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

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Enterotoxigenic *Escherichia coli* (ETEC) is a human pathogen killing especially children under the age of five in underdeveloped countries. The bacterium adheres and colonizes the host small intestine. The secreted virulence factor EtpA is a presumed cell adhesion factor that binds to flagellin at the tips of ETEC flagellae. It is secreted as part of a two-partner secretion system (TPS) for large virulence factors in Gram-negative bacteria consisting of a secreted functional passenger protein (TpsA) and a membrane-integral transporter (TpsB) to translocate the TpsA across the outer bacterial membrane. TpsAs, like EtpA, are high molecular weight proteins with repeat sequences and a conserved, N-terminal TPS domain of ~250 residues. EtpA has four 228 amino acid repeats of unknown function in its C-terminal region, with additional repeats within each 228-residue repeat.

We separated coding regions for the N-terminal TPS domain (residues 68-447) and the C- terminal repeat domain (residues 448-1767) of EtpA and simplified the latter by centrally removing three of the four repeats. We characterized both N- and C-terminal fragments by circular dichroism and solved the crystal structure of the N-terminal domain at 1.76 Å resolution. The full-length protein was modelled by AlphaFold.

The crystal structure of the TPS domain reveals a right-handed parallel β-helix with two extrahelical hairpins and an N-terminal region capped by β-strands. The conserved β-helix is presumably critical for secretion and extracellular folding of EtpA. The structural AlphaFold model of the C-terminal region indicates an extension of the β-helix. Thermal unfolding assays by circular dichroism spectroscopy indicated that both N- and C-terminal domains have similar melting temperatures of between 55 and 60°C. Unfolding of the N-terminal domain by urea is reversible but not so for the C-terminal domain. We interpret this to mean that the EtpA N-terminal domain folds autonomously during secretion, providing a template for the folding of the C-terminal domain by extending the common □-helix. Full-length EtpA has lowsolubility and degrades rapidly. 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.

Reference: Roy, K., et al., *Enterotoxigenic Escherichia coli EtpA mediates adhesion between flagella and host cells.* Nature, 2009. **457**(7229): p. 594-8.

Keywords: Infectious Diseases, Structural Biology, CD spectroscopy.