Structural and functional characterization of the secreted adhesion EtpA of enterotoxigenic *Escherichia coli*

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**Abstract**

In enterotoxigenic *Escherichia coli* (ETEC), toxin delivery is preceded by bacterial adherence and colonization of the host small intestine. The secreted adhesin EtpA promotes adherence and intestinal colonization in ETEC by binding to the tips of flagella. EtpA is part of a two-partner secretion system (TPS), a mechanism used to secrete large virulence factors in Gram-negative bacteria. TPS consist of a secretory passenger protein (TpsA) and a transporter (TpsB) that facilitates transport of TpsA across outer bacterial membrane. TpsA are high molecular weight proteins characterized by repeat sequences and share a conserved, N-terminal TPS domain of ~250 residues required for secretion and folding. EtpA is an example of a TpsA. Four consecutive 228 amino acid repeats of unknown function constitute the C-terminal region of EtpA, with additional repeats within each 208 residue repeat. We separated the N-terminal TPS domain (residues 68-441) and the C-terminal repeat domain (residues 442-1767), and simplified the latter by centrally removing three of the four repeats. We characterized both fragments by circular dichroism and the TPS domain by X-ray crystallography. To establish the interaction of both fragments with flagellin, a pulldown assay was used. We could solve the crystal structure of the N-terminal domain of EtpA at 1.76 Å resolution. The structure reveals a right-handed parallel β-helix consisting of two hairpins, an extra-helical motif and an N-terminal region capped by β-strands. As with other TPS domains, the conserved β-helix would appear to imply its importance in secretion and folding of the TpsA. Modelling the structure of the C-terminal region also yielded a β-helix indicating that EtpA has a continuous β-helical structure. Thermal unfolding followed by circular dichroism spectroscopy indicated that both N- and C-terminal domains had similar melting temperatures of between 55 and 60°C. Chemical unfolding with urea, however, showed that the N-terminal domain unfolds reversibly. This was not observed for the C-terminal domain. The EtpA N-terminus is thus presumably able to rapidly fold during secretion, providing a template that catalyses the folding of the C-terminal domain by extending the common -helix. Multiple attempts to purify full-length EtpA were discontinued due rapid degradation and low solubility of the protein. Unexpectedly, molecular pulldown assays with both N- and C-terminal domains of EtpA failed to show any interaction with flagellin implying that other factors may be involved.