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

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Biological context

Members of the Afa (Afimbrial Adhesin) family of adhesins have been isolated from Diffusely Adherent *E. coli* (DAEC) strains that are known to cause intestinal disease in humans and animals. Furthermore, a diffusely adherent adhesin has also been identified in enterohaemorrhagic 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). The focus of this work is the AfaD-III subtype from human *E. coli* isolates (Le Bouguénec et al., 1993) and is identical to the DraD invasin from uropathogenic *E. coli* (Zalewska et al., 2001). AfaD-III was found to be dispensable in the production of functional adhesins but no invasion of mucosal layers could be detected (Garcia et al., 1996). The role of AfaD-III as an invasin was further confirmed by the translocation of AfaD-coated gold particles into mucosal cells (Gounon et al., 2000; Jouve et al., 1997) via an interaction with the α5β1 integrin (Guignot et al., 2001). The presence of a chaperone and usher within the *afa* operon suggests that AfaD and AfaE adopt donor strand complementation for construction of the afimbrial sheath (Zavialov et al., 2003).

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Methods

In order to alleviate the inherent self-association properties of AfaD and facilitate isolation of soluble form a donor-strand complemented AfaD (AfaD-*dsc*) was constructed, based on the highly successful approach for FimH (Barnhart et al., 2000). A four-residue linker was added to the C-terminus of the wild-type AfaD-III sequence followed by the 16-residue N-terminal donating peptide from AfaE-III: VVPQE-DNKQ-GFTPSGTTGTTKLTVT. This construct was expressed using the pQE-30 plasmid in the M15/pREP4 *E. coli* strain (QIAGEN). ^{15}N, ^{13}C double-labelled samples of AfaD-*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 50 μM isopropyl-β-D-thiogalactopyranoside.

AfaD-*dsc* was purified in denaturing conditions (50mM sodium phosphate, pH 8.0, 0.3 M NaCl and 8 M urea) using the binding of the N-terminal hexahistidine tag (MRGSHHHHHHGS) to the Co^{2+}-agarose resin Talon (CLONTECH). Purified protein was refolded by dialysis into 50mM sodium phosphate buffer pH 7.0 and concentrated to approximately 0.5mM for NMR.

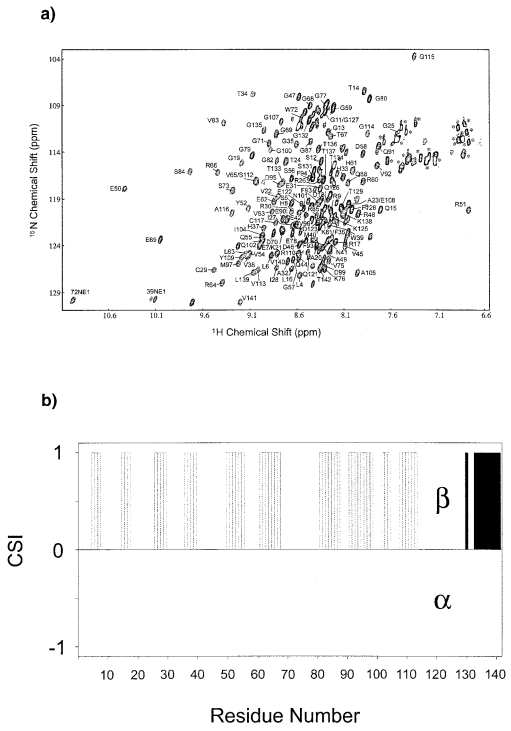

Figure 1. (a) Assigned ^{1}H-^{15}N HSQC NMR spectrum of AfaD-*dsc*. Sequential Assignments of the amides are indicated. Asterisks indicate asparagine and glutamine side chain resonances. (b) Chemical Shift Index (CSI) plot (Wishart and Sykes, 1994) of AfaD-*dsc* generated using $^{1}H_{\alpha}$, $^{13}C_{\alpha}$,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

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 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 98% of the residues from AfaD-*dsc*. Arg43 and Phe111 could not be assigned due to spectral overlap, and valines 118 and 119 due to conformational exchange in neighbouring Cys and Pro residues. The side chain assignments were assessed to be complete with the exception of overlapping resonances in regions containing the aromatic, asparagine and glutamine residues. The chemical shift data were used to identify secondary structure elements (Figure 1). These data clearly show the presence of an all β-sheet structure in AfaD-*dsc*, which is typical for fimbrial subunits. The C_{β} chemical shift of the two cysteine residues (29 and 117) indicate that they exist in a fully oxidised form within monomeric AfaD. Furthermore, NOE evidence suggest that a disulphide bond exists between these two residues. Cross-strand NOEs identify the paring of canonical β-strand F with the C-terminal extension (G strand), thereby providing the first structural evidence for the proposed 'self-complementation' (Barnhart et al., 2000).

A table of backbone and side-chain assignments is available as supplementary material and has been deposited in the BioMagResBank in Madison, WI, USA (accession code 5946).

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