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Purification and Characterization of Fimbriae from *Salmonella enteritidis*

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A human isolate of *Salmonella enteritidis* which displayed strong pellicle formation during static broth culture and mannose-sensitive hemagglutination produced fimbriae which were morphologically indistinguishable from type 1 fimbriae of members of the family *Enterobacteriaceae*. Fimbrin was purified to homogeneity, and the apparent molecular weight (M_r, 14,400) was markedly lower than that reported for the type 1 fimbrin of *Salmonella typhimurium* (M_r, 22,100). This fimbrin contained 40% hydrophobic amino acids and lacked cysteine. The sequence of the N-terminal 64 amino acids was determined, and sequence alignment revealed that although the 18 N-terminal residues of the *S. enteritidis* molecule shared considerable homology with *Escherichia coli* and *S. typhimurium* type 1 fimbrins, the *S. enteritidis* fimbrin lacked a 6- to 9-residue terminal sequence present in the other type 1 fimbrins and, after residue 18, shared little homology with the *E. coli* sequence. Antibodies raised to the purified *S. enteritidis* fimbrin bound to surface-exposed conformational epitopes on the native fimbriae and displayed pronounced serospecificity. These antibodies were used in the isolation of a nonfimbriated Tn*10* insertion mutant which was unable to hemagglutinate.

Members of the family *Enterobacteriaceae* produce a range of fimbrial types which facilitate their attachment to eucaryotic cells. These types of fimbriae have been broadly divided into two major classes, mannose sensitive and mannose resistant, based on the ability of the monosaccharide D-mannose to inhibit the adhesion of the fimbriae (4, 6, 7). Fimbriae which are mannose sensitive are referred to as common or type 1 fimbriae, and those best described are produced by *Escherichia coli* (7). These fimbriae have receptors on a wide variety of eucaryotic cells, although their role in the pathogenesis of infectious disease is unclear (7). In the case of *E. coli*, type 1 fimbriae are not homogeneous but comprise a family of fimbriae with differing subunit M_rs and different serological properties (23, 32).

The invasive enteropathogen *Salmonella typhimurium* also produces type 1 fimbriae (4). The subunits of the *S. typhimurium* type 1 fimbriae have a significantly higher subunit M_r, a different amino acid composition, and no serological cross-reactivity with the type 1 fimbriae of *E. coli* (24). Other serotypes of *Salmonella* have also been reported to produce mannose-sensitive fimbriae (5, 6), but there is no detailed biochemical information available concerning these fimbriae. One serotype commonly associated with salmonellosis in humans is *Salmonella enteritidis* (3). We isolated the mannose-sensitive fimbriae from a human isolate of *S. enteritidis* and biochemically characterized the fimbrin polypeptide subunit (M_r, 14,400). In this paper we report a rapid, straightforward purification procedure for *S. enteritidis* fimbriae along with the first reported studies on their chemical and antigenic properties.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1. Stock cultures were maintained at −70°C in 15% (vol/vol) glycerol-tryptic soy broth (GIBCO Diagnostics, Madison, Wis.). Working cultures of salmonella were grown on Luria broth (LB) agar or colonization factor agar (CFA; 10) at 37°C, and cells for fimbriae isolation and detection were grown in static CFA broth (10) at 37°C. Strains of *E. coli* producing various fimbriae types were grown under the conditions recommended for optimal fimbria production (26).

Hemagglutination. Hemagglutination (HA) was performed on glass slides. Suspensions of bacteria were prepared in

TABLE 1. Bacterial strains

Strain	Source[a]
S. enteritidis	
27655-3b	T. Wadström
S. typhimurium	
LT1	Type 1, this laboratory
S736	Type 1, J. P. Duguid
SL5166	Nonfimbrial, mannose resistant, hemagglutinin, M. Halula
S. cubana	
S211	Type 1, J. P. Duguid
S. illinois	
S1093	Type 1, J. P. Duguid
S. worthington	This laboratory
S. mikawasima	This laboratory
E. coli	
A122	Type 1, J. P. Duguid
A51	Type 1, J. P. Duguid
341	K88, T. Wadström
1475	K99, F41, T. Wadström
441	CFA I, T. Wadström
438	CFA II, T. Wadström

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phosphate-buffered saline (PBS, pH 7.4) to yield approximately 1.5×10^9 bacteria per ml. Human erythrocytes were collected immediately before use and washed three times in PBS, a 3% (vol/vol) suspension was prepared in PBS, and 20 μl was mixed with 20 μl of bacterial cells. HA usually occurred within 3 min. Sugar inhibition of HA was tested by performing HA tests in the presence of 20 μl of 0.3 M sugar.

Salt aggregation. The concentration of ammonium sulfate required to cause aggregation of bacterial cells was determined on glass slides by the procedure of Lindahl et al. (26).

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (25). Protein solubilized in sample buffer was stacked in 4.5% acrylamide (10 mA) and separated by using 12.5% acrylamide (20 mA). Molecular weight determinations were also performed after electrophoretic separation in 12.5% acrylamide gels containing 0.1% sodium dodecyl sulfate (SDS) and 8 M urea by the method of Swank and Munkres (36). Electrophoresis was done at 20 mA, constant current. Fragments of sperm whale myoglobin prepared by cleavage with CNBr in the presence of 70% (vol/vol) formic acid (15) were used as M_r standards. Protein was stained by Coomassie blue. The lipopolysaccharide (LPS) morphology in whole cell lysates was determined by the proteinase K digestion procedure of Hitchcock and Brown (17). After SDS-PAGE, gels were stained for LPS by the LPS silver staining procedure of Tsai and Frasch (39).

^{125}I radiolabeling. To radioiodinate proteins on the surface of intact cells, cells were first washed and suspended in cold PBS. A 1:10 dilution of this suspension yielded an A_{550} of 1.0. A 100-μl sample of the original suspension was then radioiodinated with the New England Nuclear Corp., Boston, Mass., radioiodination system (^{125}I), which utilizes immobilized lactoperoxidase and glucose oxidase. Enzyme beads were removed by low-speed centrifugation, and the ^{125}I-labeled cells were washed five times and suspended in 20 mM Tris hydrochloride buffer (pH 7.4). Whole cell lysates were subjected to SDS-PAGE, and radiolabeled proteins were detected by autoradiography. Purified fimbriae were radioiodinated by a modification of the chloramine-T method of Hunter and Greenwood (19).

Fimbriae purification. After 48 h of growth in static CFA broth, cells were harvested by centrifugation (4,000 × *g*, 4°C, 10 min), and suspended in cold 0.15 M ethanolamine buffer (pH 10.5), and fimbriae were separated from the cells by blending for 2 min. Cells were pelleted by centrifugation and suspended in ethanolamine buffer, and the blending and centrifugation procedures were repeated. The supernatants were combined, ammonium sulfate was added to 10% saturation, and the mixture was stirred overnight at 4°C and centrifuged (20,000 × *g*, 4°C, 10 min). The supernatant was then treated with 3 volumes of −20°C acetone, allowed to stand on ice for 10 min, and centrifuged (15,000 × *g*, 0.5°C, 10 min). The acetone was decanted, the pellet was suspended in ethanolamine buffer, and the acetone precipitation and centrifugation steps were repeated. A total of 1 ml of ice-cold ethyl ether was added to the pellet, the top of the pellet was carefully rinsed to avoid disturbing the pellet, the ether was poured off, and the precipitate was suspended in ethanolamine buffer. Crystalline ammonium sulfate was added to 40% saturation and stirred overnight at 4°C, and the precipitate was collected by centrifugation as described above. After suspension in ethanolamine buffer and centrifugation at 115,000 × *g* for 60 min at 4°C, the supernatant was dialyzed extensively against 20 mM Tris hydrochloride buffer (pH 7.5). After dialysis, the fraction was lyophilized, and the lyophilisate was suspended in 20 mM Tris hydrochloride buffer (pH 7.5) containing 0.2% SDS and centrifuged for 3 h at 115,000 × *g* at 20°C. The pellet was then suspended in ethanolamine buffer and eluted through an Extracti-Gel D column (Pierce Chemical Co., Rockford, Ill.) to remove the SDS. Fimbrin-containing fractions were combined, dialyzed against sterile distilled water, and lyophilized.

Amino acid composition analysis. The purified protein was dialyzed extensively against distilled water, lyophilized, and then hydrolyzed in 6 N HCl at 100°C for 18 h. The hydrolysates were analyzed on a Beckman 118BL amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) by using standard procedures. Cysteine was analyzed as cysteic acid, methionine was analyzed as methionine sulfone after performic acid oxidation, and tryptophan was determined spectrophotometrically by the methods of Goodwin and Morton (14) and Edelhoch (8).

N-terminal sequence analysis. The amino acid sequence analysis was performed on a Beckman Model 890C protein sequenator with a 0.1 M quadral program. Phenylthiohydantoin derivatives were isocratically separated by the procedure of Tarr (37) on octadecylsipane ultrasphere high-pressure liquid chromatography columns (Altex) fitted to a Beckman Model 332 high-pressure liquid chromatograph equipped with a Hewlett-Packard 3390A integrater (Hewlett-Packard Co., Palo Alto, Calif.).

Hydrophilicity and secondary structural predictions. The local hydrophobicity was predicted from the amino acid sequence by the method of Hopp and Woods by using hexapeptide averages (18). Secondary structural predictions were calculated from the algorithms of Chou and Fasman (2) and Garnier et al. (13).

Antibody production. Antiserum was raised in a 2-kg female New Zealand White rabbit by an injection of 50 μg of purified protein in Freund complete antigen, followed by two successive injections of 50 μg in Freund incomplete adjuvant at 3-week intervals. Antibodies to heat-stable antigens were removed by twice absorbing antisera with a homologous heat-stable antigen suspension as previously described (27). Enzyme-linked immunosorbent assay (ELISA) showed that the antiserum had a titer at A_{405} of 1:10,000. Control nonimmune serum was obtained before the first injection.

ELISA. The ELISA was essentially that of Engvall and Perlmann (9). Purified protein antigen was tested, in triplicate, at 5 μg per well; the developing antibody was alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin. A_{405} was read with an EIA model 310 ELISA reader (Biotech Instruments Inc., Burlington, Vt.). Bovine serum albumin was used as an antigen control, and nonimmune rabbit serum was used as an antiserum control.

Western blotting. After SDS-PAGE, separated components were transferred from the slab gel to 0.45-μm nitrocellulose paper (NCP) by using the methanol-Tris glycine system described by Towbin et al. (38). Electroblotting was performed in a Bio-Rad transblot apparatus (Bio-Rad Laboratories, Richmond, Calif.) for 3 h at 60 V. After blocking unreacted sites overnight in a 0.25% (wt/vol) solution of gelatin (100 bloom; Fisher Scientific Co., Pittsburgh, Pa.) in 10 mM Tris hydrochloride buffer (pH 7.4)–0.9% saline, the NCP was incubated with dilutions of antisera in Tris-gelatin-saline for 2 h at room temperature with gentle agitation. After extensive washing, the NCP was incubated with ^{125}I-radiolabeled *S. aureus* protein A (Pharmacia, Inc., Piscataway, N.J.) in Tris-gelatin-saline at 5×10^5 cpm per ml. Detection of bound radiolabeled protein A was accom-

plished by autoradiography of washed and dried NCP sheets.

Immunodot blotting was performed by transferring 10^5 cells of each strain to be tested to NCP which was then dried at 37°C for 1 h. The NCP was then blocked with Tris-gelatin-saline and reacted with antibody, and antibody-antigen complexes were detected with ^{125}I-labeled protein A as described above.

Radioimmune precipitation. ^{125}I-labeled fimbrin (100 μl, 10^6 dpm) and antifimbrin antisera (40 μl) in radioimmune precipitation buffer (0.5 M NaCl, 0.5% [vol/vol] Nonidet P-40, 0.1% [wt/vol] SDS, 2 mM Tris [pH 7.8], 1 mM EDTA, 1 mM EGTA) were incubated for 18 h at 4°C. A 50-μl portion of a 50 μg/ml suspension of protein A Sepharose (Pharmacia) (previously incubated for 1 h at 20°C in 10% [wt/vol] bovine serum albumin in PBS) was added, and after 1.5 h of incubation at 4°C, the beads were removed by centrifugation, washed twice, and then boiled in solubilization buffer. The resulting supernatant solutions were subjected to SDS-PAGE, and labeled antigen was detected by autoradiography.

Immune electron microscopy. Bacterial cells on carbon-coated, Formvar-filmed grids were reacted with antisera for 30 min. Excess antisera was removed, and the grids were washed with PBS. After the excess fluid was removed, the grids were negatively stained with 1% uranyl acetate (pH 4.2) and examined in a Zeiss Model 9 electron microscope.

Transposon mutagenesis and Tn*10* excisions. The transposable element Tn*10* (tetracycline resistance) was introduced into *S. enteritidis* at a multiplicity of infection of <1 by using the specially constructed P22 phage P22*Tc*10 (1, 21). The mixture was incubated at 37°C for 30 min to permit phage adsorption, then spread on LB agar containing 20 μg of tetracycline per ml, and the plates were incubated at 42°C for 36 h. Imprecise excisions of the Tn*10* element were selected as fusaric acid-resistant colonies (29).

Oligonucleotide probe. A mixed-sequence oligonucleotide (17-mer) based on the *S. enteritidis* fimbrin amino acid sequence from residue 25 to residue 30 was provided by M. Smith, Department of Biochemistry, University of British Columbia. The oligonucleotide was radiolabeled by [γ-^{32}P] ATP by the procedure of Whitehead et al. (43).

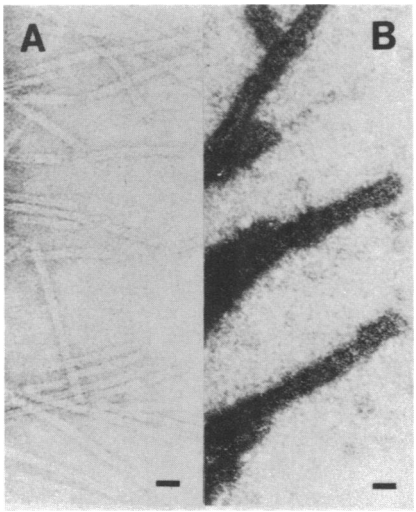

FIG. 1. Electron microscopy of fimbriae of *S. enteritidis* 27655-3b stained by 1% uranyl acetate (pH 4.2). (A) Native fimbriae. (B) Immunolabeling with 1:32 dilution of antiserum raised in rabbits to purified fimbrin. Bar, 100 nm.

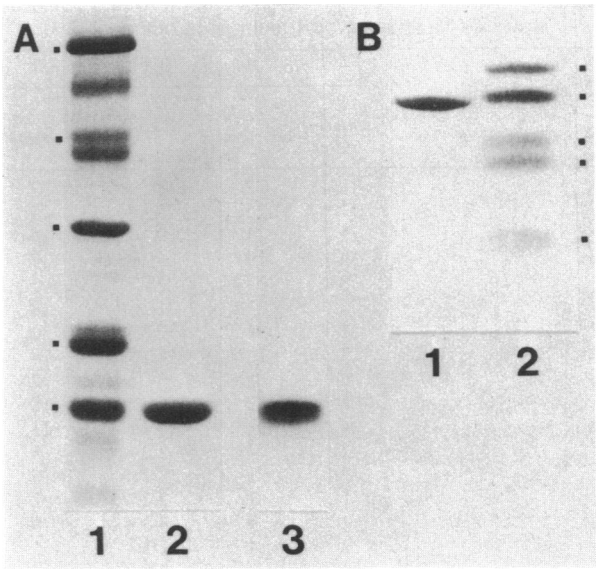

FIG. 2. Electrophoretic analysis of *S. enteritidis* 27655-3b fimbrin. (A) SDS-PAGE. Lane 1, M_r standards at 92,500, 66,200, 45,000, 31,000, 21,500, and 14,400; lane 2, purified fimbrin, staining by Coomassie blue; lane 3, Autoradiogram of fimbrin immunoprecipitated by a 1:1,000 dilution of antiserum raised in rabbits to purified fimbrin. (B) Urea-peptide gel electrophoresis. Lane 1, Purified fimbrin; lane 2, M_r standards at 17,200, 14,600, 8,200, 6,400, and 2,600; staining by Coomassie blue.

Southern transfers. DNA samples were prepared as described by Stern et al. (35), and complete restriction digests of 2 μg of chromosomal DNA were prepared by using *Bam*HI, *Eco*RI, *Sal*I, or *Pst*I (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) for 4 h at 37°C by following the directions of the manufacturer. After electrophoresis for 5 h at 100 V in 0.8% agarose gels with Tris-acetate buffer (28), electrophoretically separated restriction fragments were transferred to nitrocellulose by the procedure of Southern (34) as described by Maniatis et al. (29). The blotted filters were prehybridized for 3 h at 37°C and then hybridized against the ^{32}P-radiolabeled mixed-sequence oligonucleotide probe for 16 h at 37°C by the protocol of Whitehead et al. (43). After washes, filters were submitted to autoradiography.

RESULTS

Strain studied. *S. enteritidis* 27655 was an enterotoxin-producing strain isolated from human feces in India and, during growth on solid media, produced two variants with distinct colonial forms. The colony variants were designated 27655-3a and 27655-3b. In static liquid culture, 27655-3a produced an even turbidity, whereas variant 27655-3b produced a heavy pellicle. The cells of variant 27655-3a were apparently hydrophilic, autoaggregating in 1.5 M ammonium sulphate, whereas the cells of variant 27655-3b were apparently strongly hydrophobic, autoaggregating at 0.2 M ammonium sulfate. SDS-PAGE analysis of the LPS in proteinase K-digested whole cell lysates of both colonial variants revealed similar amounts of smooth LPS with characteristic *O*-polysaccharide chains of heterogeneous chain length (data

TABLE 2. Amino acid composition of fimbrin from *S. enteritidis* strain 27655-3b and type 1 fimbrins of other species

Amino acid	No. of residues/fimbriae subunit			
	S. enteritidis	*S. typhimurium*[a]	*E. coli*[b]	*K. pneumoniae*[c]
Asx	13	22	18/19	27
Thr	17	25	20/19	25
Ser	11	23	9/10	14
Glx	14	19	16/13	17
Pro	8	11	2/2	5
Gly	22	23	21/16	18
Ala	21	34	35/31	30
Val	13	16	14/15	18
Met	0	tr	0/0	2
Ile	5	7	5/4	8
Leu	4	12	14/10	13
Tyr	2	4	2/2	6
Phe	7	9	8/7	6
His	1	3	2/2	2
Lys	4	9	4/3	8
Arg	2	4	2/3	5
Cys	0	0	2/2	4
Trp	1	0	0/0	1
Total no. of residues/mol	145	221	174/158	209
Apparent M_r (10^3)	14.4	22.1	17.1/15.7	21.5
Hydrophobic residues (%) (V,M,I,L,A,F,W, and P)	40	40.5	45.1/43.6	39.7

[a] Data from Korhonen et al. (24).
[b] Data from Salit and Gotschlich (31)/data from Klemm (22). The data from Klemm was calculated based on DNA sequence.
[c] Data from Fader et al. (11).

not shown). Strain 27655-3b also displayed a weak ability to hemagglutinate erythrocytes of all human blood groups; this HA was inhibited by 0.1 M D-mannose but not by 0.1 M L-fucose or 0.1 M D-galactose. Electron microscopy showed that whereas cells of variant 27655-3a were nonfimbriated, *S. enteritidis* 27655-3b cells were heavily fimbriated, and these fimbriae displayed typical type 1 morphology (Fig. 1A).

Purification of fimbriae. Cells of 27655-3b were surface radiolabeled with ^{125}I by using the immobilized lactoperoxidase-glucose oxidase procedure. When whole cell lysates were fractionated by SDS-PAGE with 12.5% acrylamide gels, subsequent autoradiography revealed that a polypeptide of an apparent M_r of 14,400 was strongly radiolabeled (data not shown). SDS-PAGE analysis further indicated that this polypeptide was readily removed from the cells by homogenization and centrifugation. Electron microscopy also showed that this simple procedure removed fimbriae from the cells. For large-scale preparation, fimbriae were collected in 0.15 M ethanolamine buffer and partially purified by a simple protocol involving $(NH_4)_2SO_4$ precipitation, acetone precipitation, and differential ultracentrifugation. This preparation was contaminated with small amounts of flagella, and final purification was achieved by solubilization of the flagella in 0.2% SDS and collection of the fimbriae by ultracentrifugation. This simple protocol resulted in purification to homogeneity as assessed by Coomassie blue R staining of SDS-PAGE gels (Fig. 2A, lane 2).

Characterization of *S. enteritidis* fimbrin. SDS-PAGE analysis of the purified protein indicated an apparent M_r of 14,400 for the fimbrin subunit, and analysis by urea-peptide gel electrophoresis indicated an apparent M_r of 13,000. The

TABLE 3. N-terminal amino acid sequence of fimbrin of *S. enteritidis* strain 27655-3b and type 1 fimbrins of *E. coli* (22, 40) and *S. typhimurium* (41) aligned for greatest homology

Organism (reference)	Residue[a]
S. enteritidis	^{1}AGFVGNKA · VVQAAVTIAA QNTTSANWS · QDPGFTGPAV ·AAGQKVGTL SITATGPHNS VXIAGXQW
E. coli FimA[b] (22)	^{1}AATTVN G-T-HF-GE --N--CAVD- GSVDQTVQLG -VRTASLAQE G-TSSA--FN IQLNDCDT-Y ASK-AVAF
E. coli 1C[b] (40)	^{1}VTTVN G-T-HF-GD --N--CAVNT NSFEQTVNLG -VRSDRLKVE G-KSNP--FT IDLNECESQV SAG--IVF
S. typhimurium (41)	^{1}ADPTPTSVS G-TIHFEGK L-N--XAVST

[a] Amino acid residues are designated by the single-letter nomenclature (20). –, Residues homologous with *S. enteritidis* pilin; ·, deletion introduced into *S. enteritidis* sequence to make a better fit; X, not identified.
[b] Sequence deduced from DNA sequence.

amino acid composition of this fimbrin is shown in Table 2. Based on this composition, an M_r of 14,400 was calculated. The pilin had a calculated relative hydrophobicity of 40% (assuming V, I, L, A, F, W, and P) and lacked methionine. N-terminal amino acid sequence analysis was then performed, and the first 64 amino acids of the sequence were determined (Table 3). The predicted secondary structure of the N-terminal half of the *S. enteritidis* 27655-3b fimbrin molecule as deduced by the method of Chou and Fasman (2) and by the method of Garnier et al. (13) is shown in Fig. 3. Determination of the local average hydrophilicity along the peptide chain as a moving average of hexapeptides (18) showed that only two regions of local hydrophilicity existed in the first half of the molecule (residues 26 through 30 and residues 39 through 44), with the remainder of the first half of the molecule being distinctly hydrophobic (Fig. 3).

Antigenicity. Although Western blot experiments with purified fimbrin and polyclonal antiserum prepared in rabbits to the purified fimbrin were negative (data not shown), the antiserum could be shown to immunoprecipitate the purified protein (Fig. 2A, lane 3). Immune electron microscopy showed that the antibodies completely decorated intact native fimbriae rods on the homologous strain (Fig. 1B), confirming that the protein which had been purified was the fimbrial protein and showing that epitopes recognized by the antisera were surface exposed. The immunodot-blot assay showed that the antiserum was specific for the fimbrin of *S. enteritidis* 27655-3b, since no reaction was obtained with a variety of fimbriated *Salmonella* and *Escherichia* strains, including strains producing type 1 fimbriae (Fig. 4). The antiserum also inhibited the HA reaction of *S. enteritidis* 27655-3b (data not shown).

Transposon mutagenesis. To provide additional evidence that the fimbriae of *S. enteritidis* 27655-3b were responsible for pellicle formation and HA, in addition to contributing to the surface hydrophobicity of the strain, a nonfimbriated mutant was isolated. The transposon mutagenesis employed

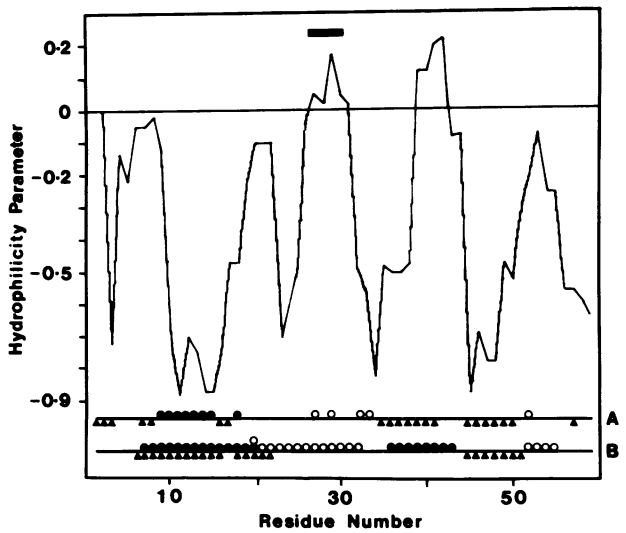

FIG. 3. Predictive analysis of the local average hydrophilicity of the N-terminal 64 residues of *S. enteritidis* 27655-3b fimbrin based on the Hopp and Woods (18) algorithm. Predictive analysis of the secondary structure by the method of Garnier et al. (A) (13) and Chou and Fasman (B) (2). ▲, β sheet; ——, random coil; ●, α-helix; ○, β-turn. Black bar, hydrophilic sequence with β-turn, representing a potential antigenic determinant (22).

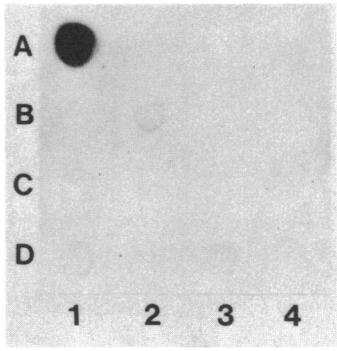

FIG. 4. Autoradiogram of immunodot-blot of bacterial cells with a 1:1,000 dilution of antiserum raised in rabbits to purified fimbrin of *S. enteritidis* 27655-3b. (A) Lane 1, *S. enteritidis* 27655-3b; lane 2, *E. coli* A122; lane 3, *E. coli* A51; lane 4, *E. coli* 341. (B) Lane 1, *E. coli* 1475; lane 2, *E. coli* 441; lane 3, *E. coli* 438; lane 4, *S. typhimurium* S736. (C) Lane 1, *S. typhimurium* LT2; lane 2, *S. typhimurium* SL51166; lane 3, *S. cubana* S211; lane 4, *S. illinois* S1093. (D) Lane 1, *S. worthington*; lane 2, *S. mikawasima*; lane 3, *S. saint-paul*; lane 4, *S. bovis morbificans*.

transduction of Tn*10* into strain 27655-3b by phage P22*Tc*10. The 956 Tc^r clones resulting from this transduction were screened for pilus production by immunodot-blot assay. One of these clones, 27655-3b-122, exhibited no reactivity with the antifimbrial antiserum, and electron microscopy confirmed the absence of fimbriae on this strain. Mutant 27655-3b-122 did not exhibit pellicle formation in static culture, was unable to hemagglutinate, and displayed a marked reduction in hydrophobicity, aggregating at 1.5 M $(NH_4)_2SO_4$ instead of 0.2 M $(NH_4)_2SO_4$ as observed with the parent. To confirm that the Fim^- phenotype was in fact due to the Tn*10* element, phage P22 was used to transduce the Tc^r marker from strain 27655-3b-122 back into the wild-type Fim^+ strain 27655-3b. A total of 30 Tc^r transductants were selected, and when tested by immunodot blotting, each failed to react with the antifimbrial antiserum, confirming that the Tn*10* insertion caused the loss of fimbriation. Furthermore, imprecise Tn*10* excisions were isolated as fusaric acid-resistant clones. As expected, these were Tc^s and afimbriate, indicating that the Tn*10* element was responsible for the Fim^- phenotype of strain 27655-3b-122. Electron microscopy of these transductants further confirmed the absence of fimbriae.

The nature of the mutation produced by the Tn*10* insertion was further investigated with a ^{32}P-radiolabeled mixed-sequence oligonucleotide probe to the *S. enteritidis* fimbrin gene. Complete restriction digests of parent and mutant DNA were prepared by using *Eco*RI and *Bam*HI, both of which have a single site in Tn*10*, and with *Sal*I and *Pst*I, which have no site in Tn*10*. Because Tn*10* is 9.2 kilobases long (21), insertion of Tn*10* into the fimbrin gene would be expected to produce an altered Southern blot profile when the restriction digests of the parent and mutant DNA were probed with the oligonucleotide. However, the results (Fig. 5) showed that the blot profiles of parent and mutant DNAs were identical for each of the restriction enzymes used, indicating that the Tn*10* insertion was in fact not in the fimbrin gene per se. This was confirmed when Southern blots of complete *Eco*RI and *Pst*I digests of DNA from three afimbriate fusaric acid-resistant Tn*10*-excision clones (122-34, 122-53, and 122-61) with the fimbrin oligonucleotide

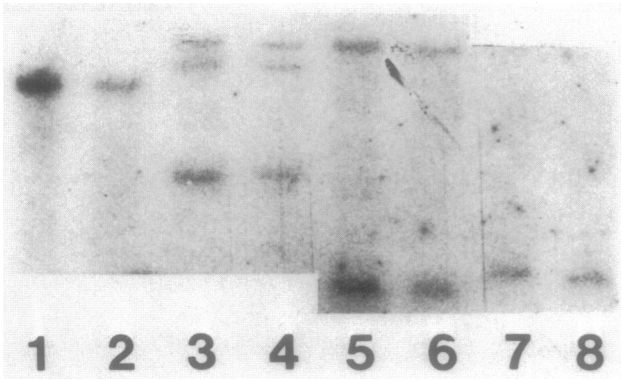

FIG. 5. Autoradiogram of Southern blot of complete restriction digests of DNA from *S. typhimurium* 27655-3b (lanes 1, 3, 5, and 7) and nonfimbriated Tn*10* insertion mutant 27655-3b-122 (lanes 2, 4, 6, and 8) with ^{32}P-radiolabeled mixed oligonucleotide probe to 27655-3b fimbrin. DNA cut by *Bam*HI (lanes 1 and 2) *Eco*RI (lanes 3 and 4), *Sal*I (lanes 5 and 6), and *Pst*I (lanes 7 and 8).

probe showed a blot profile identical to that of both the parent strain 27655-3b and the Tn*10* insertion mutant 27655-3b-122 (data not shown). The same Southern blot profile was also shown with restriction digests of DNA from the afimbriate colony variant 27655-3a, indicating that this strain was an afimbrial phase variant of 27655-3b (data not shown).

DISCUSSION

S. enteritidis fimbriae were purified by a simple, rapid procedure involving manipulations of ionic strength along with differential solubilization and ultracentrifugation in the presence of 0.2% SDS. This convenient procedure provided high yields of fimbriae of high purity. By SDS-PAGE, the M_r of the *S. enteritidis* fimbrin subunit thus purified was 14,400, which agreed with the M_r of 14,400 calculated from the amino acid composition. Interestingly, both these values were higher than the M_r of 13,000 obtained by urea-peptide gel electrophoresis and the M_r of 13,800 obtained by ultracentrifugation (unpublished data). All of these values are significantly lower than the M_r of 22,100 reported for the subunits of the type 1 fimbriae of *S. typhimurium* (24), the M_r of 21,500 reported for the type 1 fimbrin of *Klebsiella pneumoniae* (11), and are also lower than the M_r of 15,706 estimated from DNA sequence analysis for the processed *FimA* gene product in *E. coli* (22).

The amino acid composition of the *S. enteritidis* fimbrin differed from other reported type 1 fimbrin subunits (Table 2), reflecting the lower M_r of the *S. enteritidis* molecule. However, when considered on the basis of the relative proportions of various amino acid classes, the *S. enteritidis* molecule was not markedly different from other type 1 fimbrins, with hydrophobic residues comprising approximately 40% of both *Salmonella* subunits, whereas the 55.9% nonpolar amino acid content of the *S. enteritidis* fimbrin was comparable to 54.6% for the *E. coli* fimbrin. Both *Salmonella* subunits also lacked cysteine residues. In the case of the N-terminal amino acid sequence, alignment invoking a single residue deletion after residue 8 of the *S. enteritidis* molecule showed that the N-terminal 18-amino-acid sequence of the *S. enteritidis* fimbrin was highly related to the *E. coli* type 1 fimbrin sequences from residue 7 to residue 25 (Fig. 3). The sequences displayed conserved residues at positions 2, 4, 7, 9, 10, 11, 13, and 14, and, in the case of *FimA*, residue 19 (based on the *S. enteritidis* sequence) along with conservative replacements at positions 1, 8, 12, and 17. In contrast, the two *Salmonella* sequences displayed conserved residues only at positions 2, 11, 13, and 14 (based on the *S. enteritidis* sequence).

In the case of the *E. coli* and *S. typhimurium* fimbrins, the region of sequence homology occurred 6 to 9 residues downstream from the N-terminal end of the molecule. Because it is likely, especially in the case of members of the family *Enterobacteriaceae*, that all these fimbriae, including those of *S. enteritidis*, are descendents of a common ancestral gene, the absence of these 6 to 9 N-terminal residues in the *S. enteritidis* molecule points to a role for deletion in the evolution of *S. enteritidis* fimbrin. Deletion in other parts of the sequence would also account for the substantially lower M_r of this molecule compared with other type 1 fimbrins and would certainly contribute to the minimal sequence homology that appears to occur distal to residue 19 in the *S. enteritidis* sequence. Further, the observation that 145 residues per fimbrin chain allow for the production of apparently fully functional fimbriae by *S. enteritidis* suggests that in the case of *S. typhimurium*, some 76 residues are not essential to the formation of a functional fimbriae of this type. The minimal homology between the sequence distal to residue 19 and the *E. coli* sequences explains the serospecificity provided by the conformational *S. enteritidis* fimbrin epitopes and provides a compelling argument that the conserved area of primary sequence is structurally essential. *Pseudomonas aeruginosa* PAK fimbriae (33), *Neisseria gonorrhoeae* (16), *Neisseria meningitidis* (30), and *Moraxella nonliquefaciens* (12) fimbrins similarly have a conserved N-terminal hydrophobic sequence which is the only region of homology among these fimbrins. Watts et al. (42) provided excellent evidence that this sequence is buried in the native state and suggested that in these fimbriae the conserved N-terminal hydrophobic sequences are involved in subunit-subunit interactions in the native molecule. This may also hold true for type 1 fimbriae.

Application of immunodot-blot screening enabled the isolation of a Tn*10*-induced nonfimbriated mutant of *S. enteritidis* 27655-3b. Southern blot analysis of this Tn*10* mutant with a mixed sequence fimbrin gene probe indicated that the insertion was not in the fimbrin structural gene, but presumably in a gene controlling fimbrial expression. Transduction of Tn*10* back into the wild type also yielded nonfimbriated mutants. Excision of the Tn*10* element also yielded nonfimbriated mutants. Although this Tn*10* insertion was shown not to be in the structural gene, we are unable to say whether the Tn*10* insertion is in a region adjacent to the structural gene at this time. Both the Tn*10*-induced mutants and corresponding transductants displayed markedly reduced surface hydrophobicity, confirming the contribution of fimbriae to the high surface hydrophobicity of *S. enteritidis* 27655-3b. The Tn*10*-induced mutants were also HA negative, and together with the ability of antifimbrial antiserum to inhibit HA, provided strong evidence that the ability to produce HA is mediated by fimbriae in *S. enteritidis*.

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