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Conserved Hydrophobic Clusters on the Surface of the Caf1A Usher C-Terminal Domain Are Important for F1 Antigen Assembly

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The outer membrane usher protein Caf1A of the plague pathogen *Yersinia pestis* is responsible for the assembly of a major surface antigen, the F1 capsule. The F1 capsule is mainly formed by thin linear polymers of Caf1 (*c*apsular *a*ntigen *f*raction 1) protein subunits. The Caf1A usher promotes polymerization of subunits and secretion of growing polymers to the cell surface. The usher monomer (811 aa, 90.5 kDa) consists of a large transmembrane β-barrel that forms a secretion channel and three soluble domains. The periplasmic N-terminal domain binds chaperone–subunit complexes supplying new subunits for the growing fiber. The middle domain, which is structurally similar to Caf1 and other fimbrial subunits, serves as a plug that regulates the permeability of the usher. Here we describe the identification, characterization, and crystal structure of the Caf1A usher C-terminal domain ($Caf1A_C$). $Caf1A_C$ is shown to be a periplasmic domain with a seven-stranded β-barrel fold. Analysis of C-terminal truncation mutants of Caf1A demonstrated that the presence of $Caf1A_C$ is crucial for the function of the usher *in vivo*, but that it is not required for the initial binding of chaperone–subunit complexes to the usher. Two clusters of conserved hydrophobic residues on the surface of $Caf1A_C$ were found to be essential for the efficient assembly of surface polymers. These clusters are conserved between the FGL family and the FGS family of chaperone–usher systems.

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Abbreviations used: $Caf1A_C$, Caf1A usher C-terminal domain; CUP, chaperone–usher pathway; OM, outer membrane; SEC, size-exclusion chromatography; TM, transmembrane; ZW3-14, Zwittergent 3-14; wt, wild type; $rCaf1A_C$, refolded $Caf1A_C$; $PapC_C$, PapC usher C-terminal domain; IMAC, immobilized metal-ion affinity chromatography; Bicine, *N*,*N*-bis(2-hydroxyethyl)glycine; PBS, phosphate-buffered saline; PEG, polyethylene glycol; ESRF, European Synchrotron Radiation Facility; EDTA, ethylenediaminetetraacetic acid.

Introduction

The chaperone–usher pathway (CUP) is used by Gram-negative pathogens to assemble fibrillar surface organelles such as pili, fimbriae, or capsules.[1,2] These organelles have important roles as virulence factors, for dissemination, and for protection against the host immune system during infection. Many mediate the adhesion between pathogen and host, which is crucial for pathogenesis. The simplest type of surface organelle is composed of only a single type of protein subunit. These are often thin (~2 nm) flexible fibrillae that sometimes collapse into dense

capsules. The best-described example of this type is Caf1 (*c*apsular *a*ntigen *f*raction 1; also known as F1 antigen) from the plague pathogen *Yersinia pestis*. The F1 antigen is known to be important for immune evasion during infection[3] and for dissemination of *Y. pestis*.[4]

In spite of the large variation in appearance, all CUP-assembled surface organelles share the same underlying fiber structure consisting of a string of noncovalently linked Ig-like subunits.[5–7] These are constructed essentially as Ig-like β-sandwiches, but with a circular permutation that positions the sequence corresponding to the seventh C-terminal β-strand (strand G of a canonical Ig domain) at the N-terminus of the polypeptide sequence. The absence of the C-terminal G strand leaves one edge of the β-sandwich unprotected and creates a hydrophobic "acceptor cleft" on the surface of the subunits, making free subunits only marginally stable.[8,9] Organelle fibers are composed of globular modules each having an intact Ig topology generated by 'donor-strand complementation,' where each Ig module is made from two polypeptide chains and with the G strand provided by the next subunit. The resulting structure is remarkably stable.[9,10]

In the CUP, a periplasmic chaperone and an outer membrane (OM) usher operate in concert to assemble and secrete polymeric surface organelles. The chaperone binds unfolded subunits as they enter the periplasm *via* the Sec pathway,[11] assists in their folding, and forms chaperone–subunit complexes, which are passively transported to the OM. The OM usher protein binds chaperone–subunit complexes, promotes their dissociation, and promotes the polymerization of structural subunits into a linear fiber. The growing polymer is translocated to the cell surface through the secretion pore of the usher. The CUP is independent of cellular energy sources,[12] and the driving force for assembly is the folding energy preserved in the high-energy folding intermediate of the subunit in the chaperone–subunit complex.[7,10] Because chaperones trap subunits in a metastable high-energy state that serves as a substrate for assembly, they also provide an important assembly control step. Although assembly can occur even in the absence of the OM usher, it is very slow and inefficient.[13] Ushers must provide several distinct functions. For example, they must be able to catalyze donor-strand exchange, where the N-terminal donor strand of the next subunit replaces the donor strand of the chaperone. Ushers involved in the assembly of multisubunit organelles must ensure that subunit components are assembled in the right order and must allow the secretion of assembled fibers to the cell surface. Some of the details of how these complex tasks are accomplished have begun to emerge, but many questions remain unanswered.

Ushers are large (80–90 kDa) porin-like integral OM proteins. Both PapC[14–16] (P pilus) and FimD[17,18] (type 1 fimbria) ushers form 7 nm×10 nm homodimers with a ~2-nm pore in the middle area of each monomer. Such a pore is wide enough to allow translocation of folded structural subunits or their polymers through the OM. In addition to a central transmembrane (TM) β-barrel, the ushers also contain conserved N-terminal and C-terminal periplasmic domains, and a third conserved domain located in the central β-barrel region.[15,16,19,20] The N-terminal domain serves as a primary binding site for chaperone–subunit complexes supplying new subunits for the growing fiber.[19,21–23] The centrally located "plug" domain that is structurally similar to organelle subunits serves to ensure that the secretion pore is not open prior to initiation of assembly and also appears to play a role in the subunit polymerization process.[15,16,20]

Neither the function of the usher C-terminal segment nor the structural organization of this part is known to date, but the C-terminal domain of PapC has been suggested to play a role in later assembly steps.[17,18] Here we report the identification and crystal structure of the C-terminal globular domain of the Caf1A usher, which acts to assemble F1 antigen in *Y. pestis*. The 10-kDa Caf1A usher C-terminal domain ($Caf1A_C$) is crucial for the *in vivo* assembly of F1 antigen, but is not required for the initial binding of chaperone–subunit complexes to the usher. Two clusters of conserved hydrophobic residues on the surface of $Caf1A_C$ were found to be essential for the efficient F1 antigen assembly on the cell surface. Comparison of $Caf1A_C$ and PapC C-terminal domain[24] revealed a conservation of surface hydrophobic clusters between the FGL family and the FGS family of chaperone–usher systems.

Results and Discussion

Identification of the soluble Caf1A C-terminal domain

In the absence of a leader peptide, OM proteins form inclusion bodies in the bacterial cytoplasm. While analyzing inclusion bodies (accumulated during the expression of mature Caf1A usher) in *Escherichia coli* BL21 STAR/pET9dCaf1A$_{His}$, we routinely observed a much smaller polypeptide (~25 kDa) together with full-length $Caf1A_{His}$ (91.3 kDa). After the solubilization of Caf1A inclusion bodies in 8 M urea, the small fragment copurified with the C-terminal His-tagged full-length Caf1A on Ni-NTA chromatography (Fig. 1a), suggesting that the small fragment is derived from the C-terminal segment of the Caf1A usher. After refolding in the presence of 0.5% Zwittergent 3-14