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Letter to the Editor: Complete resonance assignments of bundlin (BfpA) from the bundle-forming pilus of enteropathogenic *Escherichia coli*

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Biological context

Enteropathogenic *Escherichia coli* (EPEC) causes acute and persistent neonatal diarrhea in developing countries (reviewed in Frankel et al., 1998). The production of a type IVB fimbria in EPEC, known as the bundle-forming pilus (BFP), is required for the formation of large discrete EPEC microcolonies on the surface of epithelial cells – a phenotype characterized as localized adherence (LA) (Donnenberg et al., 1992). In addition, BFP is crucial for EPEC virulence, antigenicity and autoaggregation (Bieber et al., 1998). Bundlin, the product of the *bfpA* gene, is the pilin protein that constitutes the only known structural subunit of BFP filaments (Donnenberg et al., 1997, Blank et al., 2000). Pre-bundlin contains a hydrophilic leader sequence that is cleaved by a prepilin peptidase BfpP to produce the mature protein. The focus of this communication is the globular 'head' domain of the bundlin that is encoded by the α1 allele (Blank et al., 2000).

Methods and experiments

In order to alleviate the solubility problems of this membrane-associated protein, the hydrophobic N-terminus of the protein was omitted. The C-terminal end of *bfpA-α1* (encoding residues 38–193 of the mature protein) was cloned into pET39b+ to form a fusion protein with DsbA. The vector was engineered by inverse PCR to remove the codons for mature DsbA leaving behind the *dsbA* signal sequence (necessary for targeting bundlin to the periplasm and an N-terminal histidine). Cultures were grown in 1L Silantes OD2 CN media to mid-log phase (OD_{600} 0.5) and induced with 1 mM IPTG overnight. Cells were lysed using a French press and the clarified lysate was affinity purified with nickel nitriloacetic acid (Ni-NTA) agarose using a one batch purification step followed by purification using gel filtration.

Purified $^{13}C/^{15}N$-labelled bundlin protein was concentrated to approximately 0.5 mM into 20 mM sodium phosphate buffer pH 5.2. NMR experiments were performed at 303K on a 500 MHz four channel Bruker DRX500 spectrometer equipped with a z-shielded gradient triple resonance cryoprobe. ^{1}H chemical shifts were referenced to internal TSP and ^{13}C and ^{15}N chemical shifts were referenced indirectly to TSP using the absolute frequency ratios. Sequence-specific backbone ^{1}HN, ^{15}N, $^{13}C'$, $^{13}C\alpha$ and $^{13}C\beta$ chemical shifts were determined using standard triple resonance methods (for review see Sattler et al., 1999). The ^{1}H and ^{13}C chemical shifts of side chains, except for aromatic rings, were assigned using 3D HBHA(CBCACO)NH, 3D H(C)CH-TOCSY (Kay et al., 1993) and (H)CC(CO)NH-TOCSY (Sattler et al., 1999). Assignments for the aromatic side-chains were obtained from a 3D ^{13}C-edited NOESY-HSQC recorded at 800 MHz in conjunction with a ^{1}H-^{13}C HSQC experiment. A 3D ^{15}N-edited NOESY-HSQC experiment enabled the sequential assignment to be confirmed and the structured regions in bundlin to be identified. Spectra were processed with NMRPipe

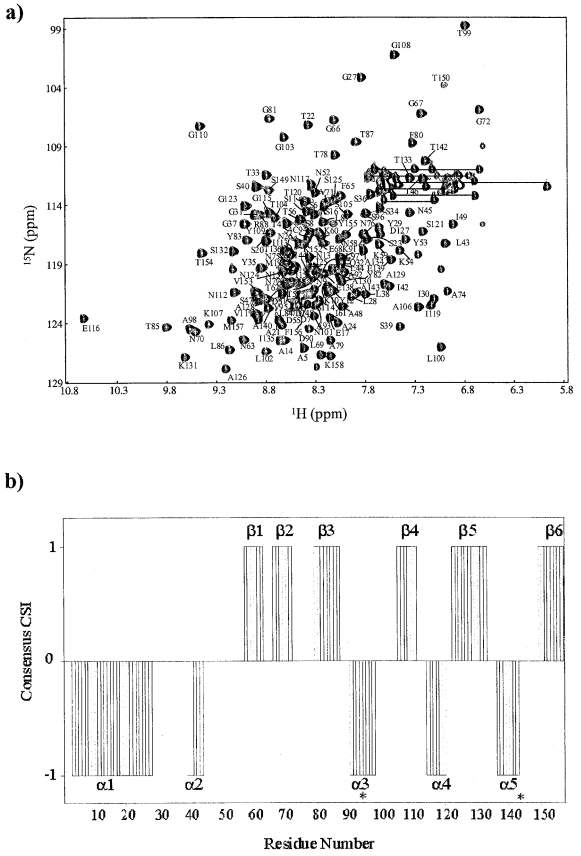

Figure 1. a) Assigned ^{1}H-^{15}N HSQC NMR spectrum of bundlin at 303K. Unassigned side-chain resonance pairs are connected by horizontal lines. b) Consensus chemical shift index for bundlin. α-helices and β-strands are labelled accordingly and the asterisks identify the two cysteine residues. The figure was created using Jplot (C. Jeremy Craven, University of Sheffield).

(Delaglio et al., 1995) and analysed using NMRView (Johnson and Blevins, 1994).

Extent of assignments

Backbone assignments in bundlin, illustrated in the ^{1}H-^{15}N HSQC (Figure 1a), are essentially complete (>96%) except for the three first amino acids. In addition, Lys145, Asn146 and Thr147 were not observed due to conformational exchange in the vicinity of Cys144. The side chain assignments were also assessed to be complete, except for the NH_2 moieties of asparagines and glutamines. The C_β chemical shifts of Cys94 and Cys144 are indicative of a disulfide bond between the two cysteines. The consensus chemical shift index (Wishart and Sykes, 1994) indicates that BfpA is an α/β protein, characterized by 5 helices and 6 β-strands (Figure 1b). Furthermore, distinctive NOEs between NH and H_α signify the presence of both anti-parallel and parallel β-strands. Data suggest that the topology of bundlin presents some similarities to that of the Toxin-Coregulated Pilus (TcpA) from *Vibrio cholerae* (Craig et al., 2003).

The chemical shifts have been deposited in the BioMagResBank database (*http://www.bmrb.wisc.edu*) under the accession code 6003

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