Interaction with Type IV Pili Induces Structural Changes in the Bacterial Outer Membrane Secretin PilQ*

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Type IV pili are cell surface organelles found on many Gram-negative bacteria. They mediate a variety of functions, including adhesion, twitching motility, and competence for DNA uptake. The type IV pilus is a helical polymer of pilin protein subunits and is capable of rapid polymerization or depolymerization, generating large motor forces in the process. Here we show that a specific interaction between the outer membrane secretin PilQ and the type IV pilus fiber can be detected by far-Western analysis and sucrose density gradient centrifugation. Transmission electron microscopy of preparations of purified pili, to which the purified PilQ oligomer had been added, showed that PilQ was uniquely located at one end of the pilus fiber, effectively forming a “mallet-type” structure. Determination of the three-dimensional structure of the PilQ-type IV pilus complex at 26-Å resolution showed that the cavity within the protein complex was filled. Comparison with a previously determined structure of PilQ at 12-Å resolution indicated that binding of the pilus fiber induced a dissociation of the “cap” feature and lateral movement of the “arms” of the PilQ oligomer. The results demonstrate that the PilQ structure exhibits a dynamic response to the binding of its transported substrate and suggest that the secretin could play an active role in type IV pili assembly as well as secretion.

To establish either a commensal or pathogenic relationship with its human host, Neisseria meningitidis develops specific cell-cell contacts and, to this end, exploits a diverse arsenal of adhesive proteins located on the cell surface (1, 2). During tissue colonization many pathogenic Gram-negative bacteria display long adhesive fibers, termed type IV pili, from the cell surface that mediate cellular attachment to epithelial tissue receptors (3–5). Pili are also involved in several other bacterial processes, including bacterial auto-agglutination (6), variation of target tissue specificity (7), and natural competence for DNA uptake (8, 9). Recent data have demonstrated that type IV pili are retracted and that this retraction process is responsible for twitching motility of neisserial cells on solid and mucosal surfaces (10, 11). Type IV pili are long (>1–5 μm), thin (60–70 Å), mechanically strong polymeric fibers containing 500–2000 subunits of the major pilin protein, PilE (12). The assembly of pilin into pili, as well as pilus disassembly, is controlled by a complex interacting apparatus of up to 30 proteins (13–15), which is similar to the apparatus for secretion of proteins of the general (type II) secretory pathway (16, 17).

Prior to pilus assembly PilE is processed through the cytoplasmic membrane by the pre-pilin peptidase PilD, removing a hydrophobic leader peptide and methylating the N-terminal amino acid en route (13). At this stage PilE is located in the periplasm but is thought to remain tethered to the inner membrane by a single transmembrane a-helix located at its N terminus (18). It is hypothesized that this phenomenon creates a “pool” of accessible pilin subunits associated with the inner membrane. Several other neisserial proteins, termed pseudopilins or minor pilins, share homology within their N-terminal regions with the PilE subunit and are also required for correct pilus expression, although they are expressed at low levels compared with PilE (19). A number of these minor pilins are thought to be integrated into the growing pilus (20). An adhesin protein (PilC) has been shown to bind to the host epithelial receptors CD46 (21) and C4B (22). PilC also plays a role in the assembly process and a minor pilin, PilV, has been implicated in its functional presentation (20). Furthermore, type IV pilus retraction in Neisseria appears to be regulated by PilC (23). Other essential components for the biosynthesis and expression of type IV pili include a cytoplasmic protein, PilF, and a polytopic inner membrane protein, PilG, of unknown functions (24, 25). PilF, PilT, and PilU contain a consensus ATP binding motif, are homologues of GspE (a member of the AAA chaperone/mechanico-enzyme family) and presumably drive pilus assembly/disassembly by ATP hydrolysis (26, 27). The cytoplasmic protein PilT is dispensable for pilus assembly but essential for retraction (28). Despite individual characterization of some of these components, their function and coordinate regulation in pilus assembly, extrusion, and retraction are poorly understood.

The branch point between the assembly and retraction of pili through the outer membrane occurs at the PilQ oligomer. PilQ is an antigenically conserved, abundant outer membrane protein that is essential for meningococcal pilus expression (29). It is a member of the general secretory pathway secretin superfamily, members of which translocate a variety of macromolecules across the outer membrane (16, 29, 30). Double knock-out mutants of pilT and pilQ form long, membrane-covered pilus-like structures that fill the periplasm (31). PilQ subunits form a heat- and SDS-stable complex, which requires the PilP lipoprotein for functional oligomer assembly (32). The N-terminal part of neisserial PilQ contains two to seven copies of unique octapeptides, termed small basic repeats (29). The outer membrane protein Omp85 has also been shown to affect the
assembly of the PilQ oligomer into the outer membrane (33). Images of isolated bacterial secretions have been recorded by transmission electron microscopy, and “donut-like” ring projections surrounding a central cavity have been observed (34, 35), with dimensions that vary considerably among secretions and appear to be related to the specific translocated substrate. The three-dimensional (3-D)1 structure of the PilQ oligomer from N. meningitidis has been determined using single particle averaging, initially on samples prepared in conventional negative stain (36) and, more recently, at the higher resolution of 12 Å using cryo-negative stain (37). The structure is dominated by a large central cavity, which is closed at both ends by “plug” and “cap” features, and four “arm-like” features, which form the sides. The PilQ oligomer exhibits 4-fold rotational symmetry with 12-fold quasi-symmetry (37). It is hard to rationalize this unusual structure as a passive pore within the outer membrane, acting as a conduit for a pilus fiber already assembled in the periplasm. We demonstrate here that type IV pili, purified from meningococci, spontaneously interact with the meningococcal PilQ oligomer in a highly specific fashion. Substantial conformational changes occur in the cap and arm portions of the PilQ oligomer upon association with type IV pili. These findings suggest that PilQ does not function solely as a passive pore for passage of pili through the outer membrane but is capable of a dynamic response upon interaction with the secreted substrate.

MATERIALS AND METHODS

**Bacterial Strains and Constructs**—Meningococcal strain M1080 was grown overnight on 5% blood agar in an atmosphere containing 5% CO₂ before harvesting. The fragments encoding the full-length, N-terminal and central portions of the PilQ gene were cloned into the vector pQE-30 (Qiagen, Germany), and the C-terminal portion of the pilQ gene was cloned into vector pET28b (+) (Novagen). Both vectors encode a polyhistidine tag. All proteins were overexpressed in Escherichia coli ER2566 (New England Biolabs) (see Table I). The recombinant PilQ proteins were expressed in E. coli as inclusion bodies and were subsequently solubilized using 8 M urea in phosphate-buffered saline. The recombinant proteins were affinity-purified using Ni-NTA-agarose (Qiagen, Germany) under denaturing conditions in 8 M urea. After immediate buffer exchange on a PD10 column (Amersham Biosciences) into 50 mM NaH₂PO₄ (pH 7.5), they were subsequently exchanged into exchange chromatography using a Resource Q column (Amersham Biosciences) before dialysis against 50 mM NaH₂PO₄ (pH 7.5). The recombinant OpaD and HmbR proteins from N. meningitidis strain MC58 were expressed in E. coli as inclusion bodies, solubilized with 6 M guanidine HCl, refolded by rapid dialysis, and purified by ion exchange and size exclusion chromatography. Recombinant OpaC was obtained as described previously (38). The plasmid pMP121 was generously provided by Matthias Froesch (39) and used to generate a negative mutant of N. meningitidis strain M1080 expressing truncated lipooligosaccharide. The pilE-negative Neisseria gonorrhoeae N400 was generously provided by Mike Koomey (31). Genomic DNA from this strain was used to generate a pilE-negative N. meningitidis M1080 strain.

**Purification of Native and Histidine Tag PilQ Oligomer**—The native PilQ oligomer was isolated from meningococci using a purification procedure described previously, employing the detergent Zwittergent 3–10 (36). For the generation of PilQ histidine-tagged in the N-terminal small basic repeat region, splicing by overlapping extension PCR and non-selective transformation of the PCR product into N. meningitidis strain M1080 were employed. The native histidine tag PilQ oligomer was isolated from meningococcal outer membranes as previously described (36).

**Purification of Meningococcal Type IV Pili Fibers**—Type IV pili were purified from the meningococcal cell surface using ammonium sulfate precipitation of a shearing fraction (40). Meningococcal cells derived from four heavily streaked Petri dishes were vortexed for 1 min in 10 ml of 0.15 M ethanolamine buffer (pH 10.5), and cellular debris was removed by centrifugation at 14,000 × g for 15 min. Pilus fibers were precipitated at room temperature for 30 min by addition of one-tenth volume of ammonium sulfate-saturated (0.15 M) ethanolamine buffer and collected by centrifugation at 14,000 × g for 15 min. Pili were subsequently washed twice with 50 mM Tris-buffered saline.

**SDS-PAGE and Far-Western Assay**—1–2 μg of the purified recombinant PilQ proteins, purified native type IV pili fibers, neisserial outer membrane proteins (OpaD, OpaC, and HmbR), and BSA were separated by SDS-PAGE, and the proteins were transferred onto a Hybond-C extra nitrocellulose blotting membrane (Amersham Biosciences) in blotting buffer (25 mM Tris-HCl, 190 mM glycine, 20% methanol, pH 8.9). For the far-Western analysis, the membrane was briefly washed twice with cold renaturing buffer (10 mM Tris-HCl, 100 mM NaCl, 0.5% BSA, 0.25% gelatin, 0.2% Triton X-100, 5 mM 2-mercaptoethanol, pH 7.5), and the proteins were renatured by overnight incubation at 4 °C in the same buffer. For the detection of type IV pili fiber binding to PilQ fragments, the membrane was incubated for 3 h with purified PilQ fragments and exposed to anti-PilQ polyclonal rabbit antiserum at a dilution of 1:1000. For immunoblotting of purified protein, anti-PilQ and type IV pili-specific rabbit polyclonal antiserum was used at dilutions of 1:2000 and 1:1000, respectively.

**Formation of PilQ-Type IV Pilus Fiber Complex and Sample Preparation for TEM**—Type IV pili were selected into 200-Å2 boxes (43), and the resulting single particle averages were subjected to 2-D crystallographic package CRISP (45). The resulting fast Fourier transforms were noisy because of the inherent low contrast of pilus fibers, but a characteristic helical cross-diffraction pattern and first order ladder lines were indexed (46) allowing the range of helical pitch of the pili to be determined.

**Negative Staining**—Samples were adsorbed to freshly glow-discharged carbon-coated copper grids (No. 400) prior to negative staining.

1 The abbreviations used are: 3-D, three-dimensional; 2-D, two-dimensional; TEM, transmission electron microscopy; BSA, bovine serum albumin; CTF, contrast transfer function; Ni-NTA, nickel-nitrilotriacetate acid.
Ni-NTA-Gold Labeling—PilQ-type IV pilus complexes were prepared as described above. 20 μl of the PilQ-type IV pilus complex was incubated with 3.3 μl (1nM) of Ni-NTA-nanogold (Nanoprobes) for 18 h at room temperature. Samples were then negatively stained with 6% (w/v) ammonium molybdate (pH 6.8)/1% (w/v) trehalose.

**Image Analysis and Volume Calculations of the PilQ-Type IV Pilus Complex Using Negative Stain and the Random Conical Tilt Method**—Table II summarizes all additional information pertinent to TEM low dose data collection, micrograph scanning, and volume back projection used in this study. The PilQ oligomer was found to interact with pili of different fiber lengths, and, as with free pili, we observed that there was an intrinsic tendency for fibers to bend along their length. To calculate an accurate 3-D structure of the PilQ-type IV pilus complex, allowance was made for the bending observed along the pilus fiber length by treating the complex as a single particle, selected into a box 342 × 342 Å, with a determined center at the PilQ-pilus interface. The CTF of each micrograph was examined, and appropriate CTF phase corrections were applied to the entire particle data-set. Aligned particles were grouped using correspondence analysis and hierarchical ascendant classification, and a 2-D projection map was generated from the major class average. 3-D volumes of interacting PilQ-pilus complexes were subsequently calculated using the random conical reconstruction method (47–50) executed using the software packages SPIDER and WEB. The same procedures have been described previously in detail for the 3-D structure calculation of the PilQ oligomer (36).

**Symmetry Analysis**—Density map correlation coefficients were calculated by rotation of the unsymmetrized 3-D map about the long axis of the pilus fiber using MAPROT (51), and the density map correlation coefficient was calculated using OVERLAPMAP (52), both from the CCP4 suite (53).

**RESULTS**

**The Meningococcal PilQ Monomer Interacts Directly and Specifically with Pili**—PilQ from *N. meningitidis* is required for pilus biogenesis (29) but has not been shown to form a direct interaction with its secreted substrate. We employed far-Western analysis to assess the association between purified type IV pili and recombinant fragments of PilQ, which covered the N-terminal, central, and C-terminal portions of the protein (Fig. 1A and Table I). The binding of three other neisserial outer membrane proteins to type IV pili were also examined as controls, specifically the adhesins OpcA and OpaD, and the heme receptor HmbR. For the far-Western analysis, denatured PilQ fragments in the SDS-PAGE gel were blotted onto the protein-containing membrane overlaid with purified type IV pilus fiber preparation and subsequently detected with anti-type IV pilus antibody; b, Coomassie Blue-stained gel; c, immunoblot detected using polyclonal antibody against purified type IV pilus; d, immunoblot detected using polyclonal antibody against PilQ. Lane 1, purified type IV pilus fibers from *N. meningitidis* strain M1080; lane 2, recombinant PilQ, full-length protein; lane 3, recombinant N-terminal PilQ protein; lane 4, recombinant central region of the PilQ protein; lane 5, recombinant C-terminal PilQ protein; lane 6, neisserial outer membrane protein OpcA; lane 7, neisserial outer membrane protein OpaD; lane 8, the hemoglobin receptor HmbR; and lane 9, BSA.

**Fig. 1. Interaction of recombinant meningococcal PilQ with type IV pili.**

A, schematic diagram to indicate the regions of the pilQ gene covered by the four recombinant proteins used in this study. The five small arrows within the N-terminal region of PilQ indicate the location of the small basic repeats described previously (29), and H represents the position of the His6 tag. B, the interaction between recombinant PilQ and natively purified pili studied by far-Western analysis. The four panels a–d contain gels of the same samples in each lane, but detected with different reagents. a, far-Western blot (protein-containing membrane overlaid with purified type IV pilus fiber preparation and subsequently detected with anti-type IV pilus antibody); b, Coomassie Blue-stained gel; c, immunoblot detected using polyclonal antibody against purified type IV pilus; d, immunoblot detected using polyclonal antibody against PilQ. Lane 1, purified type IV pilus fibers from *N. meningitidis* strain M1080; lane 2, recombinant PilQ, full-length protein; lane 3, recombinant N-terminal PilQ protein; lane 4, recombinant central region of the PilQ protein; lane 5, recombinant C-terminal PilQ protein; lane 6, neisserial outer membrane protein OpcA; lane 7, neisserial outer membrane protein OpaD; lane 8, the hemoglobin receptor HmbR; and lane 9, BSA.
supporting membrane and a “renaturation” step was introduced, which involved incubating the membrane overnight. When the experiment was repeated without this renaturation step the PilQ-pilus interaction was much weaker, suggesting that the conformation adopted by the PilQ proteins was less structured under these conditions. The far-Western analysis was repeated several times, and the pilus interactions with the C- and N-terminal fragments of the monomer PilQ were detected each time. In the converse experiment, when the membrane from a PilE gel sample was overlaid with PilQ and anti-PilQ antibodies, a reciprocal interaction of the N- and C-terminal PilQ fragments with PilE was detected (data not shown). Although the PilQ and pilus proteins are predicted to have different charges (Table I), this observation provides evidence that the interaction detected here is dependent on the formation of conformational epitopes and excludes a non-specific attraction that is purely electrostatic in origin. The sample of pilus protein showed several bands (lane 1), a feature that indicates the tendency of pilin subunits for self-association, even in the presence of SDS. In addition to the major product at 80 kDa, the full-length PilQ preparation also contained a 33-kDa fragment, presumably generated by limited proteolysis during isolation (lane 2). This pattern of proteolytic degradation is similar to that seen previously for meningococcal PilQ (29) and secretins from other organisms (54, 55). Treatment of identical blots with anti-PilQ and anti-PilE polyclonal antibodies showed that the PilQ and pilus preparations were free of cross-contamination (Fig. 1B, gels c and d).

The interaction between the PilQ oligomer and pili was examined in more detail using sucrose density gradient centrifugation. Native PilQ oligomer, purified from a pilE-negative meningococcal strain, was mixed with pili, applied to a sucrose density gradient and centrifuged for 18 h at 245,000 × g in an SW-41 rotor. After centrifugation, the gradients were fractionated from the bottom. Fractions were assayed for PilQ and pilus content by enzyme-linked immunosorbent assay using polyclonal antibodies specific for PilQ (●) and PilE (○). A, purified PilQ oligomer and type IV pili were pre-mixed. B, purified PilQ oligomer and type IV pili assayed separately.

**TABLE I** Properties of recombinant PilQ and native PilE proteins used in this study.

| Gene product | Amino acid position in M1080 PilQ | Length (residues) | Mass (kDa) | pI |
|--------------|----------------------------------|------------------|------------|---|
| Full-lengtha | 25–761                           | 749              | 80.6       | 9.34 |
| N-terminal   | 25–354                           | 363              | 38.7       | 9.77 |
| C-terminal   | 352–761                          | 420              | 45.5       | 8.32 |
| Central region | 218–478                         | 276              | 30.6       | 8.98 |
| PilE         | NAb                             | 162              | 17.2       | 5.66 |

a Recombinant PilQ constructs were prepared using the pilQ gene from *N. meningitidis* strain M1080 (accession number AJ564200).

b NA, not applicable.

Sucrose density gradient analysis of the interaction between the PilQ oligomer and type IV pili. The sedimentation of the PilQ oligomer and purified type IV pilus fibers were analyzed by centrifugation through a sucrose gradient. Samples were loaded on top of 12-mL sucrose gradients and centrifuged for 18 h at 245,000 × g in an SW-41 rotor. After centrifugation, the gradients were fractionated from the bottom. Fractions were assayed for PilQ and pilus content by enzyme-linked immunosorbent assay using polyclonal antibodies specific for PilQ (●) and PilE (○). A, purified PilQ oligomer and type IV pili were pre-mixed. B, purified PilQ oligomer and type IV pili assayed separately.

**Fig. 2.** Sucrose density gradient analysis of the interaction between the PilQ oligomer and type IV pili. The sedimentation of the PilQ oligomer and purified type IV pilus fibers were analyzed by centrifugation through a sucrose gradient. Samples were loaded on top of 12-mL sucrose gradients and centrifuged for 18 h at 245,000 × g in an SW-41 rotor. After centrifugation, the gradients were fractionated from the bottom. Fractions were assayed for PilQ and pilus content by enzyme-linked immunosorbent assay using polyclonal antibodies specific for PilQ (●) and PilE (○). A, purified PilQ oligomer and type IV pili were pre-mixed. B, purified PilQ oligomer and type IV pili assayed separately.
out of over 54 separate micrographs recorded and ~1000 such complexes observed, the pilus fibers were only ever found to be terminated by the protein complex at one end. Structures with a “dumbbell-like” appearance, corresponding to the protein mass located at both ends of a pilus, were never observed. Similarly, the protein mass was never observed along the length of a pilus fiber. The dimensions of the object, and its complete absence from preparations of pili without the PilQ oligomer, suggested that these mallet-like complexes represented the PilQ oligomer liganded with a pilus. These projections would therefore correspond to a side-on view of the PilQ oligomer bound to a nascent pilus fiber. It should also be noted that many of the pili observed following incubation with the PilQ oligomer were reduced in length compared with the control sample. We attribute this observation to the inclusion of 0.1% (w/v) Zwittergent 3-10 in the PilQ sample buffer. Treatment with the mild detergent 8-Octylglucoside and variation in the buffer pH have been shown previously to dissociate intact pili and solubilize pilin subunits (56). For clarity, we will refer to the structure of a PilQ oligomer bound to the end of a type IV pilus fiber as a “PilQ-type IV pilus complex.” To examine the structure of the PilQ-type IV pilus complex in more detail, a projection map was obtained by averaging several hundred particles. The projection structure of the major class average confirmed that the PilQ oligomer adopted a defined, homogeneous, structure at the end of the pilus (Fig. 4), with a width of 140 Å and a height of 150 Å. Density corresponding to the pilus can be seen emerging from the top of the central mass of the particle, but gradually becomes less distinct further away because the flexibility of the pilus fiber increasingly blurs the projection. The identity of the mass at the end of the pilus fiber was confirmed as being PilQ by the incorporation of a polyhistidine sequence tag into PilQ within the polymorphic small basic repeat region (29). The PilQ oligomer, with the polyhistidine sequence tag incorporated, was purified and examined by TEM in the presence of Ni-NTA-gold particles, which bind selectively to the polyhistidine tag (Fig. 5). The TEM data were recorded using ammonium molybdate/trehalose, rather than uranyl acetate, as a negative stain, because the relatively large stain granules of uranyl acetate can obscure visualization of the 18-Å gold particles. The PilQ mass is selectively labeled with gold particles, which were generally located in a central location within the PilQ mass, or toward the distal end of the complex. This result was confirmed by use of an antibody raised against the N-terminal domain of PilQ, which was used to immunogold label the PilQ-type IV pilus complexes and produced similar results (data not shown).
3-D Structural Analysis of the PilQ-Type IV Pilus Complex—A 3-D volume of the PilQ-type IV pilus complex was determined to 26-Å resolution, by collecting data from 607 pairs of tilted and untitled particles (Table II). A section of the top of the unsymmetrized 3-D volume, orientated to correspond to a view down the long axis of the emerging pilus fiber, is shown in Fig. 6A, using sky blue and red netting to delineate stain-excluding protein density. A distinct feature of the PilQ-type IV pilus complex was a 4-fold rotational symmetry, readily apparent when viewed from this orientation. This observation was confirmed by examining the rotational symmetry in the calculated 3-D structure (Fig. 6B). Peak values of the map correlation coefficient were observed at 90° intervals, consistent with the complex having 4-fold rotational symmetry about the axis of the pilus fiber. The structure of the PilQ oligomer alone also shows a pronounced 4-fold symmetry (37). This observation therefore provides further independent evidence that the mass at the end of the type IV pilus is indeed the PilQ oligomer and the recognition of the end of the pilus fiber is specific.

Interpretation of the Different Conformational States of PilQ—Fig. 7 compares different views of the PilQ-type IV pilus complex with the structure of the PilQ complex determined previously from cryo-negative stain (37). Both structures have been aligned on the basis of structural similarity. Symmetry averaging can increase the signal-to-noise ratio of a density map (47), and so both structures are shown with 4-fold rotational symmetry applied. It should be appreciated that application of rotational symmetry averaging to the PilQ-type IV pilus complex only improves the data in the regions of the volume corresponding to the PilQ oligomer, because there is an inherent symmetry mismatch between it and the helical symmetry of the pilus fiber. To allow a comparison between the structures, they were filtered to the lowest resolution obtained (26 Å). When viewed from the base of the complex, as though the pilus were extending away from the viewer, both structures have similar square dimensions and appearance. The principal difference discernable in this view is the four “blade-like” structures, which protrude from the main bulk of the PilQ-type IV pilus complex (Fig. 7, far left). These blade structures are clearly visible from the side views and extend over the vertical height of the complex covered by the arms and cap regions of the unliganded PilQ oligomer.

A view of a section through the center reveals that, in contrast to the structure of the PilQ oligomer alone, the interior of the PilQ-type IV pilus complex is inaccessible to stain and therefore apparently filled. The 3-D difference map (Fig. 7, far right) shows that the main difference between the PilQ oligomer alone and the PilQ-type IV pilus complex lies in the occupancy of the central cavity. We suggest that these structural changes could be explained by an outward movement of the arms and dissociation of the cap feature of the PilQ oligomer on binding to the pilus. A variance map of the unliganded PilQ oligomer showed that the arms and cap regions were the most dynamic portions of the structure (37). Assuming a density of 0.7 g/cm³, we estimate that the volume of the PilQ-type IV pilus complex (excluding the pilus “stalk”) corresponds to a mass of 950–1140 kDa when contoured at 2 and 1 Å above the mean density, respectively. These values are about 15% higher than those calculated previously for the PilQ oligomer alone (37) and are consistent with the proposition that the cavity of the PilQ oligomer has become filled upon association with the pilus.

The non-symmetrized density map of the PilQ-type IV pilus complex showed features consistent with a helix emerging from the mass of the PilQ oligomer, with a pitch of ~40 Å (Fig. 8) in good agreement with the value determined from unliganded pili. Although it is not possible, at the limit of the resolution of the density map determined here, to distinguish contributions arising from pilus versus the PilQ oligomer in the PilQ-type IV pilus complex, it is reasonable to infer that this external feature does indeed correspond to the type IV pilus.

**DISCUSSION**

The results presented above provide evidence for the specific recognition and binding of type IV pili by the outer membrane secretin PilQ. It has also been suggested that such binding causes a structural change in the PilQ oligomer. Specificity of the interaction between the PilQ oligomer and type IV pili is implied by several observations: PilQ particles were found to orientate in a structurally defined manner relative to the type IV pilus fiber. They were also found at only one end of the fiber. Single particle averaging of the PilQ oligomer-type IV pilus complex produced a well defined 3-D structure that showed specific structural changes in part of PilQ. It seems unlikely that the observed interaction between the PilQ oligomer and type IV pili could be nonspecific. A nonspecific interaction would be manifested by the binding of multiple PilQ oligomers to single pilus fibers, which was not observed. Nonspecific binding is also generally associated with low binding affinity; the results obtained by immunoblotting and sucrose gradient analysis suggest a high affinity interaction between PilQ and the type IV pili.

Kooi and co-workers (31) have provided evidence from phenotypic observations of *N. gonorrhoeae* mutants that pilus assembly and translocation to the outer membrane are distinct steps in biogenesis. A key observation made by these authors was that a pilQ mutants was non-piliated but that a pilT-pilQ double mutant was able to produce long pilus-like structures inside the periplasm, although the latter mutant was defective...
in growth. This observation led to the proposal that pili are pre-assembled in the periplasm and then pass through the PilQ oligomer in an assembled state. There are challenges, however, inherent in an attempt to reconcile this model with the 3-D structure of PilQ (37). There is no obvious pore through the PilQ oligomer, and the size of the channel required (diameter 65 Å) would be difficult to form while retaining the structural integrity of the PilQ complex. An assessment of the physiological relevance of our observations is complicated by the multi-component nature of the type IV pilus biogenesis apparatus: here we have described the *in vitro* reconstitution of an interaction between just two proteins, which are presumably derived from a secretory complex that is likely to be composed of many more (16, 58). It is possible, therefore, that we may be observing a complex between the PilQ oligomer and type IV pili whose existence is transitory *in vivo* and may be very difficult to detect in the fully functioning pilus biogenesis machinery. The value of being able to observe the formation of such complexes *in vitro* is self-evident. It is a limitation, however, of this approach that it is not easy to discern whether the structural forms of the PilQ oligomer observed here are intermediates in the assembly or disassembly processes, any “static” stages, or in all these phases. The results of our experiments do demonstrate, however, that the PilQ oligomer is capable of a dynamic behavior that has not been observed in any secretin to date and that might inform the type of model that could be proposed for neisserial pilus biogenesis.

Previous studies on secretins from type II secretion systems have found only indirect evidence for an interaction between secretins and their secreted substrates (55, 59). It has been shown that the type II pullulanase secreton from *Klebsiella oxytoca* is capable of assembling a pseudopilin, PulG, into pilus-like structures (60). In the case of the type IV pilus, such an interaction acquires an added importance, because it suggests a means whereby the pili could be anchored to the outer membrane. Elegant work using laser tweezers has shown that pili are capable of sustaining powerful retractile forces (11, 27). The way in which the base of the pilus fiber is secured to the
bacterium is unclear, but an interaction of the base of the fiber with PilQ could provide a plausible explanation for the stability of this interface. Image analysis of Type III secretion system needle complexes, purified intact from Salmonella and Shigella, has demonstrated that the complexes have a distinct tripartite structure: an inner membrane interface, a secreted outer membrane ring, and containing the secretin. Together, these structures form a basal plate connected to an extracellular tube of varying length (61). The function of the secretins within the needle complex has been probed by experiments that remove the secretin components completely by mechanical disruption. Electron microscopy studies of these depleted complexes reveal that the tube section actually extends further than the inner membrane base (62). In this context, the secretin apparently fulfills a “conduit-like” role, despite being in regular contact with a filament structure. By contrast, the general secretory pathway secretins (PulD, OutD, and XcpQ) are directly responsible for the outer membrane transport of one or several substrates (34, 35), and intimate substrate anchoring by the secretin would have no practical role in these systems. A possible “anchor” role for the PilQ oligomer would therefore represent a unique adaptation among the members of the secretin superfamily. This discussion has made the tacit assumption that the PilQ-type IV pilus complex is orientated facing outwards, i.e., with the emerging pilus fiber projected externally. To date, it has not been possible to resolve the issue of the orientation of the complex unambiguously. Nevertheless, the structural constraints on the passage of the pilus fiber through PilQ, referred to above, would remain whatever the orientation.

To date, structural studies on secretins have not addressed the interaction with their secreted substrates. It may be the case that the structures of secretins without other components of their respective secretion systems are in some ways misleading if their structure changes substantially during the dynamic secretion process. It is clear from structures of the PilQ oligomer (37) and the pIV secretin (63) that the cavity within the protein is blocked, requiring a large scale structural change to permit passage of the substrate. We suggest, therefore, that future structural studies will need to take account of conformational changes that occur on interaction with other components of the secretion machinery.

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