NOTES

Thin Aggregative Fimbriae from Diarrheagenic *Escherichia coli*

S. KAREN COLLINSON,¹ LEVENTE EMÖDY,² TREVOR J. TRUST,¹ AND WILLIAM W. KAY^{1*}

Department of Biochemistry and Microbiology and Canadian Bacterial Diseases Network, Petch Building,
P.O. Box 3055, University of Victoria, Victoria, British Columbia V8W 3P6, Canada,¹ and
Institute of Microbiology, University Medical School, H-7643 Pécs, Hungary²

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Four strains of diarrheagenic *Escherichia coli* originally isolated from distinct geographic regions were found to produce unusual thin aggregative fimbriae requiring depolymerization in formic acid prior to analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoelectron microscopy of native fimbriae and Western blot (immunoblot) analysis of the corresponding 18-kDa fimbrins showed that these *E. coli* fimbriae were serologically cross-reactive with SEF 17 (*Salmonella enteritidis* fimbriae with a fimbrin molecular mass of 17 kDa). The *E. coli* and *S. enteritidis* fimbrins had similar total amino acid compositions and highly conserved N-terminal amino acid sequences. These results indicate that *E. coli* and *S. enteritidis* produce biochemically related, aggregative fimbriae which constitute a new type of intergenerically distributed fimbriae for which we propose the descriptive name GVVPQ fimbriae on the basis of the conserved N-terminal amino acid sequence.

Successful bacterial pathogens present to their susceptible host complex cell surfaces which contain components enabling the bacteria to initially associate with and subsequently adhere to specific host tissues (2, 21). The attachment of enteropathogens to gastrointestinal epithelial cells is presumed to be essential if these bacteria are to avoid host clearance mechanisms and compete with normal flora for appropriate niches (2, 15). However, the specific attachment mechanism(s) of many enteropathogens in vivo has not been clearly established (2, 14). Numerous enteropathogens, including enterotoxigenic *Escherichia coli* (23, 26), enteroadherent aggregative *E. coli* (39), enteropathogenic *E. coli* (17, 32), *Vibrio cholerae* (36), *Yersinia enterocolitica* (1, 35), *Shigella flexneri* (11), and *Salmonella* spp. (1, 9, 29, 35, 37), produce adhesive fimbriae which extend from the bacterial cell surface as thin (2 to 7 nm), filamentous structures. Some fimbriae have been shown to bind specific carbohydrate moieties on glycoconjugate receptors on eukaryotic cell surfaces (2) and are therefore possible virulence factors. Importantly, certain host-adapted, noninvasive enterotoxigenic *E. coli* strains possess plasmid-encoded fimbriae which mediate bacterial attachment to intestinal epithelial cells essential for virulence (2, 16). Moreover, the excellent antigenic nature of these fimbriae has resulted in the successful commercialization of vaccines based on purified fimbriae for the protection of young domestic animals from enterotoxigenic *E. coli* infections (23).

Recently, aggregative diarrheagenic *E. coli* strains have been described (33, 39), as have hydrophobic *E. coli* strains capable of binding tissue matrix proteins (13, 27, 28). However, the nature of the aggregative phenotype and the mechanism by which the *E. coli* strains bind to tissue matrix proteins are unknown. Concurrently, we have been studying an extremely aggregative *Salmonella enteritidis* strain,

27655-3b (9, 29), which also binds certain tissue matrix proteins (5, 8a) and produces unusual thin, aggregative fimbriae designated SEF 17 (*S. enteritidis* fimbriae composed of 17-kDa fimbrin) that are extremely insoluble and recalcitrant to purification by standard techniques for fimbriae (9). Moreover, SEF 17 confer on *S. enteritidis* a distinctive rough, aggregative colonial morphology and the ability to bind fibronectin (8a). In view of the fact that SEF 17 are not detected by standard techniques, certain aggregative *E. coli* strains were examined for SEF 17-like fimbriae. This study confirms that aggregative *E. coli* strains produce fimbriae which are morphologically, biochemically, and immunologically related to the SEF 17 originally discovered on *S. enteritidis*.

Identification of aggregative fimbriae on *E. coli*. The bacterial strains used in this study (Table 1) were grown at 37°C for 24 h on solid T medium (9) or T medium supplemented with 100 µg of Congo red (Sigma Chemical Co., St. Louis, Mo.) per ml prior to sterilization. Immunoelectron microscopy showed that several aggregative *E. coli* strains possessed distinctive fimbriae which were specifically decorated with protein A-gold following incubation of the cells with serum from rabbits immunized with SEF 17 purified from *S. enteritidis* (9) (Fig. 1). However, the fimbriae were not immunolabelled by preimmune serum (Fig. 1C and H). These *E. coli* fimbriae were thin (2 to 4 nm) and were associated with the *E. coli* cells as diffuse aggregates (Fig. 1F), denser clumps (Fig. 1D), or encasements (Fig. 1A), although cell-free fimbrial aggregates were also present. Each strain exhibited this full range of fimbrial distribution, including many fimbria-free cells (Fig. 1A). The immunological cross-reactivity of these *E. coli* fimbriae with SEF 17 of *S. enteritidis* was unexpected since serologically related fimbriae of these two genera of enteropathogens have not been previously detected (24). This finding raised the possibility that these fimbriae were also biochemically related and prompted our further investigation.

* Corresponding author.

TABLE 1. Bacterial strains used in this study and their fimbriae

Strain	Source or reference	Fimbriae produced		Colony morphology ^a	CR binding ^b
		Various ^c	GVVPQ ^d		
<i>E. coli</i>					
11775	ATCC ^e	ND	—	NAg	—
C600	ATCC	Curli (30)	—	NAg	—
HB101	7	Type I (12)	—	NAg	—
E1049a-13	T. Wadström	CFA/I	—	NAg	—
438 Hf	T. Wadström	CFA/II	—	NAg	—
B41M	J. Morris	F41 (10)	—	NAg	—
HM1475	J. Morris	K99	—	NAg	—
Vietnam I/1	T. Pál	ND	+	Ag	+
Gambia 3	28	ND	+	Ag	+
NG7C	13	Curli (13)	+	Ag	+
NG7C/1	13	ND	+/-	NAg	—
Viet G	T. Pál	ND	+	Ag	+
<i>S. enteritidis</i> 27655-3b	T. Wadström	SEF 14, SEF 21 (29)	SEF 17 (9)	Ag	+

^a Colonial morphology on cells grown on T medium: Ag, aggregative (rough); NAg, nonaggregative (mucoid).

^b Congo red (CR) binding by cells grown on T medium: +, cells bind Congo red, red colony; —, cells do not bind Congo red, pink colony.

^c CFA, colonization factor antigen; ND, not determined.

^d Presence of GVVPQ fimbriae determined by Western blot analysis of cell digests using immune serum to purified SEF 17 of *S. enteritidis* 27655-3b as the primary antibody. +, present; +/-, decreased amounts; —, not detected.

^e ATCC, American Type Culture Collection.

Biochemical characterization of aggregative fimbriae from *E. coli*. The fimbriae were purified from the aggregative *E. coli* strains Vietnam I/1, Viet G, NG7C, and Gambia 3 as previously described for SEF 17 of *S. enteritidis* (9). To facilitate routine screening of bacteria for the aggregative fimbriae subunit, whole cells were digested in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (25) containing 0.2 M glycine (pH 1.8) for 10 min at 100°C, since the aggregative fimbriae were resistant to depolymerization even when boiled in this acidified SDS-PAGE sample buffer. The insoluble cellular material was recovered by centrifugation (16,000 × g, 5 min), washed twice in distilled H₂O, and then acidified with formic acid prior to electrophoresis (9). After they were depolymerized, the fimbriae were analyzed by SDS-PAGE (25), and a major protein band with an apparent molecular mass of 18 kDa was observed by Coomassie blue staining (data not shown). Fimbriae monomers electrophoretically transferred to nitrocellulose were detected by Western blotting (immunoblotting) with rabbit immune serum raised to SEF 17 purified from *S. enteritidis* (9) as the first antibody followed by labelling with goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase. Immunoblots were developed with 5-bromo-4-chloro-3-indolylphosphate and nitro blue tetrazolium. Western blot analysis confirmed that the aggregative fimbriae required depolymerization in formic acid prior to electrophoresis and that the 18-kDa fimbrins isolated from *E. coli* Vietnam I/1, Viet G, NG7C, and Gambia 3 were immunologically cross-reactive with the 17-kDa fimbrin of SEF 17 (Fig. 2). This result also verified that these proteins were the fimbrin subunits of the aggregative *E. coli* fimbriae observed by immunoelectron microscopy in Fig. 1.

Amino acid sequence and compositional analyses. N-terminal amino acid sequencing was performed directly on approximately 1 to 10 µg of Immobilon-bound 18-kDa fimbrins as previously described (9). The first 10 N-terminal amino acid residues of the 18-kDa fimbrins isolated from *E. coli* Vietnam I/1, Viet G, NG7C, and Gambia 3 were conserved (Table 2). Moreover, these fimbrin sequences were very

similar to those of SEF 17, with the exception being a conservative substitution of the tyrosine residue at position 6 of the *E. coli* fimbrins with a tryptophan residue in the *S. enteritidis* fimbrin (Table 2). A FASTA computer search (31) and a literature survey which included reports about fimbriae from various *E. coli* strains (3, 6, 10, 16–19, 30) did not reveal any other fimbrial protein with an N terminus similar to that of the 18-kDa fimbrins or SEF 17. Furthermore, other thin fimbriae for which N-terminal sequence data are unavailable were not reported to require acid depolymerization of the fimbriae prior to electrophoresis (1, 8, 20, 38, 40).

Total amino acids of fimbriae purified from Vietnam I/1, Viet G, NG7C, and Gambia 3 were obtained from approximately 1 to 5 µg of Immobilon-bound 18-kDa fimbrin samples hydrolyzed in gaseous HCl (165°C, 1 h) and analyzed with an Applied Biosystems Model 420 amino acid derivatizer analyzer. The amino acid compositions of the 18-kDa fimbrins from these four strains were found to be quite similar to each other and to that of the SEF 17 fimbrin of *S. enteritidis*; the percentages of basic, potentially acidic, hydrophobic, aromatic, and polar uncharged amino acids were comparable (Table 3). The main difference between the compositions of the *E. coli* 18-kDa fimbrins and SEF 17 fimbrin was the presence of additional glycine residues in the former which probably accounted for their slightly increased molecular mass. The unusual abundance of the small amino acids serine (S), glycine (G), and alanine (A), from 36 to 47%, suggests a high degree of β-structure in all of these proteins.

Clearly, these *E. coli* strains produced fimbriae which were morphologically, biochemically, and serologically related to SEF 17 of *S. enteritidis*. As a general reference to this type of fimbriae, which are produced by at least two genera of important enteropathogens, we propose the descriptive name GVVPQ fimbriae on the basis of their conserved fimbrin N-terminal amino acid sequence.

Distribution of aggregative fimbriae. The *E. coli* strains Vietnam I/1, Viet G, NG7C, and Gambia 3, which produced the aggregative GVVPQ fimbriae, also exhibited aggregative

FIG. 1. Electron micrographs of negatively stained, immunogold-labelled, aggregative *E. coli* strains. (A to C) Gambia 3; (D and E) NG7C; (F to H) Viet G. Cells were incubated with immune serum against SEF 17 (A, B, and D to G) or with preimmune serum (C and H) prior to labelling with protein A-gold. Bars: A, C, and F, 1 μ m; B, D, E, G, and H, 250 nm.

FIG. 1—Continued.

FIG. 2. Western blot analysis of aggregative fimbriae isolated from digested *S. enteritidis* and *E. coli* cells. Lanes: 1 and 2, SEF 17 from *S. enteritidis* 27655-3b; 3 and 4, aggregative fimbriae from *E. coli* Viet G; 5 and 6, aggregative fimbriae from *E. coli* NG7C; 7 and 8, aggregative fimbriae from *E. coli* Gambia 3; 9 and 10, aggregative fimbriae from *E. coli* Vietnam I/1. The fimbriae were either untreated (lanes 1, 3, 5, 7, and 9) or pretreated with 90% formic acid (lanes 2, 4, 6, 8, and 10) prior to electrophoresis. The Western blot was developed after incubation with rabbit polyclonal immune serum raised against SEF 17 of *S. enteritidis* 27655-3b. The molecular masses of prestained protein standards (Bethesda Research Laboratories, Gaithersburg, Md.), β -lactoglobulin (18.4 kDa) and lysozyme (15.4 kDa), are indicated on the left.

colonial morphology, whereas the nonaggregative *E. coli* strains exhibited smooth colonial morphology (Table 1). The sole exception was the nonaggregative spontaneous variant of strain NG7C, designated NG7C/1, which produced small amounts of GVVPO fimbriae (Table 1). Lipopolysaccharide (LPS) analysis of proteinase K-digested cells indicated that *E. coli* C600 and HM1475 produced little or no LPS O chain whereas the other strains possessed normal amounts of LPS O chain (data not shown). These obvious alterations in LPS did not correlate with the aggregative colonial morphology. However, the ability of *E. coli* strains to bind the hydrophobic dye Congo red correlated with their autoaggregative phenotype and their ability to produce large amounts of GVVPO fimbriae (Table 1). These results suggest that the GVVPO fimbriae are associated not only with a distinctive aggregative colonial morphology but also with Congo red binding. This is consistent with our recent studies of SEF 17-deficient *S. enteritidis* mutants, which lose their aggregative colonial morphology and their ability to bind Congo red (8a). Similarly, Congo red binding apparently correlates with the ability of NG7C to produce curli (13), suggesting that other *E. coli* fimbriae may also promote Congo red binding. Congo red binding has been correlated with virulent strains of *Yersinia*, *Shigella*, and *Aeromonas*, whereas their avirulent counterparts do not bind Congo red (4, 22, 34). The Congo red-binding surface component of *Aeromonas salmonicida* has been identified as the regular surface array protein, and this protein is an established virulence factor (22). Although it is premature to speculate that the GVVPO fimbriae may be a virulence factor, it may be significant that the GVVPO fimbriae, SEF 17, confer on *S. enteritidis* the

TABLE 2. N-terminal amino acid sequence of 17-kDa and 18-kDa fimbriae isolated from aggregative fimbriae of *S. enteritidis* and *E. coli*, respectively

Organism	Fimbrin	N-terminal amino acid sequence ^a
<i>E. coli</i>	Vietnam I/1	G V V P Q Y G G G G N-
	Gambia 3	G V V P Q Y G G G G N-
	NG7C	G V V P Q Y G G G G N-
	Viet G	G V V P Q Y G G G G H-
<i>S. enteritidis</i> ^b	SEF 17	G V V P Q W G G G G N-

^a See Table 3 for the three-letter code corresponding to the single-letter amino acid code.

^b *S. enteritidis* 27655-3b fimbrin N-terminal amino acid sequence previously determined (9).

TABLE 3. Total amino acid analysis of 17-kDa and 18-kDa fimbriae isolated from aggregative fimbriae of *S. enteritidis* and *E. coli*, respectively

Amino acid residue	No. of residues per fimbrin subunit ^a				
	<i>E. coli</i>				<i>S. enteritidis</i> 27655-3b SEF 17 ^b
	Vietnam I/1	Gambia 3	NG7C	Viet G	
D or N (Asp or Asn)	37	34	24	16	31
T (Thr)	10	10	11	9	11
S (Ser)	14	13	10	8	13
E or Q (Glu or Gln)	19	17	16	25	17
P (Pro)	2	2	2	2	3
G (Gly)	39	39	56	43	26
A (Ala)	15	16	25	25	19
C (Cys)	ND ^c	ND	ND	ND	0
V (Val)	11	11	16	20	10
M (Met)	0	0	0	0	1
I (Ile)	4	5	5	6	6
L (Leu)	9	10	15	18	7
Y (Tyr)	5	5	3	2	6
F (Phe)	4	4	6	5	3
H (His)	4	4	2	2	1
K (Lys)	4	4	2	2	3
R (Arg)	3	4	2	2	4
W (Trp)	ND	ND	ND	ND	1 ^d
Total	180	178	195	185	162
% Hydrophobic ^e	28	30	37	42	34
% Acidic	31	29	21	22	30
% Polar uncharged ^e	38	38	41	34	35
% Basic	6	7	3	3	5
% Aromatic	5	5	5	4	6
% S, G, or A	38	38	47	41	36

^a The molar ratio of each amino acid was determined from the compositional analysis, and the alanine content was adjusted for a fimbrin molecular mass approximating 18 kDa, the apparent molecular mass of the fimbrin subunit as determined by SDS-PAGE.

^b Data from Collinson et al. (9).

^c ND, not determined.

^d Tryptophan analysis was not done, but the N-terminal amino acid sequence contains one tryptophan residue.

^e % hydrophobic determined by assuming P, A, V, M, I, L, Y, and F are hydrophobic; % polar uncharged determined by assuming G, S, T, C, and Y are polar uncharged.

ability to bind Congo red and fibronectin (8a) and that *E. coli* NG7C and Gambia 3, which also bind Congo red and various tissue matrix proteins including fibronectin (13, 27, 28), are now known to produce GVVPO fimbriae. It remains to be determined whether GVVPO fimbriae are involved in bacterial colonization of host epithelial tissues and whether they are advantageous to enteropathogens faced with the challenge of establishing an intestinal infection.

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