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Letter to the Editor: Backbone ^{1}H, ^{13}C and ^{15}N assignments of the 25 kDa SPRY domain-containing SOCS box protein 2 (SSB-2)

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Biological context

The suppressors of cytokine signalling (SOCS) family of proteins contain a N-terminal protein–protein interaction domain followed by a conserved 40-residue motif known as the SOCS box (Hilton et al., 1998). The SOCS box mediates proteolytic degradation via recruitment of an E3 ubiquitin ligase complex (Alexander and Hilton, 2004), while the domains N-terminal to the SOCS box are thought to determine the targets for ubiquitination and subsequent degradation (Kile et al., 2002). SSB-2 (SPRY domain-containing *S*OCS box protein 2) is one of a sub-family of four proteins composed of a C-terminal SOCS box, a central domain known as the SPRY domain, and a variable N-terminal region (Hilton et al., 1998).

The SPRY domain is prevalent in many genomes, with over 130 proteins known to contain this domain in man and at least five in yeast. It was originally discovered in *SP*la and *RY*anodine receptors (Ponting et al., 1997), but its function was not defined. Since then it has been shown to function by mediating protein–protein interactions, as for the SPRY domain of RanBPM, which specifically binds regions of the androgen receptor (Rao et al., 2002). Although over 450 distinct proteins contain a SPRY domain, the structure of this domain has yet to be solved. The SPRY domain represents a unique domain with low sequence similarity to other known protein domains as determined by amino acid sequence alignment (www.ncbi.nim.nih.gov/BLAST). On the other hand, studies based on theoretical secondary structure suggest that the SPRY domain may have an immunoglobulin-like fold (Seto et al., 1999). No entries were found in the BioMagResBank (www.bmrb.wisc.edu) database with any significant sequence similarity to the SSB-2 protein. With such a large number of proteins utilising this conserved domain, it is anticipated that structural analysis will help us understand not only the mode of action of SSB proteins, but also SPRY domain-mediated interactions in general.

Methods and experiments

The SSB-2 construct used in this study included almost all the native sequence of mouse SSB-2 except for the SOCS box and the first 11 residues (residues 12–224, SWISS-PROT accession number O88838). This sequence, together with six residues at the N-terminus (GSSARQ, numbered 6–11) and seven at the C-terminus (TRRIHRD, numbered 225–231), both originating from the vector, gave a construct of 226 residues in total. This was expressed as a GST fusion protein in BL21 (DE3) *Escherichia coli*. Bacteria were grown in M9 minimal media and supplemented with either ^{15}N NH_4Cl (99%, 1 g l^{-1}) and/or $^{13}C_6$-glucose (99%, 4 g l^{-1}). 70% $^{2}H_2O$ was used in the M9 medium to produce random fractionally deuterated protein. The GST fusion protein was purified from clarified cell lysates using Glutathione Sepharose 4B (Amersham Biosciences), then cleaved *in situ* using biotinylated thrombin (Pierce). Thrombin was removed using Immobilized Streptavidin (Pierce). The cleaved protein was then concentrated and further purified by gel filtration using a Superdex 200 column (Amersham Biosciences). Samples of SSB-2 for NMR included uniformly ^{15}N-labelled (0.4 mM), uniformly ^{13}C, ^{15}N-enriched (0.6 mM) and uniformly ^{13}C, ^{15}N and random ^{2}H-labelled (1.0 mM)

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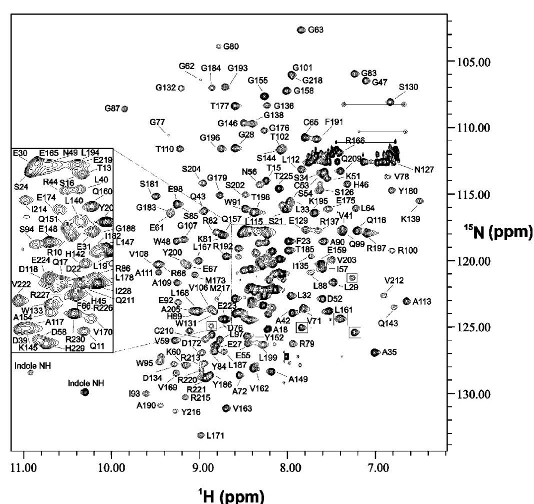

Figure 1. 2D ^{1}H–^{15}N HSQC spectrum of the SPRY domain-containing protein SSB-2 recorded using Bruker Avance500 spectrometer with a cryoprobe at 22 °C. The spectrum was acquired from uniformly ^{15}N labelled protein (0.4 mM, pH 7.0) with a data matrix of 2048 × 256 and 8 scans per t_1 increment in ^{15}N dimension. Folded resonances arising from Arg side-chains are indicated in square boxes.

proteins in H_2O containing 5% ^2H_2O, 10 mM sodium phosphate, 50 mM sodium chloride, 2 mM EDTA and 0.02% (w/v) sodium azide at pH 7.0. Shigemi NMR tubes (catalogue no BMS-005B, Shigemi Inc.) were used for both double- and triple-labelled samples.

Spectra were recorded largely at 22 °C on a Bruker Avance500 spectrometer using a cryoprobe. The ^{1}H chemical shifts were referenced indirectly to TMS at 0 ppm via the H_2O signal, and the ^{13}C and ^{15}N chemical shifts were referenced indirectly using absolute frequency ratios (Wishart et al., 1995). NMR data were processed in XWINNMR (Version 3.5, Bruker) and analysed using XEASY software (Version 1.3; Bartels et al., 1995). Backbone assignments were achieved largely from standard triple resonance HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB and CBCA(CO)NH spectra (Sattler et al., 1999), while a 3D ^{15}N NOESY–HSQC spectrum with a mixing time of 120 ms was also used to identify NH(i)–NH(i + 1) through-space nuclear Overhauser enhancement connectivities.

Extent of assignments and data deposition

A ^{1}H–^{15}N HSQC spectrum recorded on the ^{15}N-labelled sample is shown in Figure 1. Backbone amide 1H, ^{15}N resonances of SSB-2 (12–224) were assigned for 178 out of 197 non-proline residues. Backbone resonance assignments were not obtained for residues S12, R68–R69, Q73–T75, H103–A104, H119–G125, S128, V206–G208 due to spectral overlap or peak absence. Missing peaks are presumed to be from residues in intermediate conformational exchange and consequently broadened beyond detection. Assignments for ^{13}C', $^{13}C^{\alpha}$ and $13C^{\beta}$ of SSB-2 were obtained for 208, 204 and 164 residues, respectively. Backbone assignments were also made for vector-derived residues ARQ and TRRIHR at the N- and C-termini, respectively. From the assigned backbone chemical shifts, one α-helix and nine β-strands were identified for SSB-2 (12–224) using PsiCSI (Hung and Samudrala, 2003). The α-helix and first two β-strands are located in the N-terminus of SSB-2, with the remaining seven β-strands in the SPRY domain, which agrees moderately well with the previous prediction of an immunoglobulin-like fold containing up to 10 β-strands (Seto et al., 1999). The backbone chemical shifts of SSB-2 have been deposited in the BioMagResBank (www.bmrb.wisc.edu) under accession number BMRB-6311.

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