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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N resonance assignments of the EscJ protein, a structural component of the Type III secretion system of enteropathogenic *E. coli* (EPEC)

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Biological context

Enteropathogenic *Escherichia coli* (EPEC) strains are an important cause of infantile diarrhoea in the tropics (Nataro and Kaper, 1998). Infection is characterised by the so-called attaching/effacing (A/E) lesion. The effacement is the result of cytoskeletal rearrangement brought about by EPEC-secreted factor proteins that reorganise structural host cell proteins such as actin, cytokeratins and myosin (Rosenshine et al., 1996; Batchelor et al., 2004;). Secretion is attributed to the Type III secretion system (TTSS), a structure spanning both bacterial membranes that bears remarkable similarity to the bacterial flagellar export system (Hueck, 1998). The EPEC Esc proteins, homologous to the *Yersinia* Ysc proteins, are amongst those proteins that constitute the structural components of the TTSS. EscJ represents another structural component from the EPEC TTSS that is believed to span the periplasmic space connecting EscV and EscC within the inner and outer membranes, respectively. EscJ is a homologue of Ysc J from the TTSS of *Yersinia* (Hueck, 1998) and it also has sequence homology with the N-terminus of FliF from the flagellum. Although EscJ is associated with lipid, either directly or by interaction with EscC or EscV, it represents the only soluble component of the cell-wall-associated TTSS apparatus.

Methods

EscJ sequence 21–190 (170 residues, minus N-terminal signal peptide (Hueck, 1998)) was cloned into plasmid pET-28a (6 × His-Tag) and expressed in BL21 DE3 cells. Cultures were grown at 37 °C, until OD reached ~0.5. EscJ expression was induced with a final concentration of 0.4 mM IPTG, and growth at 30 °C for 4 h. Labelling with ^{13}C and ^{15}N was obtained by expressing EscJ in minimal media supplemented with 0.7 g/l [^{15}N]NH_4Cl and 2g/l [^{13}C]D-glucose. Perdeuteration with $^{13}C/^{15}N$ labelling was achieved by expressing EscJ in triple-labelled medium OD1 CDN (Silantes).

EscJ was purified by affinity chromatography, utilising the Co^{2+} TALON resin (Becton–Dickinson). For NMR, the pooled fractions were buffer exchanged with sodium phosphate buffer (pH 7.2) and concentrated to a final protein concentration of ~250 μM.

All experiments were carried out at 303 K. Triple resonance experiments included the HNCA, HN(CO)CA, CBCA(CO)NH, HNCACB, HNCO and HN(CA)CO (reviewed in Sattler et al., 1999). Most of the above were recorded in their deuterated-optimised form (Kay et al., 1994; Yamazaki et al., 1994a, b; Shan et al., 1996) on deuterated

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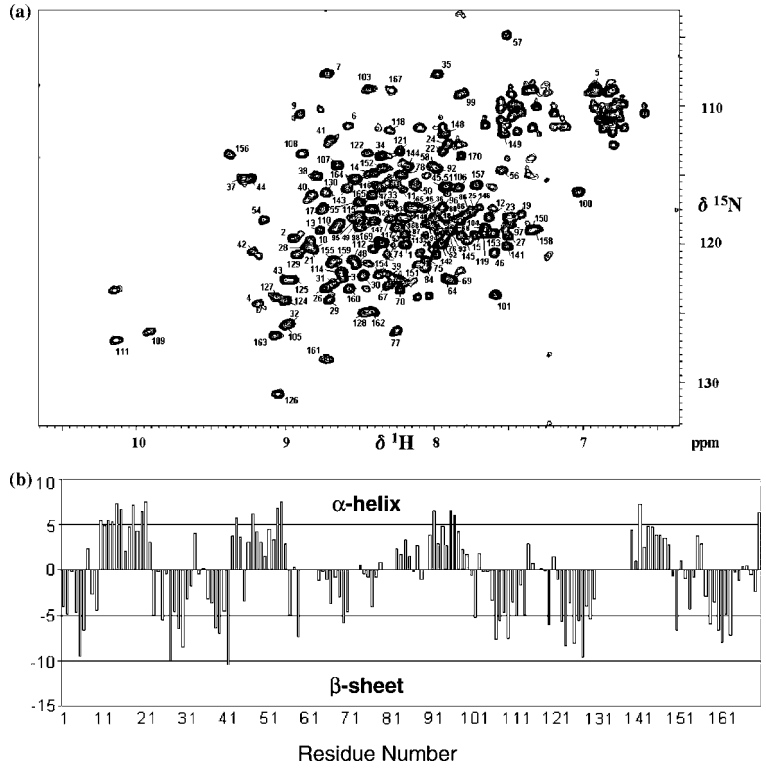

Figure 1. (a) Assigned ^{1}H–^{15}N HSQC NMR spectra of the EscJ. Sequential assignments of the backbone amides are indicated. (b) Consensus plot of the deviations from random coil $^{1}H_{\alpha}$, $^{13}C_{\alpha}$, $^{13}C_{\beta}$and $^{13}C'$ chemical shifts.

EscJ to complete backbone resonance assignment. Subsequently, the HBHA(CBCACO)NH and HCCH-TOCSY (reviewed in Sattler et al., 1999) recorded on the non-deuterated sample facilitated the assignment of Hα and Hβ.

Extent of assignments and data deposition

Despite the unusually low number of aromatic residues (3% in total) and the associated poor chemical shift dispersion, >90% of ^{1}H, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$, ^{13}CO and $^{1}H\alpha/\beta$ resonances could be assigned using the triple labelled $^{2}H/^{13}C/^{15}N$ sample. The presence of conformational exchange within regions spanning 60–62 and 80–81, and a paucity of NOEs between the N-terminal and C-terminal halves of EscJ suggest the presence of two domains connected by a linker between residues 55 and 85. The consensus plot of chemical shift deviations from random coil values indicates that the linker region possesses no regular secondary structure and each domain is composed a mixed α/β topology (Figure 1b). The assignments have been deposited in the BioMagResBank (BMRB) at Madison, WI, U.S.A., with accession number 6252.

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