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Letter to the Editor: Complete resonance assignments of a 'donor-strand complemented' AfaE: The afimbrial adhesin from Diffusely Adherent *E. coli*

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Biological context

Members of the Afa (Afimbrial Adhesin) family of adhesins have been isolated from *Escherichia coli* strains that are able to cause both urinary tract (uropathogenic *E. coli* or UPEC) and intestinal infections (Diffusely Adherent *E. coli* or DAEC) in humans and animals (Lalioui et al., 1999). Furthermore, a diffusely adherent adhesin has also been identified in enterohaemorragic and enteropathogenic *E. coli* (Keller et al., 2002). The afimbrial sheath is composed of two proteins from the *afa* operon, AfaE and AfaD, that have different roles in host cell interactions. AfaE and AfaD are directly linked to virulence, co-localise at the outer membrane and perform the roles of adhesin and invasin, respectively (Jouve et al., 1997). AfaE mediates adhesion to epithelial cells by recognising the receptor, decay-accelerating factor (DAF or CD55) (Nowicki et al., 2001). This in turn induces the increased surface expression of MHC class I-related MICA molecules present in the intestinal epithelium (Tieng et al., 2002). The presence of the chaperone protein within the *afa* operons suggests that AfaD and AfaE adopt donor strand complementation for the construction of the afimbrial sheath (Zavialov et al., 2003). The focus of this work is on the AfaE-III subtype from human *E. coli* isolates (Le Bouguénec et al., 1993).

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Methods

In order to alleviate the inherent self-association properties of AfaE-III and facilitate isolation of a soluble form, a 'donor-strand complemented' AfaE-III (AfaE-*dsc*) was constructed, based on the highly successful approach for FimH (Barnhart et al., 2000). The first 16 N-terminal amino acids were removed from mature AfaE-III (GFTPSGTTGTTKLTVT) and inserted at the C-terminus after a four-residue linker (DNKQ). This construct, AfaE-*dsc*, was expressed cytoplasmically using the pRSETA plasmid (Promega) in the BL21(DE3) *E. coli* strain (Novagen).

The expressed fragments were purified under denaturing conditions (50 mM sodium phosphate, pH 8.0, 0.3 M NaCl and 6 M guanidine hydrochloride) using an N-terminal hexahistidine tag which is separated from the body of the protein by a thrombin cleavage site. The purified protein was refolded and subsequently readily concentrated to mM concentrations. ^{15}N, ^{13}C double-labelled samples of AfaE-*dsc* were produced in minimal media, containing 0.07% $^{15}NH_4Cl$ and 0.2% ^{13}C-glucose, supplemented with 50 μg ml^{-1} ampicillin. Protein expression was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside. Purified proteins were dialysed into 20 mM sodium acetate buffer at pH 5.3 and concentrated to approximately 0.8 mM for NMR.

The majority of NMR spectra were recorded at 303 K on a 500 MHz four-channel Bruker DRX500 spectrometer equipped with a z-shielded gradient triple resonance cryoprobe. Sequence-specific backbone ^{1}HN, ^{15}N, ^{13}Cα and ^{13}Cβ were determined using standard triple resonance methods (for review

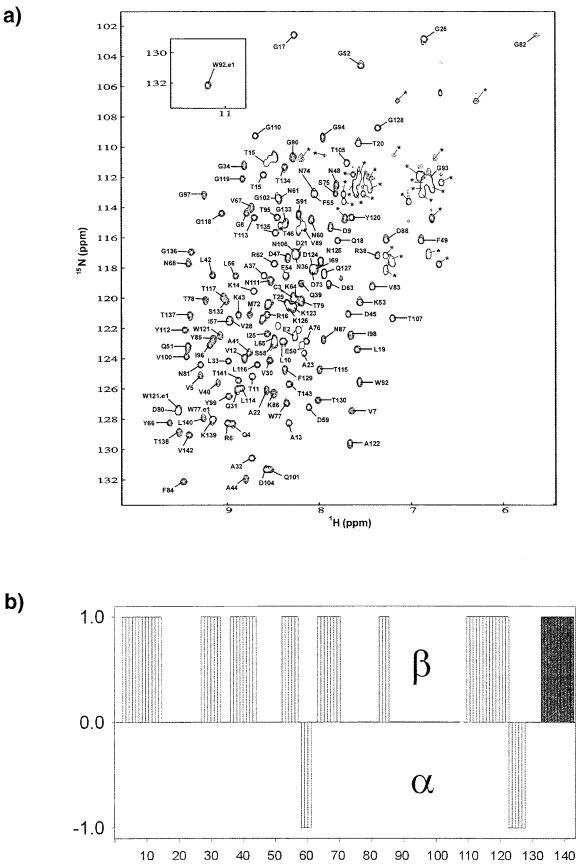

Figure 1. (a) Assigned ^{1}H-^{15}N HSQC NMR spectrum of AfaE-dsc. Sequential Assignments of the amides are indicated. (b) Chemical Shift Index (CSI) plot (Wishart and Sykes, 1994) of dsc-AfaE generated using $^{1}H_{\alpha}$, $^{13}C_{\alpha}$, $^{13}C_{\beta}$ and $^{13}C'$ chemical shifts. The secondary structural elements are indicated. The self-complemented strand is shaded dark grey.

see Sattler et al., 1999). H_{α} and H_{β} assignments were obtained using an HBHA(CBCACO)NH experiment (Sattler et al., 1999). All triple resonance experiments employed constant-time evolution in the ^{15}N dimension, whereas in the CBCA(CO)NH and HBHA(CBCACO)NH experiments both indirect dimensions were recorded in constant-time mode. The side chain assignments were achieved using HCCH-total correlation (TOCSY) spectroscopy and an (H)CC(CO)NH TOCSY (Sattler et al., 1999).

Extent of assignments

Using the standard triple-resonance assignment methodology, backbone assignments could be made for 100% of residues from AfaE-*dsc*. The side chain assignments were assessed to be complete with the exception of some overlapping resonances in arginine side chains. The chemical shift data were used to identify secondary structure elements (Figure 1). These data clearly show the presence of a predominantly β-sheet structure in AfaE-*dsc*, which is typical for fimbrial subunits. The C_{β} chemical shift of the two cysteine residues (3 and 35) indicate that they exist in a fully oxidised form within monomeric AfaE. Furthermore, there is NOE evidence suggesting that the two cysteines are adjacent within the domain and likely form a disulphide bond. Cross-strand NOEs identify that the canonical β-strand F pairs with the donor-strand in the predicted manner, thereby providing the first structural evidence for the proposed 'self-complementation' (Barnhart et al., 2000).

A table of complete backbone and side-chain assignments is available as supplementary material and has been deposited in the BioMagResBank in Madison, WI, USA (accession code 5947).

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