The crystal structure of the chaperone-major subunit complex FaeE-FaeG gives insight in the biogenesis of F4 fimbriae from enterotoxigenic *E. coli*

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Binding of bacterial pathogens to host cells is typically mediated by adhesins. These are located on the bacterial surface in polymeric, proteinaceous appendages called fimbriae or pili, or in non-pilus structures [1]. The most prevalent assembly pathway for these adhesive structures is the chaperone/usher pathway [2]. Regardless of their ultrastructure, all known adhesive structures dependent on the chaperone/usher pathway are assembled via a donor strand complementation/exchange mechanism [3-5].

F4 expressing enterotoxigenic *Escherichia coli* cause diarrhea in neonatal and nearly weaned piglets. The fimbriae are mainly composed of the major subunit FaeG, which also contains the adhesive properties.

In 2007 we reported on the structure of a plant expressed FaeG dimer [6]. The crystal structure of the chaperone-major subunit FaeE-FaeG now allows us to get a better insight into the biogenesis of F4 fimbriae.

As we reported in 2005 the F4 chaperone FaeE crystallizes in three different crystal forms [7], all revealing a dimeric FaeE structure (Figure 1.a). The monomeric FaeE shows the typical boomerang shaped structure containing two immunoglobulin domains as seen for all periplasmic chaperones. In the first domain strands A_1', A_1", B_1, C_1, D_1', D_1", E_1, F_1 and G_1 are organised in two sheets, one containing strands A_1', B_1, D_1' and E_1, the other strands C_1, D_1", F_1 and G_1. The second domain also comprises two sheets: one with strains A_2, B_2 and D_2, and one with strands C_2, E_2 and F_2. A α-helix links strands C_2 and E_2. FaeE dimerization occurs through extensive main chain hydrogen bonding between the G_1 strands of both FaeE monomers.

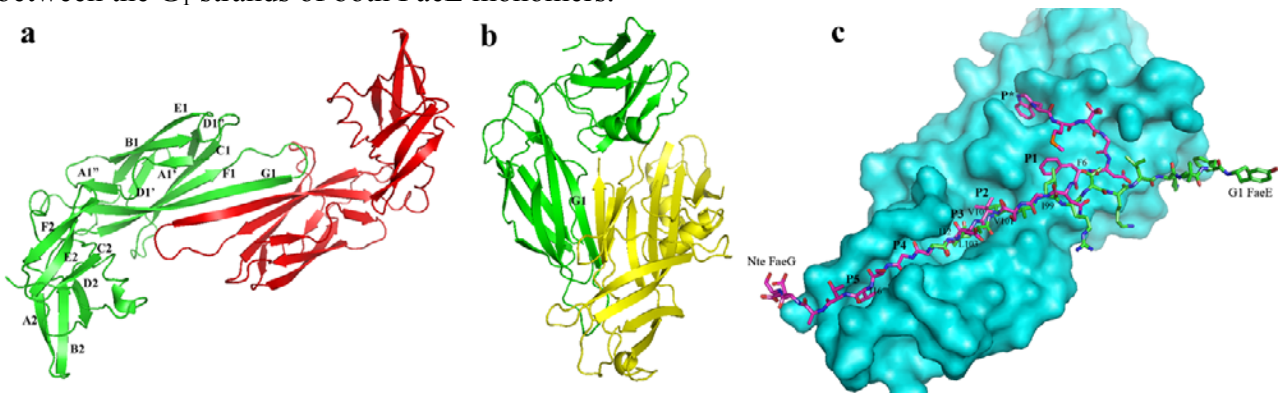

Figure 1. a. Cartoon presentation of the FaeE dimer. Strands are labeled in one monomer. b. Cartoon presentation of the FaeE-FaeG complex showing FaeE in green and FaeG in yellow. The chaperones G1 strand is labeled. c. Comparison of the interaction of the G1 strand of the FaeE and the Nte of FaeG with the core of FaeG. The FaeG core is shown in surface presentation, in cyan. The G1 strand (green) and the Nte (magenta) are shown as sticks. The G1 strand, the Nte, the pockets in the groove on FaeG and the hydrophobic residues of which the side chains are buried in the pockets are labeled

During the conserved donor strand complementation mechanism the chaperone FaeE interacts with the major subunit FaeG via the G1 strand and the G1-F1 loop, the C-terminus of the subunit is

anchored by residu Arg8 of the chaperone (Figure 1.b). In addition to 10 main chain hydrogen bonds with the F strand of FaeG, the side chains of Leu103, Val 101 and Ile99 on the chaperones G1 strand are buried into the hydrophobic pockets in the groove on the subunits surface.

From the structure of the plant expressed FaeG dimer we concluded that residues 1 to 17 of FaeG form the N-terminal extension (Nte) involved in the donor strand exchange mechanism. Residues 6-17 form the G-strand that aligns antiparallel with the F strand. In addition to 19 main chain hydrogen bonds, the Nte interacts with the FaeG core through a pattern of alternating hydrophobic residues. The side chains of residues Phe6, Val10, Ile12 and Ile16 are buried in the core of FaeG. Moreover, burial of residue Trp1 in a shallow pocket on the surface of FaeG anchors the Nte in the FaeG core.

Major differences can be seen when comparing the FaeE-FaeG structure with the self complementing dimer model of plant expressed FaeG (Figure 1.c). First, the G1 strand of FaeE makes a smaller number of contacts with the FaeG core than the Nte. Secondly, the G1 strand of FaeE binds the hydrophobic groove on the surface of FaeG in a different register as does the Nte of FaeG (Figure 1.c). Based on the nomenclature used in the Pap system [8], the pockets in the hydrophobic groove on FaeG were numbered P1 to P5. The G1 strand of FaeE occupies P1 to P3 pockets while the Nte occupies the P1, P2, P3 and P5 pockets. The P4 pocket stays unoccupied in both complexes: in the Nte the P4 residue is a glycine, in the G1 strand the side chain of Ser97 rather points away from the hydrophobic groove. The Trp1 residue of the Nte is buried in extra pocket P*. This pocket only becomes available after donor strand exchange, marking another difference between the structures.

Based on the length of the loop between their F_1 and G_1 strands fimbrial chaperones were devided into FGL and FGS chaperones. FGS chaperones are involved in the biosynthesis of monoadhesive fimbriae. These show a complex multisubunit structure, presenting the adhesin at the tip. FGL chaperones help the assembly of polyadhesive structures consisting of mainly one subunit. Recently Zavialov classified the F4 and related F5 fimbriae as an intermediate family of adhesive fibers, FGS chaperone assembled polyadhesive fibers. The structural details from the chaperone-major subunit complex thus give us more insights into the biogenesis of this class of adhesive fibers.

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