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NMR Structure of the *Escherichia coli* Type 1 Pilus Subunit FimF and Its Interactions with Other Pilus Subunits

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Type 1 pili from uropathogenic *Escherichia coli* strains mediate bacterial attachment to target receptors on the host tissue. They are composed of up to 3000 copies of the subunit FimA, which form the stiff, helical pilus rod, and the subunits FimF, FimG, and FimH, which form the linear tip fibrillum. All subunits in the pilus interact via donor strand complementation, in which the incomplete immunoglobulin-like fold of each subunit is complemented by insertion of an N-terminal extension from the following subunit. We determined the NMR structure of a monomeric, self-complemented variant of FimF, $FimF_F$, which has a second FimF donor strand segment fused to its C-terminus that enables intramolecular complementation of the FimF fold. NMR studies on bimolecular complexes between $FimF_F$ and donor strand-depleted variants of FimF and FimG revealed that the relative orientations of neighboring domains in the tip fibrillum cover a wide range. The data provide strong support for the intrinsic flexibility of the tip fibrillum. They lend further support to the hypothesis that this flexibility would significantly increase the probability that the adhesin at the distal end of the fibrillum successfully targets host cell receptors.

Keywords: donor strand complementation; FimF; protein–protein interactions; type 1 pili; tip fibrillum

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Introduction

A crucial event in the onset of a bacterial infection is the specific binding of bacteria to host cells. Numerous bacteria use adhesive surface organelles to accomplish this initial event of infection. These organelles can be classified according to their assembly pathway. Adhesive pili or fimbriae from Gram-negative bacteria represent the best-characterized group of adhesive organelles, which are assembled *in vivo* via the "chaperone–usher" pathway.[1–3] At least 37 adhesive surface filaments that belong to this class have been identified.[4] Most thoroughly investigated are type 1 pili from uropathogenic *Escherichia coli* strains, which cause cystitis,[5,6] P pili from *E. coli*, which are involved in the development of pyelonephritis and cystitis,[7] Afa/Dr fimbriae from *E. coli* strains, which cause chronic diarrheal and recurrent urinary tract infections,[8] and the F1 antigen on *Yersinia pestis*, which causes plague.[9]

This work focused on type 1 pili from uropathogenic *E. coli* strains, which are outstandingly stable filamentous structures and are composed of a pilus rod carrying a peripheral tip fibrillum (Fig. 1). The pilus rod is about 7 nm wide and up to 2 μm long and consists of 500–3000 copies of the main structural subunit FimA. These assemble into a right-

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Abbreviations used: 2D, two-dimensional; HSQC, heteronuclear single quantum coherence; NOE, nuclear Overhauser enhancement; NOESY, nuclear Overhauser enhancement spectroscopy; PRE, paramagnetic relaxation enhancement.

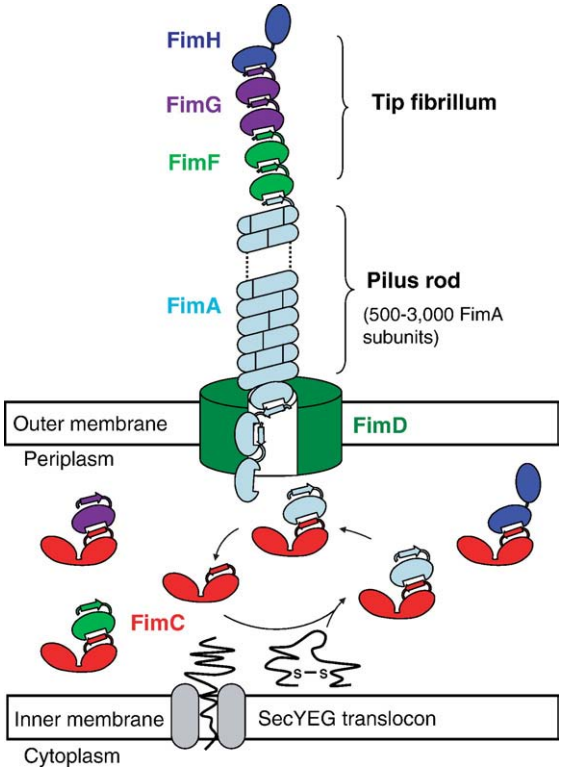

Fig. 1. Subunit composition and *in vivo* assembly of type 1 pili from *E. coli* via the "chaperone–usher pathway." Type 1 pili consist of up to 3000 protein subunits that interact via donor strand complementation (see the text for details). A single *E. coli* cell may bear more than 100 pili, which vary in length and are up to 2 μM long. The main structural pilus subunit FimA oligomerizes to a right-handed helical quaternary structure with about 3.4 subunits per turn, termed the pilus rod. In contrast to FimA, subunits FimF, FimG, and FimH, which form the tip fibrillum, are arranged linearly.[10] A single copy of the adhesin FimH, which contains an additional lectin domain for binding of target receptors, is located at the distal end of the tip fibrillum, and one or several copies of FimG and FimF connect FimH to the pilus rod.[10] The pilus is anchored to the outer bacterial membrane via the assembly platform ("usher") FimD. All structural pilus subunits are secretory proteins that fold in the periplasm and form 1:1 complexes with the pilus chaperone FimC. FimC–subunit complexes dissociate at the assembly platform, which mediates translocation of folded monomeric subunits through the outer membrane (adapted from Ref. 4).

handed helical quaternary structure, with ~3.4 subunits per turn.[10] The adhesin FimH at the tip of the pilus is linked to the pilus rod via one or several copies of subunits FimF and FimG, which assemble linearly and form the tip fibrillum.[10] Deletion of the gene encoding FimF or FimG decreases the number or increases the length of pili displayed on the cell surface, respectively, indicating that both subunits are involved in the regulation of pilus assembly.[11] During pilus biogenesis, all structural pilus subunits fold in the periplasm and form transient complexes with the periplasmic chaperone FimC.[12] The chaperone–subunit complexes dissociate upon contact with the assembly platform in the outer membrane, FimD, which mediates translocation of the folded pilus subunits to the cell surface.[13,14]

High stability of type 1 pili against denaturation at high temperature or in the presence of denaturants[15] is based on strong interactions between the subunits by "donor strand complementation." The individual structural pilus subunits have an incomplete immunoglobulin-like fold in which the seventh and last β-strand is missing. In addition, they possess an N-terminal extension that acts as a donor strand in the quaternary structure of the pilus and completes the fold of the previous subunit. This mechanism is reminiscent of "domain swapping"[16] and strongly contributes to the stability of subunit–subunit interactions in the pilus.[2,17–19] In contrast to all structural type 1 pilus subunits, the adhesin FimH consists of two domains. The C-terminal FimH domain shares the immunoglobulin-like fold with the other subunits and interacts with the subunit FimG via donor strand complementation. The N-terminal FimH domain, responsible for binding of the pilus to target cells, recognizes mannose units in the surface glycoprotein uroplakin Ia of epithelial cells from the urinary tract.[20,21] In addition, bacterial attachment via FimH can mediate internalization and persistence of the bacteria in host cells and thus promote evasion from the extracellular host defense.[5,22]

Comparisons of the structures of different chaperone–subunit complexes[17–19,23–25] and subunits complemented by a donor strand of a subunit[19,23–29] have revealed that the chaperone keeps the subunit in an "open" assembly-competent conformation. Upon incorporation into the pilus, the subunits undergo transition to a more compact and thermodynamically more stable conformation. The difference in subunit stability between the chaperone-bound form and the subunit-bound form is supposed to be the driving force for pilus assembly.

Understanding the requirements for the specificity of subunit–subunit interactions in the quaternary structure of adhesive pili requires structural information on the contacts between neighboring subunits. In this context, the available structures so far are the X-ray structure of the ternary complex between the chaperone Caf1M and two Caf1 subunits of F1 fimbriae from *Y. pestis*,[19] that of the ternary complex between the P pilus chaperone PapD and two copies of the main structural subunit PapA,[25] and the solution structure of the complex between subunits AfaD and AfaE from Afa/Dr fibrils.[27] Although it remains unclear whether the chaperone interactions with one of the subunits prevent further subunit–subunit contacts in the Caf1M/$(Caf1)_2$ and PapD/$(PapA)_2$ complexes, the NMR structure of the AfaD/AfaE complex revealed strong intersubunit interactions that give rise to a well-defined relative orientation of the subunits in the complex.

The type 1 pilus rod is stabilized not only by intermolecular donor strand complementation between neighboring subunits but also by multiple