The fatty acid and retinol-binding (FAR) proteins are a family of unusual helix-rich lipid binding proteins found exclusively in nematodes, and are secreted by a range of parasites of humans, animals and plants. Na-FAR-1 is from the parasitic nematode *Necator americanus*, an intestinal blood-feeding parasite of humans. Sequence-specific $^1$H, $^{13}$C and $^{15}$N resonance assignments have been obtained for the recombinant 170 amino acid protein, using three-dimensional triple-resonance heteronuclear magnetic resonance experiments. Backbone assignments have been obtained for the recombinant 170 amino acid protein, using three-dimensional triple-resonance heteronuclear magnetic resonance experiments. Backbone assignments have been obtained for 99.3 % of the non-proline HN/N pairs (146 out of 147). The amide resonance of T45 was not observed, probably due to rapid exchange with solvent water. A total of 96.9 % of backbone resonances were identified, while 97.7 % assignment of amino acid sidechain protons is complete. All Hα(166), Hβ(250) and Hγ(160) and 98.4 % of the Hδ (126 out of 128) atoms were assigned. In addition, 99.4 % Cα (154 out of 155) and 99.3 % Cβ (143 out of 144) resonances have been assigned. No resonances were observed for the NH groups of R93 Nε2, arginine, Nγ1H2, Nε3H2, histidine Nδ1H3, Nε4H3 and lysine Nε3H3. Na-FAR-1 has a similar overall arrangement of α-helices to Ce-FAR-7 of the free-living *Caenorhabditis elegans*, but with an extra C-terminal helix.

**Keywords**  Parasitic nematode · *Necator americanus* · Fatty-acid and retinol-binding protein · Na-FAR-1 · NMR

**Biological context**

Over 500 million people living in tropical and subtropical regions are infected with human hookworms, *Necator americanus* being the most prevalent. The infective larvae burrow into skin and develop to adulthood in the intestine, where they feed on blood and tissue. The infection causes anaemia and growth stunting, the effects being most severe in children and women of child bearing age (Hotez et al. 2004).

Fatty acid and retinol-binding (FAR) proteins are α-helix-rich proteins of around 20 kDa that are produced at different life cycle stages of nematodes and have been observed to be secreted by adult parasites (Basavaraju et al. 2003; Kennedy et al. 1997; Prior et al. 2001). It is hypothesized that FARs may play a role in host-parasite interaction and pathogenesis by sequestering or delivering lipid mediators, thereby affecting the local tissue environment of the host, and compromising immune and inflammatory defences (Bradley et al. 2001). FAR proteins are already used as diagnostic tools (Burbelo et al. 2009), and have also been shown to induce immunity against one parasite infection (Fairfax et al. 2009). They are, therefore,
attractive potential targets for drug or vaccine development because they have no structural counterparts in mammals (Basavaraju et al. 2003).

Na-FAR-1 has been identified in a transcriptomic survey of *N. americanus* (Daub et al. 2000), and we here report resonance assignments of its bacterial recombinant form. The only structure of a FAR currently available is a crystal structure of Ce-FAR-7 of the free living nematode *Caenorhabditis elegans* (Jordanova et al. 2009). This FAR has seven α-helices and comprises two binding sites that could accommodate different types of ligands. We find that Na-FAR-1 has a similar overall arrangement of regular secondary structure elements, but with an extra helical region at the C-terminus.

**Methods and experiments**

Recombinant Na-FAR-1 was expressed in BL21 (λDE3) *Escherichia coli* cells using [13C, 15N]-labelled M9 minimal medium. The His-tagged fusion protein was purified by nickel affinity and gel filtration chromatographies. The removal of copurifying ligands from the bacterial expression system was achieved by reverse-phase high performance liquid chromatography (RP-HPLC) with a C8 stationary phase and water/acetonitrile/trifluoroacetic acid mobile phase. The protein was refolded in aqueous buffer and concentrated to approximately 0.5 mM in 50 mM sodium phosphate pH 7.2, 50 mM NaCl. D2O was added to a final concentration of 10 % (v/v) prior to data acquisition. All spectra were recorded at 311 K on a Bruker Avance 600 MHz spectrometer equipped with a TCI cryoprobe.

Sequence-specific resonance assignment of the Na-FAR-1 backbone was accomplished with the aid of 2D 15N-HSQC, 3D HNCACB, 3D CBCA(CO)NH (Muhandiram and Kay 1994), 3D HNCO (Kay et al. 1994) and 3D HNCACO spectra. The majority of aliphatic sidechain carbon and proton resonances were located by navigating from the backbone data using 3D (H)(C)(O)NH-TOCSY, 3D HBHA(CBCA)NH (Wang et al. 1994) and 3D H(C)(O)NH-TOCSY spectra (Grzesiek and Bax 1992). Remaining aliphatic resonances were identified using 3D HcCH-TOCSY and 3D hCCH-TOCSY (Kay et al. 1993), this latter proving particularly useful for assignment of lysine sidechain CδH and CɛH groups that were too overlapped in both 1H and 13C dimensions to be resolved in other experiments. A proportion of aromatic sidechain 13C/1H signals (histidine Hδ1, tyrosine Hδ2, and phenylalanine Hα,γ) were assigned using 2D HBCBCGCDH and 2D HBCBCGCDCEHE spectra (Yamazaki et al. 1993) and the remainder were identified from the 13C-edited [1H,1H]-NOESY spectrum. NMR spectra were processed using AZARA (http://www.bio.cam.ac.uk/azara) and analysed with CCPN analysis (Vranken et al. 2005).

**Extent of assignments and data deposition**

All Na-FAR-1 polypeptide backbone resonances were assigned, with the exception of the 15 His-tag N-terminal residues (MGSSHHHHHHSSGHH). Excluding the His-tag residues, backbone resonance assignments have been obtained for 99.3 % of the non-proline HN/N pairs (146 out of 147). The amide resonance of T45 was not observed, probably due to rapid exchange with solvent water. Figure 1 shows the 1H-15N HSQC spectrum with the assigned crosspeaks. A total of 96.9 % of backbone resonances were identified, while 97.7 % assignment of amino acid sidechain protons is complete. All Hx(166), Hβ(250) and Hγ(160) and 98.4 % of the H6 (126 out of 128) atoms were assigned. In addition, 99.4 % Cα (154 out of 155) and 99.3 % Cβ (143 out of 144) resonances have been assigned. No resonances were observed for the following NHn groups: R93 Nδ2, arginine, Nη1H2, Nη2H2, histidine Nδ1Hδ1, Nη1Hη, and lysine Nε2H3.

Chemical shift index (CSI) (Wishart and Sykes 1994) and DANGLE secondary structure analysis (Cheung et al. 2010) of Na-FAR-1 revealed a α-helix pattern consistent with the previously reported C. elegans FAR protein, with an additional α-helix segment at the C-terminus (Fig. 2).

The 1H, 13C and 15N chemical shift assignments have been deposited with the BioMagResBank database (http://www.bmrb.wisc.edu), accession number 18637.

![Fig. 1](image-url) Two-dimensional 1H-15N HSQC spectrum of recombinant Na-FAR-1 showing the backbone amide resonance assignments. Crosspeaks have been labelled with the single letter amino acid code along with the native sequence specific number.
Fig. 2 Na-FAR-1 CSI consensus values (bars) and secondary structure elements (bottom) alignment. Negative CSI indicate α-helical segments which are shown as boxes below.

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