

## FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the *Enterobacteriaceae*

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**ABSTRACT** Type 1 pili are heteropolymeric mannose-binding fibers produced by all members of the *Enterobacteriaceae* family. The bulk of the fiber is composed of FimA. Two macromolecular complexes responsible for mediating an interaction with mannose-containing receptors were purified from *fimA*<sup>-</sup> *Escherichia coli* by mannose affinity chromatography and ion-exchange chromatography. One complex contained only the mannose-binding adhesin, FimH, associated with FimG, a minor component of the type 1 pilus. In the other complex the FimG–FimH moiety was loosely associated with a chaperone–minor subunit complex (FimC–FimF), possibly representing an intermediate in tip fibrilla assembly. The FimC chaperone has also been shown to form a preassembly complex with FimH that has been purified and characterized previously. Purified FimC did not bind to the FimG–FimH complex but did recognize FimH dissociated from the FimG–FimH complex. Quick-freeze deep-etch electron microscopy revealed that the FimG–FimH complex had a thin fibrillar architecture. High-resolution electron microscopy of type 1 pili revealed that a 16-nm fibrillar tip structure with an architecture identical to that of the FimG–FimH complex was joined end-to-end to the pilus rod. In a *fimH*<sup>-</sup> deletion mutant, the tip fibrillae joined to pilus rods were ≈3 nm in length. The full-length tip fibrilla was restored by complementation with the *fimH* gene in trans. The bipartite nature of the type 1 pilus was also demonstrated on pili purified from clinical isolates of members of the *Enterobacteriaceae* family arguing that it is a conserved feature of the type 1 pilus.

Species of bacteria belonging to the family *Enterobacteriaceae* play a significant role in human disease and have been associated with cystitis, pyelonephritis, pneumoniae, meningitis, bacteremia, and diarrheal disease among others (1). The restricted range of hosts, tissues, and cell types that bacteria are able to colonize is determined, at least in part, by a stereochemical fit between microbial adhesins and complementary receptor architectures on host cell surfaces (2). Type 1 pili bear the mannose-binding FimH adhesin, which is serologically conserved throughout the *Enterobacteriaceae* (3). The affinity of the FimH lectin for mannosylated proteins provides a number of host targets for type 1 fimbriated bacteria, including human buccal cells, proximal tubular cells of the kidney (2, 4), epithelial cells in the bladder, lung, and intestine (2), and various inflammatory cells (5). Bloch *et al.* have shown (6) a role for type 1 pili in oropharyngeal colonization in neonatal rats and transmission of *Escherichia coli* K1 among littermates. Additional receptors for the type 1 pilus have been reported to include a 65-kDa protein of guinea pig erythrocytes (7), the leukocyte adhesin molecules CD11 and CD18 (8), and the Tamm–Horsfall glycoprotein of human urine (2); recently, type 1 pilated bacteria have been shown to interact with the

basement-membrane component laminin (9) and with fibronectin (10). Sokurenko *et al.* (10) demonstrated that the FimH adhesin of the type 1 pilus represents a family of proteins which bind to various targets, including mannan and fibronectin.

In addition to the FimH adhesin there are two minor components in the pilus, FimF and FimG, which have been reported to play important roles in the regulation of pilus biogenesis (11–14). FimA, the major subunit of the type 1 pilus, is arranged in a tight right-handed helical rod (15). Assembly of type 1 pili depends on two proteins that are not a part of the final structure: FimC and FimD (16–18). FimC is a member of the immunoglobulin-like periplasmic chaperone family (17). Pilus chaperones, such as FimC, are thought to recognize specific carboxyl-terminal motifs on pilus subunits, forming chaperone–subunit complexes (19) that are targeted to outer membrane assembly sites (20). FimD belongs to the outer membrane usher family of proteins.

We report here the purification and analysis of the assembly of the mannose-binding moiety of the type 1 pilus. High-resolution electron microscopy revealed that the FimH adhesin molecule is a component of a fibrillar tip structure which was identified at the distal ends of pilus rods. The incorporation of FimH into the tip fibrilla proceeds from a FimC–FimH complex so that a surface on FimH recognized by the FimC chaperone becomes inaccessible following polymerization into the pilus.

### MATERIALS AND METHODS

**Bacterial Strains and Genetic Constructs.** *E. coli* ORN103 (18) was used as a host for recombinant plasmids in order to express and purify type 1 pili. The clinical isolates of *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Citrobacter freundii* from patients with urinary tract infections have been described (3), as has the clinical isolate of *E. coli* (J96) (18). The plasmids used in this study, pJP5 (type 1 operon, *fimC*<sup>-</sup>) (17), pJP4 (*fimC*) (17), pLS101 (*papD*) (21), pHA9 (type 1 operon, *fimA*<sup>-</sup>) (22), pSH2 (type 1 operon) (18), and pUT2002 (*fimH* deletion) (23) have all been described. The plasmid pHJ20, containing the *fimH* coding sequence, was constructed by subcloning *fimH* as a *Pvu* II fragment from pSH2 into pMMB66 (20).

**Purification of the Adhesin Complex/Tip Fibrillae.** Type 1 tip fibrillae were purified from 6 liters of ORN103/pHA9 grown for 18 hr in Luria broth. The cell pellet (12 g) was resuspended in 150 ml of buffer P (75 mM NaCl/0.5 mM Tris-HCl, pH 8.0) and subjected to shearing in a Waring Blendor (five 2-min pulses on the highest setting, with cooling on ice between each pulse). The supernatant, following centrifugation to remove cells and debris, was concentrated by precipitation with ammonium sulfate at 50% saturation; the precipitate was then dialyzed into buffer P. Binding to a slurry

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of mannose-linked Sepharose beads (gift of P. Stahl, Washington University Medical School) was allowed to proceed overnight at 4°C with gentle rocking. After exhaustive washing with 1× PBS (0.12 M NaCl/2.7 mM KCl/10 mM phosphate, pH 7.4), bound material was eluted with 15% methyl  $\alpha$ -D-mannopyranoside (Sigma) at room temperature. The eluate from the mannose chromatography was dialyzed into 20 mM Bis-Tris (Sigma) at pH 6.5, applied to a Spherogel TSK DEAE 5PW column (Beckman), and eluted with a linear gradient of 0–0.5 M NaCl in 20 mM Bis-Tris at pH 6.5. Peak fractions were analyzed by SDS/PAGE. The FimG–FimH complex was eluted at 0.25–0.3 M NaCl. Preparation of samples for high-resolution quick-freeze deep-etch electron microscopy was as described (24).

**Purification of Pili.** Type 1 pilus expression was induced in the recombinant strains of ORN103 as described (17). Induction of type 1 pili for purification from the clinical isolates was performed by static passage in Luria broth. Pili were purified from bacteria by shearing from the bacterial surface as described above for fibrillae and collected by precipitation with MgCl<sub>2</sub> (25).

## RESULTS

**Purification of the Adhesive Determinant of the Type 1 Pilus.** Hultgren *et al.* (22) showed previously that bacteria harboring the *fimA* deletion plasmid (pHA9) were capable of hemagglutinating guinea pig erythrocytes in a mannose-sensitive fashion; therefore the adhesin moiety, FimH, must have been exposed in a receptor-binding conformation on the bacterial surface. We undertook studies to purify the adhesive determinant in the absence of the FimA rod in order to perform a detailed structural and functional analysis on the adhesin complex. Transposon mutagenesis demonstrated that mannose-sensitive hemagglutination by ORN103/pHA9 was dependent on two of the known *fim* genes: *fimC* and *fimD* (22). We report that the adhesive determinant could be harvested from ORN103/pHA9 by shearing the bacteria in a Waring Blender—the method typically used to harvest type 1 pili.

After ammonium sulfate precipitation and dialysis the crude preparation was applied in batch to a slurry of mannose-Sepharose beads. The high-affinity mannose analog methyl  $\alpha$ -D-mannopyranoside was used to elute the adhesive determinant from the beads; this method was previously used to identify a FimC–FimH complex (17). SDS/PAGE of the partially purified mannose-binding determinant showed six prominent bands (Fig. 1A, lane 1). Amino-terminal sequencing and Western blot analysis confirmed the identity of the 14-, 16-, and 30-kDa proteins as FimG, FimF, and FimH, respectively (C.H.J. and S.J.H., unpublished data). Two additional Fim proteins copurified with the adhesin complex: FimC, the periplasmic chaperone, and a truncate of the FimH adhesin molecule. The identity of both proteins was confirmed by amino-terminal sequence analysis. The FimH truncate (FimH<sub>t</sub>) has the same amino terminus as mature FimH and binds to mannose (A.V.N. and S.J.H., unpublished data). In a non-denaturing PAGE system the mannose-purified adhesin complex ran as a series of three bands; all three of which reacted with anti-FimF, anti-FimG, and anti-FimH antibodies, confirming that FimF, FimG, and FimH are components of a distinct oligomeric complex(es) (C.H.J. and S.J.H., unpublished data). Cosedimentation in a 10–30% sucrose gradient also indicated that FimF, FimG, and FimH interact as a distinct complex (C.H.J. and S.J.H., unpublished data). Further purification of the adhesin complex by HPLC ion-exchange chromatography resulted in the separation of FimH<sub>t</sub> and the ~95-kDa contaminant from the adhesin complex (Fig. 1A). Interestingly, chromatography also resolved the complex into two populations: one contained FimC, FimF, FimG, and FimH (Fig. 1A, lane 2), and the other was composed of only FimG and FimH (lane 3). The purified FimG–FimH complex bound to the mannose-Sepharose beads and was specifically eluted with methyl  $\alpha$ -D-mannopyranoside, confirming that FimH in the complex exists in a receptor-binding conformation (Fig. 1B).

Quick-freeze deep-etch electron microscopy of the FimG–FimH complex revealed thin fibrilla-like architectures (Fig. 1C Lower). The FimG–FimH complex that was associated with

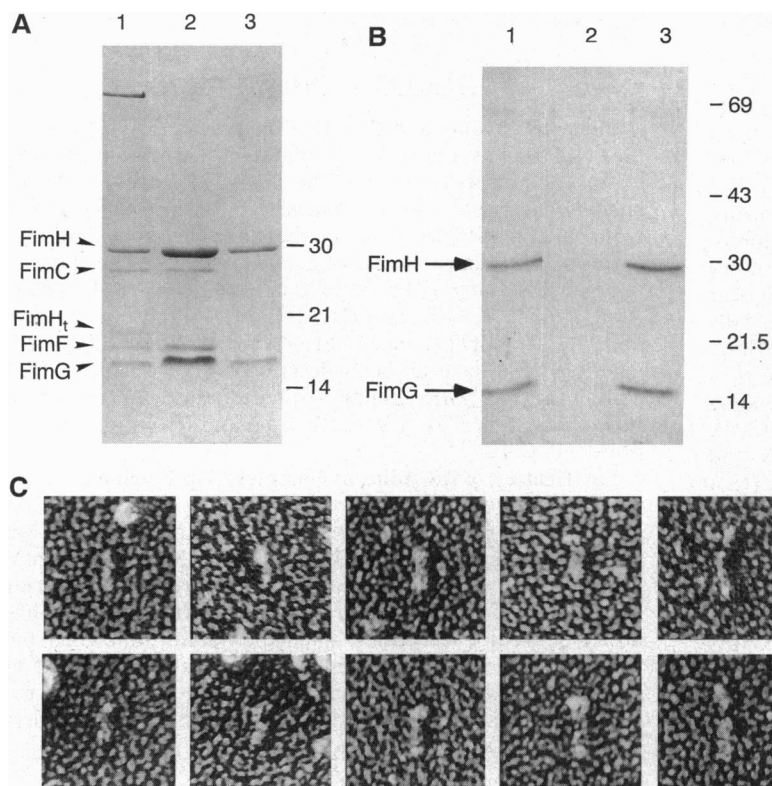


FIG. 1. Composition of type 1 adhesin determinant.

(A) Type 1 fibrillae obtained from *E. coli* and purified by mannose-Sepharose affinity chromatography were analyzed by SDS/PAGE. Lane 1 shows the 15% (wt/vol) methyl  $\alpha$ -D-mannopyranoside eluate from mannose-Sepharose. This material was further purified by HPLC ion-exchange chromatography. Lanes 2 and 3 show representative fractions from an early and a late chromatographic peak, respectively. The identities of the five proteins in the fibrillae are indicated and were verified by amino-terminal sequence analysis and Western blotting (C.H.J. and S.J.H., unpublished data). FimH<sub>t</sub> is an amino-terminal mannose-binding truncate of FimH (A.V.N. and S.J.H., unpublished data). (B) HPLC-purified FimG–FimH complex was applied to a mannose-Sepharose slurry and incubated 12 hr at 4°C with rocking. The slurry was washed five times with 1× PBS and eluted with 15% methyl  $\alpha$ -D-mannopyranoside in 1× PBS. Starting material, the first wash, and the eluate were analyzed by SDS/PAGE (lanes 1–3, respectively). (C) Quick-freeze deep-etch electron microscopic analysis of purified type 1 tip fibrillae (24) from the first chromatographic peak (Upper) (see A, lane 2, for protein composition) and structures composed of the FimG–FimH complex (Lower) (see A, lane 3). ( $\times 390,000$ .)

FimF and FimC had an identical fibrilla-like architecture (Fig. 1C Upper).

**Composite Architecture of Pili.** The identification of a putative structural component responsible for mediating mannose-sensitive hemagglutination by type 1 pili was reminiscent of the recently described P pilus tip fibrilla. The P pilus of pyelonephritic *E. coli* was the first pilus structure shown to bear a composite architecture (25). These structures (Fig. 2A) are composed of a flexible, open helical fiber linked end-to-end to the major pilus rod. The tip fibrilla of the P pilus is composed mostly of repeating monomers of PapE (25), with three additional minor components: PapF, PapK, and PapG. PapF and PapK have critical functions in biogenesis as adapter proteins that join the adhesin to the fibrilla and the fibrilla to the rod, respectively (26). PapG is the P pilus adhesin molecule, which is located at the distal end of the fibrilla (25).

High-resolution quick-freeze deep-etch electron microscopy of purified type 1 pili from clinical isolates of *E. coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, as well as recombinant strains of *E. coli*, revealed fibrillar tip structures joined end-to-end to the type 1 pilus rod (Fig. 2 B–G) which were identical in length (16 nm), width (3 nm), and overall morphology to the affinity-purified structures from ORN103/pHA9. We refer to the type 1 mannose-binding adhesive determinant as the type 1 tip fibrilla, suggesting a function analogous to that of the P pilus tip fibrilla. The type 1 tip fibrilla is a stubby (16 nm) structure in comparison to the P tip fibrilla (40–60 nm) (Fig. 2, compare A and B). The structure of the type 1 fibrilla is consistent with the lack of a subunit, such as PapE, that is capable of self-polymerization into a fiber (25). Interestingly, the P pilus-associated periplasmic chaperone, PapD (21), which shares 50% amino acid homology with FimC, is able to bind and assemble type 1

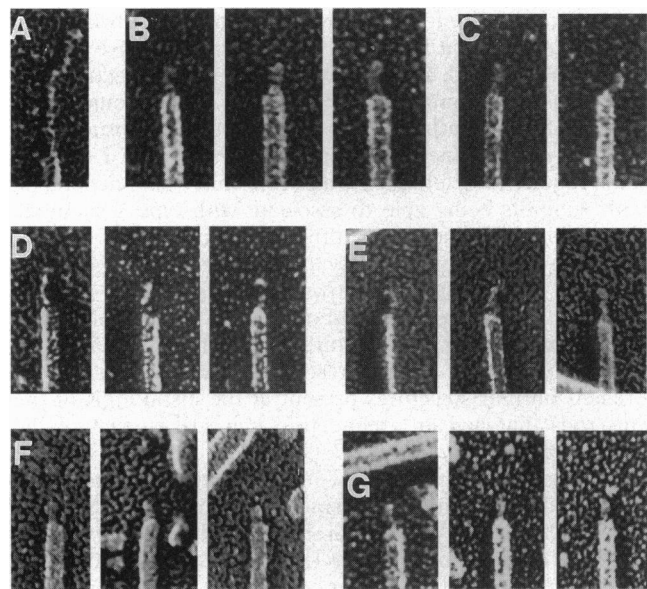


FIG. 2. High-resolution quick-freeze deep-etch electron microscopic analysis of P and type 1 fibrillar structures (24). Each box contains a single tip fibrilla joined to a pilus rod; these examples are representative of a population of pili in several random fields. (A) Purified P pili demonstrating the P pilus fibrillar structure, reported previously by Kuehn *et al.* (25). (B and C) Type 1 pili purified from recombinant strains of *E. coli*: ORN103/pJP5/pJP4 (*fimC*) (B) and ORN103/pJP5/pLS101 (*papD*) (C). Plasmid pJP5 (17) contains the type 1 operon bearing a *Xho* 1 linker in the *fimC* locus that can be complemented in trans by either *fimC* or *papD*, on plasmids pJP4 and pLS101, respectively (17). (D–F) Type 1 pili were also examined from: *Citrobacter freundii* clinical isolate (3) (D), *Klebsiella pneumoniae* clinical isolate (3) (E), *Enterobacter cloacae* clinical isolate (3) (F), and *E. coli* clinical isolate J96 (18) (G). ( $\times 240,000$ .)

subunits into composite type 1 fibers that are indistinguishable from those assembled by the FimC chaperone (Fig. 2C). The ability of two different chaperones to assemble FimA, FimF, FimG, and FimH into identical composite structures argues for a common structure/function relationship between the chaperones and the proteins with which the chaperones interact.

**The Major Component of the Fibrilla Is FimH.** We investigated the effect of a deletion of the *fimH* gene on the structure (length and overall morphology) of the tip fibrilla. Type 1 pili were purified from ORN103/pUT2002 (23). The *fimH* mutation has no effect on the expression or morphology of type 1 pili, although the pili fail to direct mannose-sensitive hemagglutination (23). High-resolution electron microscopy revealed that the tip fibrillae produced by ORN103/pUT2002 are reproducibly shorter (3 nm) than the tip fibrillae produced by ORN103/pSH2 (16 nm) (Fig. 3 Left and Center). Complementation of the *fimH* deletion by pHJ20, which contains the *fimH* gene under control of the *tac* promoter restores wild-type mannose-sensitive hemagglutination (data not shown) and results in fibrillae having a wild-type length and morphology (Fig. 3 Right).

**Tip Fibrilla Biogenesis.** We used the purified tip complexes and purified FimC in two assays to investigate the hypothesis that surfaces on subunits recognized by the chaperone are no longer available after subunit–subunit association—i.e., the uncapped surface may facilitate subunit–subunit interactions. The FimC chaperone, purified by our standard protocol, binds to a peptide corresponding to the carboxyl terminus of FimH in an *in vitro* chaperone binding assay (M. J. Kuehn and S.J.H., unpublished data). The ability of pure FimC, present in 100-fold excess, to bind to the purified FimG–FimH complex was investigated. FimC was incubated with the FimG–FimH complex (25°C, 30 min) before applying it to a slurry of mannose-Sepharose beads. Unbound protein was washed off the beads and the bound material was eluted with methyl  $\alpha$ -D-mannopyranoside. FimC was not eluted with the FimG–FimH complex, but instead was present in the unbound fraction, arguing that FimC did not bind to the FimG–FimH complex. Similarly, when FimC was applied to a mannose-Sepharose slurry that already contained bound FimG–FimH complex, it was found in the unbound fraction and was not coeluted with the FimG–FimH complex (data not shown).

In another assay we investigated the ability of FimC to bind to FimF, FimG, and FimH before and after their dissociation from the tip macromolecular complex (Fig. 4B). Subunit–subunit associations are thought to be relatively stable in SDS at 25°C but are dissociated at 95°C (27). FimCFGH mannose-purified tip complexes were mixed with SDS loading buffer and incubated at either 25°C (Fig. 4B, lanes 1 and 3) or 95°C

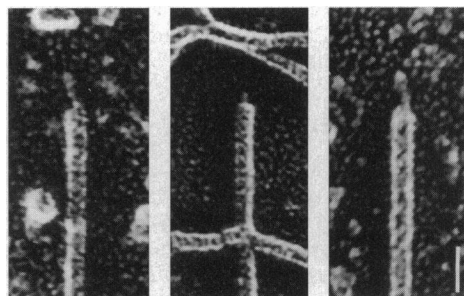


FIG. 3. The bulk of the tip fibrilla is composed of FimH as determined by quick-freeze deep-etch electron microscopy (24). (Left) Wild-type type 1 pili purified from ORN103/pSH2 possessed tip fibrillae that had an average length of 16 nm ( $n = 22$ ). (Center) Type 1 pili missing FimH purified from ORN103/pUT2002 ( $\Delta$ *fimH*) were dramatically shorter; the average length was 3 nm ( $n = 25$ ). (Right) Complementation of the  $\Delta$ *fimH* mutation with *fimH* in trans from ORN103/pUT2002/pHJ20 (*fimH*) restored the average lengths of the tip fibrillae to 16 nm ( $n = 10$ ). ( $\times 300,000$ ; bar = 20 nm.)

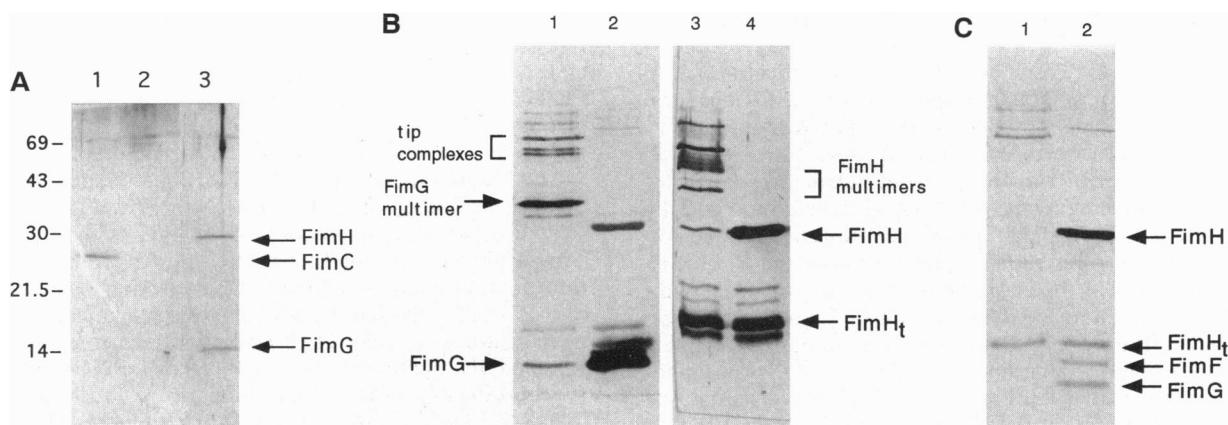


FIG. 4. FimC binds dissociated subunits but not subunits associated in the tip fibrilla in *in vitro* chaperone binding assays. (A) Coomassie blue-stained SDS/polyacrylamide gel of three mannose-Sepharose chromatographic fractions of purified FimG-FimH complex that was pre-incubated with a 100-fold excess of purified FimC (10  $\mu$ g). Lane 1, material that failed to bind mannose-Sepharose slurry; lane 2, slurry containing bound proteins was washed five times, and the final wash was loaded; lane 3, bound proteins eluted with methyl  $\alpha$ -D-mannopyranoside. (B) Western blot of mannose-Sepharose-purified FimCFGH tip complexes after fractionation by SDS/PAGE. Samples were incubated at 25°C (lanes 1 and 3) or 95°C (lanes 2 and 4) for 5 min in SDS loading buffer prior to loading on the gel. After electrophoresis and transfer to poly(vinylidene difluoride) filters, the filters were probed with anti-FimG antiserum (lanes 1 and 2) or anti-FimH antiserum (lanes 3 and 4). We have noted crossreactivity of the FimG antiserum with FimH. (C) Western blot of FimCFGH tips that were treated as in B, probed with biotinylated FimC (28), and developed with avidin-alkaline phosphatase conjugate. The tips were incubated at 25°C and 95°C in SDS sample buffer (lanes 1 and 2, respectively) prior to loading. The gel in C was run slightly further than the gels in B; note relative positions of FimH and FimH<sub>t</sub>.

(lanes 2 and 4) for 5 min prior to separation by SDS/PAGE and transfer to poly(vinylidene difluoride) paper in two identical blots for Western blot analysis. FimG and FimH were present in the high molecular weight complexes at 25°C (lanes 1 and 3) but were present as distinct free proteins at 95°C (lanes 2 and 4). Small amounts of monomeric FimG and FimH were also present at 25°C. Anti-FimG antibodies (lanes 1 and 2) identified a different banding pattern than anti-FimH antibodies (lanes 3 and 4) in the 25°C samples. This may have been due to partial dissociation of the tip fibrillae into different complexes at 25°C, including FimH oligomers, FimG oligomers, and FimG-FimH oligomers. FimC was dissociated from all complexes at both temperatures (data not shown). Biotinylated FimC (28) was used to probe a blot similar to that shown in Fig. 4B and the binding reaction was developed with avidin-alkaline phosphatase conjugate (Fig. 4C). Biotinylated FimC bound to free FimH in the 95°C sample (Fig. 4C, lane 2) but bound weakly or not at all to FimH in the high molecular weight complexes in the 25°C samples (lane 1). Similarly, FimC bound to FimF and FimG only after the proteins were dissociated from their respective complexes at 95°C (lane 2). The FimH<sub>t</sub> molecule was not a part of any high molecular weight complex (Fig. 4B, lanes 3 and 4; Fig. 4C). These results argue that the sites for FimC are no longer available in the FimFGH complex, in agreement with the finding in the experiment shown in Fig. 4A.

## DISCUSSION

All *Enterobacteriaceae* are capable of expressing type 1 pili. The identification of a type 1 tip fibrilla, taken together with an earlier report on the P pilus fibrilla (25), suggests that tip fibrillae are a feature common to most pilus structures. Type 1 tip fibrillae are composed minimally of FimG and the type 1 mannose-binding adhesin molecule, FimH. A FimF component is probably part of a mature fibrilla as well. Previous genetic studies have indicated a role for FimF and FimG in biogenesis of the type 1 rod (12, 13). By analogy to the studies on P pilus biogenesis (25, 26), in which initiator/adaptor proteins were found to be components of the P fibrilla, localization of FimG and possibly FimF to the tip is consistent with a role for these molecules in pilus biogenesis. However, our findings do not rule out additional locations of the minor

subunits or the FimH adhesin in the pilus structure. For example, a previous study localized the FimH adhesin in lateral sites along the pilus shaft (29).

The proposed role of the P pilus tip fibrilla is to localize the PapG adhesin in a flexible location in order to enhance the interaction of the adhesin with host receptors (25, 26). The major component of the P fibrilla is PapE, which is able to self-associate into an open helical fiber (25). The stubbiness and apparent lack of flexibility of the type 1 tip fibrilla (Fig. 1 B-G) suggests that the type 1 fibrilla does not have a PapE homologue. Instead, type 1 fibrillae have an architecture that resembles *papE* mutants of P pili (25). We attempted to incorporate PapE into type 1 tip fibrillae but were unsuccessful (data not shown). Incorporation of PapE into type 1 fibrillae would require an interaction with FimC and would depend on PapE subunits being able to associate with type 1 subunits. Presumably, PapE does not fulfill these criteria.

Mannose-Sepharose affinity chromatography of extracts from *fimA*<sup>-</sup>, mannose-sensitive hemagglutination-positive cells resulted in the purification of a FimG-FimH moiety that had a thin fibrillar architecture (Fig. 1). Examination of purified type 1 pili by high-resolution electron microscopy revealed fibrillar structures present at the distal ends of the pilus rods that had an architecture identical to that of the FimG-FimH moiety (Fig. 2 B-G). In the absence of FimH there was a significant decrease in the length (16 nm to 3 nm) of the fibrillae present at the distal ends of the type 1 pilus rods (Fig. 3). The most likely interpretation of these results is that FimH is a component of the tip fibrilla and that it is joined to FimG. Although we have been unable to show directly that FimH forms a multimeric structure, it appears that FimH is present in the tip in more than one copy. Alternatively, FimH and FimG may interact to form a short heteropolymer.

The assembly of pili requires the conversion of chaperone-subunit complexes into pili across the outer membrane (20). Dissociation of the chaperone from the subunit probably exposes interactive surfaces that drive high-affinity subunit-subunit interactions and the assembly of pili. It is interesting, considering our model of pilus biogenesis (20), that we purified a chaperone-subunit complex loosely associated with the FimG-FimH complex (Fig. 1). It seems likely that this macromolecular complex represents an assembly intermediate that occurs prior to the putative incorporation of FimF into the tip

fibrilla; however, we have been unable to detect a FimFGH complex in our assays. HPLC ion-exchange chromatography dissociated the FimC–FimF complex from the FimG–FimH tip fibrilla. Similarly, the FimG–FimH complex was stable in SDS at 25°C but the FimC–FimF complex was dissociated (data not shown). At 95°C in SDS both the subunit–subunit and chaperone–subunit complexes were dissociated (Fig. 4B). Although the FimG and FimH antisera did not react in all cases with the same higher molecular weight species in Fig. 4B, it is clear that FimG and FimH are associated in complexes that are to some degree SDS-stable and these complexes are weakly or not at all recognized by FimC. The SDS treatment at 25°C may perturb the FimG–FimH complex to some degree, resulting in complexes containing only FimG or FimH. In another pilus system, the P pilus, PapA (the major subunit) forms SDS-stable complexes that are multimers of PapA and the formation of the complexes is dependent on the PapD chaperone (27). Therefore, there is some precedent for the SDS-stable complexes we have identified.

The inability of purified FimC to bind the FimG–FimH complex (Fig. 4) suggests that once subunits are polymerized into the fibrilla complex, the chaperone binding surfaces are lost. An essential step in pilus biogenesis is removal of the chaperone, which presumably results in the uncapping of the interactive surfaces that drive subunit–subunit interactions. A conformational change in the subunit upon polymerization of the subunit into the pilus might mask or alter the surface recognized by the chaperone, resulting in chaperone dissociation and a possible mechanism to prevent rebinding of the chaperone to subunits at the base of the fiber. This would put in place a mechanism to ensure that a subunit surface is available for an incoming complex and not blocked by the chaperone, which would inhibit pilus assembly. This may explain why overexpression of a chaperone does not inhibit pilus assembly (21).

The identification of the discrete tip fibrilla structure provides another target for vaccine development, as early attempts to use whole pili were not successful (30). The fibrilla is conserved throughout the *Enterobacteriaceae*, a bacterial family that causes significant pathology in the human host. The FimH molecules from *Klebsiella pneumoniae* and *E. coli* are 89% similar and 83% identical in amino acid sequence (31). Furthermore, the FimH molecule is serologically conserved throughout the *Enterobacteriaceae* (3, 14); in contrast, the FimA component varies significantly in sequence and antigenicity (14). Purification of the FimH lectin in the fibrilla, separate from the FimA rod, will allow testing of this bacterial adhesin as a vaccine candidate.

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