. Electron micrograph of purified fimbriae from strain CSH50. ×72,000.

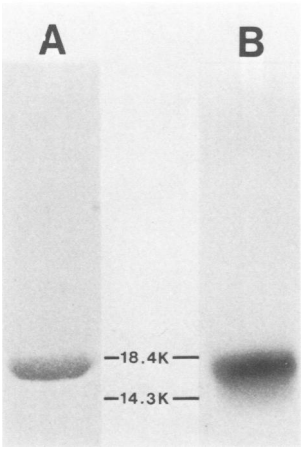

FIG. 4. SDS-PAGE analysis of strain CSH50 fimbriae and material immunoprecipitated by hyperimmune sera raised against purified fimbriae. Lane A is overloaded with 80 μg of purified fimbrial protein from strain CSH50 and stained with Coomassie blue R-250. Lane B shows [^{3}H]leucine-labeled material from strain CSH50 precipitated by antifimbrial serum and detected by fluorography.

two fimbrial preparations migrated at different apparent molecular weights in SDS-PAGE: 17K for CSH50 and 19K for VL-2. The two fimbrial types, while both stable in urea, differed in their ease of entering an SDS-polyacrylamide gel. Heating VL-2 fimbriae at 100°C for 10 min in SDS sample buffer allowed much of the protein to enter the gel while no CSH50 fimbriae entered the gel even after 20 min at 100°C (not shown). Amino-terminal amino acid sequencing in duplicate of the first 26 residues of CSH50 fimbriae revealed a sequence identical to that previously described (13), except for substitution of alanine for aspartic acid at residue 24, the addition of serine at residue 21, and the additions of aspartic acid and alanine at residues 25 and 26, respectively.

Immune response. Agglutination titrations of sera employed isogenic strains CSH50 (Pil$^+$) and VL-361(Pil$^-$) to determine if antibody to fimbriae was present in the preimmune serum and if there was a rise in agglutinating antibody to nonfimbrial antigenic determinants present on the Pil$^-$ strain (Table 1). Although strain CSH50 showed a slightly higher titer than strain VL-361 with the preimmune serum, it demonstrated a marked rise after immunization that was not seen with strain VL-361. Figure 4, lane B, demonstrates that only a protein with the same apparent molecular weight as fimbriae was immunoprecipitated when cells were labeled with [^{3}H]leucine (similar results were obtained with ^{3}H-labeled mixed amino acids). Lastly, indirect immunoferritin labeling of fimbriate bacteria showed that the antiserum bound to fimbriae and not to flagella or the cell surface (Fig. 5).

ELISA inhibition. An ELISA was developed with CSH50 fimbriae to quantitate cell-associated fimbriae. We found that fimbrial preparations, particularly when not freshly isolated, coated microtiter plate wells poorly and unevenly. Treatment of fimbriae with probe sonication (not shown) or PEG resulted in greatly increased coating efficiency (Fig. 6). We used PEG for coating because of its ease of use and reproducibility. The efficiency of PEG increased little above a concentration of 0.5% (data not shown). All further experiments used 0.5% PEG and 5 μg of fimbriae per ml.

Measurement of cell-associated fimbriae by ELISA inhibition was performed as follows. Serial dilutions of cell cultures were prepared in the fimbriae-coated microtiter plates. Antiserum (1:3,000, final dilution) was then added to the wells, and the mixtures were incubated for 2 h. Increased amounts of Pil$^+$ bacteria or purified fimbriae caused decreased titers in the ELISA (Fig. 7a and c). In contrast, Pil$^-$ strain VL-361 did not inhibit the assay at any concentration of bacteria, demonstrating the fimbria-specific nature of the assay. Also, VL-2 showed little cross-reactivity in this system. When the data for inhibition were plotted as a double-reciprocal plot (Fig. 7b and d), we found a linear relationship between 1/percent inhibition and 1/cells (or 1/fimbriae). In the case of inhibition with either CSH50 fimbriae or intact CSH50 cells, the y-intercept occurred at 0.01 (1/100%), demonstrating that inhibition was competitive. Moreover, since the curves generated by CSH50 fimbriae and CSH50 bacteria were superimposable, it could be assumed that the affinities of cell-free and cell-associated fimbriae for antibody were the same. Thus, a standard curve generated by known amounts of pure fimbriae permitted a simple assay for determining the amount of antigenically active fimbriae on the surface of intact cells used in the inhibition assay. For example, the amount of fimbriae on cells giving 50% inhibition was considered to be the same as

TABLE 1. Agglutination titers of preimmune and immune rabbit serum[a]

Strain	Agglutination titer for:	
	Preimmune	Immune
CSH50	1:8	1:16,384
VL-361[b]	1:1	1:1

[a] A 3-week bleeding after immunization with purified type 1 fimbriae.

[b] Strain VL-361 is isogenic to strain CSH50 but lacks fimbriae.

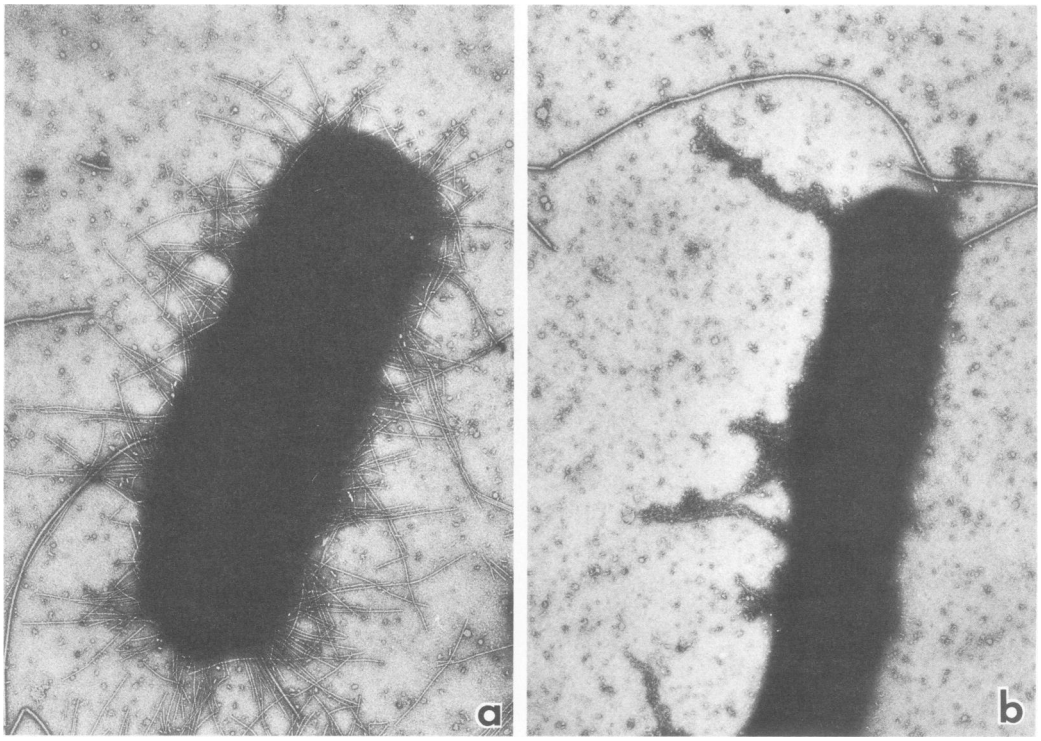

FIG. 5. Indirect immunoferritin labeling of fimbriae. (a) Electron micrograph of strain CSH50 treated sequentially with a 1:64 dilution of preimmune rabbit serum and ferritin-labeled goat anti-rabbit IgG. (b) Electron micrograph of strain CSH50 treated sequentially with a 1:128 dilution of rabbit antifimbrial serum and ferritin-labeled goat anti-rabbit IgG. Ferritin molecules (black dots) can be seen coating the fimbriae but not the flagella or cell wall. ×29,260.

the amount of purified fimbriae giving 50% inhibition (7.6 ng in this experiment), as calculated from linear regression of these lines. We estimated that CSH50 grown at 37°C in static BHI contained 10 ng of fimbriae per 10^6 cells. In a reconstitution experiment, when VL-361 was mixed 1:1 with CSH50, the amount dropped to 5.3 ng per 10^6 cells, approximately half that of CSH50 alone.

DISCUSSION

The variety of fimbrial isolation procedures currently in use suggests that fimbriae present

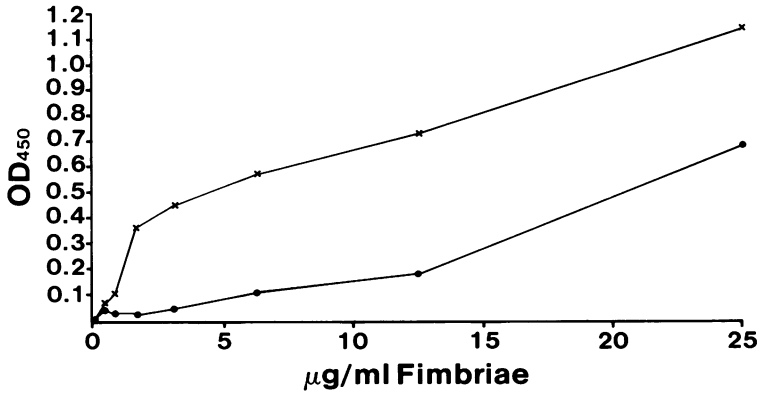

FIG. 6. Effect of PEG on the coating efficiency of strain CSH50 fimbriae to microtiter wells. A 150-μl amount of 1 M carbonate buffer, pH 9.6, containing the indicated concentration of fimbriae was incubated overnight at 30°C in the presence (x) or absence (•) of 1% PEG. OD_{450}, Optical density at 450 nm.

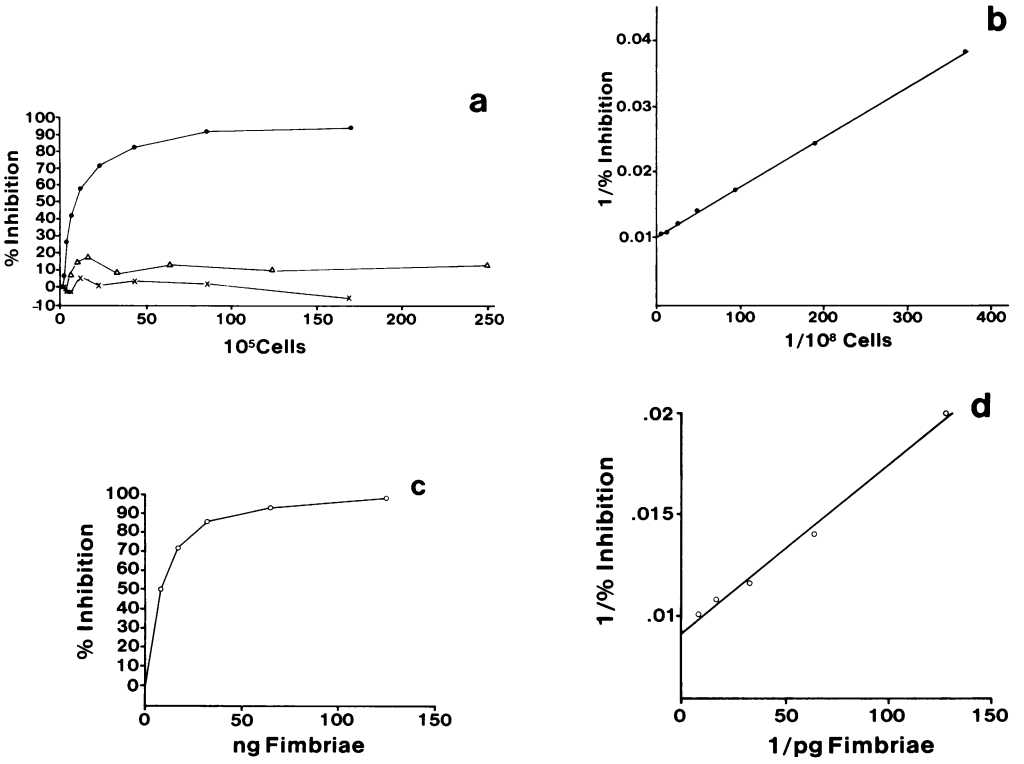

FIG. 7. ELISA inhibition test. Inhibition of the ELISA was performed by incubating for 2 h 50 μl of serial dilutions of whole cells of strains CSH50, VL-361, and VL-2 or purified CSH50 fimbriae with 100 μl of a 1:2,000 dilution of antifimbrial antiserum in microtiter wells that were coated with CSH50 fimbriae. (a) Inhibition by whole cells. Only strain CSH50 (●) completely inhibits the ELISA. Little inhibition can be seen with strain VL-2 (Δ) and none by the nonfimbriate strain VL-361 (x). (b) The data from (a) obtained with strain CSH50 replotted as 1/percent inhibition versus $1/10^8$ cells; $r = 0.9997$. (c) Inhibition by the homologous purified CSH50 fimbriae. (d) The data from (c) replotted as in (b); $r = 0.996$.

on different bacteria often differ physiochemically. In the case of strain VL-2, none of the published purification procedures proved adequate. In fact, the $MgCl_2$ precipitation method of Brinton (2), although suitable for the K-12 strain CSH50, increased the flagellar contamination of strain VL-2. Similarly, the method of ammonium sulfate precipitation as used by Salit and Gotschlich (26) resulted in poor yields and significant protein contamination. The fimbriae that were purified were often irreversibly aggregated.

The simple procedure used in this communication proved effective for the two strains under study. The method depends on the marked differential resistance of fimbriae, compared with other surface proteins, to disaggregation in 5 M urea, as shown by Salit and Gotschlich (26) and Korhonen et al. (16). Flagella, in particular, disaggregate readily under these conditions (18). In the case of type 1 fimbriae from a K-12 strain of *E. coli*, we found that we could significantly shorten the Korhonen method and sacrifice neither yield nor antigenic purity.

Although fimbriae from the clinical isolate VL-2 and the laboratory strain CSH50 were similar morphologically and functionally, they displayed some important differences. First, CSH50 fimbriae were easily aggregated by Mg^{2+}, whereas VL-2 fimbriae were not. Second, most VL-2 fimbrial protein would enter a polyacrylamide gel if boiled for 10 min in SDS sample buffer, whereas CSH50 fimbrial protein would not, even after 20 min of boiling. Third, VL-2 fimbrial protein migrated with an apparent subunit molecular weight of 19K during SDS-PAGE, whereas CSH50 fimbrial protein migrated at 17K under identical conditions. Fourth, VL-2 showed little cross-reactivity with CSH50 in the ELISA inhibition assay. Also, amino acid sequences of the fimbriae of strains CSH50 and B_{am} (13) differed slightly. These results strengthen the view that fimbriae are structurally and antigenically heterogeneous. As an additional example, in the case of gonococcal fimbriae, Schoolnik et al. (26a) demonstrated the presence

of two major domains: one, a structurally conserved, immunorecessive binding region and the other, a hypervariable, immunodominant nonbinding region. Presumably, immunological pressure in nature has selected a variety of mutations coding for amino acids unimportant for ligand-receptor interaction but crucial for antigenic recognition. In contrast, we have shown that under other conditions, such as exposure of bacteria to an antibiotic that causes protein mistranslation, the binding domain can be altered as well (9).

Perhaps the most important aspect of the present study is the development of a simple but precise method to quantitate fimbriae on *E. coli* surfaces. Previous methods have suffered from tediousness, as in counting fimbriae on bacteria as observed by electron microscopy, or from imprecision, as in the agglutination assays. For example, agglutination titers have never, to our knowledge, been demonstrated to be linearly related to the amount of fimbriae present in a given culture. The process of agglutination may depend in a complex fashion on the length or number of fimbriae per cell. Another drawback to the morphological analysis of fimbriation is that it determines the proportion of phase-positive cells in a culture rather than the amount of fimbriae produced (2).

Two previous methods of quantitation of fimbriae deserve comment. The first, by Bar-Shavit and co-workers (1), utilized [^{14}C]mannan binding to intact bacteria to quantitate active ligand-binding sites on the fimbriae. The second, by Isaacson (14), utilized an enzyme-linked antibody centrifuge assay for detection of K99 fimbrial antigen. Neither method employs a solid support for the binding protein apart from intact bacteria, so that establishment of a standard curve with measured amounts of pure fimbriae is not feasible. Our method is an improvement in its ability to quantitate on an absolute rather than on a relative scale, without resorting to radioisotopes (3).

We have already used our ELISA inhibition test to complement results from our operon fusion assay (7, 7a, 8a). While the former detects total fimbrial protein synthesized, the latter determines rates of phase variation. We have found, for instance, that growth of *E. coli* in glucose does not result in catabolite repression of fimbrial synthesis as previously believed (8), nor does it change the proportion of phase-positive cells through genetic control mechanisms. In addition to its use as a probe of the regulation of fimbrial synthesis, we believe that the ELISA inhibition method will permit analysis of antigenic cross-reactivity between heterologous fimbriae as well as the characterization of antigenic domains within fimbrial subunits.

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