NMR assignment of the *Rhodobacter sphaeroides* fasciclin-1 domain protein (Fdp)

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Abstract

We report the almost complete assignment of ^{1}H, ^{13}C and ^{15}N nuclei in the 137-residue his-tagged fasciclin domain protein (Fdp) from *Rhodobacter sphaeroides*. Fdp is homologous to fasciclin I domains, including *Drosophila* FAS1 and *M. tuberculosis* MPB70 and plays a role in cell adhesion.

Key words: fasciclin, NMR assignments, adhesion

Biological context

The fasciclin I family of proteins is found in a wide range of vertebrates, invertebrates and microorganisms. Members generally function as cell-surface proteins involved in homophilic cell adhesion or symbiosis. One of the most well known examples is *Drosophila melanogaster* FAS1, which guides developing axons to target neurons or muscle cells (Elkins *et al*., 1990; McAllister et al., 1992; Clout et al., 2003). In

mammals the Fas1 domain of human transforming growth factor-β-induced gene product (βig-h3) contributes to corneal epithelial cell adhesion through interaction with $\alpha_3\beta_1$ integrin (Kim *et al*., 2000). It has been proposed that the fasciclin protein periostin, which is secreted in high levels in ovarian cancer cells, functions as a ligand for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins in order to support adhesion and migration of ovarian epithelial cells (Gillan *et al*., 2002). FEEL-1, a scavenger receptor with *in vitro* bacteria-binding and angiogenesis modulating activities is another human Fasciclin I protein (Adachi and Tsujimoto, 2002). Fasciclin I-like domains also occur in plant proteins including the *Arabidopsis thaliana* SOS5 protein required for normal cell expansion (Shi *et al*., 2003). One of the most well-characterised microbial fasciclin proteins is MPB70 from *Mycobacterium bovis* (Nagai *et al*., 1981), closely related to *M. tuberculosis* MBP70 (Carr et al., 2003). This is a prominent secreted and highly antigenic protein (Wiker *et al.,* 1991). *Rhodobacter sphaeroides* Fdp is unusually a single-domain protein and is likely to have a role in bacterial adhesion, making it a target for study of bacterial virulence.

Methods and experiments

The *fdp* gene is located on chromosome 1 of *R. sphaeroides* as ORF RSP1409 (http://genome.ornl.gov/microbial/rsh). The *fdp* region 57 to 470 (relative to ATG, where A is position 1), which lacks the region encoding the signal peptide region, was cloned into the expression vector pET14b for protein overproduction with a 21-residue N-terminal $[His]_6$-tag. Induction by IPTG and expression was carried out in M9 minimal medium, using ^{15}N-ammonium chloride and ^{13}C-glucose. The NMR samples contained 1-2 mM protein in 50 mM $NaPO_4$ (pH 7.0), 0.03% NaN_3 in 10%:90% D_2O:H_2O.

NMR experiments were performed on Bruker DRX 500 and 800 spectrometers (operating at 500 and 800 MHz respectively for ^{1}H) at 295 K. Spectra were processed and analysed using Felix 2000 (Accelrys Inc., San Diego). The 2D ^{15}N HSQC and 3D triple-resonance CBCA(CO)NH, HNCA, HNCACB, HNCO, and HN(CA)CO spectra were used to obtain the backbone resonance assignments. CCH and HCCH-TOCSY spectra were used for the side-chain resonance assignments. The assignment was confirmed and completed with 3D NOESY. The secondary structure of Fdp was obtained using chemical shift and NOE data. The Fdp NMR structure is composed of 6 helical regions corresponding to residues 5-11 (α_1), 19-25 (α_2), 27-32 (α_3), 43-50 (α_4), 53-58 (α_5) and 60-74 (α_6), a three-stranded β sheet corresponding to residues 36-41 (β_1), 75-78 (β_2) and 124-129 (β_7) and a four-stranded β-sheet corresponding to residues 88-93 (β_3), 97-103 (β_4), 108-110 (β_5) and 112-114 (β_6) of the mature protein.

Assignments and data deposition

Almost complete assignments for the backbone and side-chain resonances of Fdp were obtained (136/137 HN, 136/137 Cα, 130/137 C', 118/120 Cβ excluding the poly-$[His]_6$-tag) (Figure 1). Fdp is degraded relatively rapidly, such that after several days of acquisition of NMR spectra, additional peaks start to appear. These peaks appear in positions characteristic of random coil peptides, and it is therefore suspected that there is slow proteolysis of the protein occurring, despite the addition of protease inhibitor cocktail. As a result separate samples of Fdp were purified independently for the recording of each of the backbone, side-chain and NOESY spectra and used immediately. The resonance assignments and the chemical shift values for Fdp have been deposited in the BioMagResBank under the accession number 6312.

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Figure 1. $^{1}H,^{15}N$ HSQC spectrum of *Rhodobacter sphaeroides* Fdp in 90% H_2O/10% D_2O, 295K, pH 7.0, 50 mM phosphate. Backbone resonance assignments are indicated by the one-letter amino acid code.