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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignment of the human Spred2 EVH1 domain

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Biological context

EVH1 (*Drosophila Enabled* (Ena)/Vasodilator-stimulated phosphoprotein (VASP) homology 1) domains are protein interaction modules which target their multi-domain host proteins to specific sites of action where they are involved in regulating cellular processes as diverse as cytoskeletal re-organisation, synaptic transmission, proliferation and differentiation. Based on sequence conservation, four different subclasses of EVH1/WH1 domains have been identified. High resolution structures of six representatives from the three major classes comprising the cytoskeletal Ena/VASP proteins, the synaptic terminal Homer/Vesl proteins and the Wiskott-Aldrich syndrome (WAS) proteins have been solved (for reviews see Ball et al. (2002); Callebaut et al. (1998)). However, no structural information on the fourth, less closely related class comprising the *Drosophila* AE33 and the mammalian Spred proteins is yet available. Whereas the *Drosophila* gene was cloned some time ago as a probable transcription factor target regulating photoreceptor cell development in the fly, Spred proteins (Sprouty-related EVH1 domain-containing protein) have only recently been described as negative regulators of growth-factor induced mitogen-activated protein kinase (MAPK) pathways (Wakioka et al., 2001).

Three paralogues of the Spred protein have been identified in mouse and human to date, with Spred2 being the most ubiquitously expressed isoform (Kato et al., 2003). They consist of three distinct domains, namely the N-terminal EVH1 domain, a unique KBD (c-Kit kinase binding) domain and a C-terminal SPR (Sprouty related) domain. The cysteine-rich SPR domain involved in membrane localization was first described in the context of Sprouty proteins, which have been shown to be both positive and negative regulators of MAP kinase pathways (for review see, Christofori, 2003). Both EVH1 and SPR domains were found to be necessary for inhibition of the MAP kinase pathway by Spred proteins (Wakioka et al., 2001). The specific function of the Spred2 EVH1 domain is unknown so far and no interaction partner has yet been identified. Here we describe the expression, purification and resonance assignment of the human Spred2 EVH1 domain as a first step towards a detailed molecular understanding of its function in comparison to the other structurally well-characterized members of the EVH1 domain repertoire (Ball et al., 2000).

Methods and experiments

The DNA sequence encoding the human Spred2 (SwissProt accession code Q924S7) EVH1 domain (residues 1-124) was cloned from a human fetal spleen cDNA library (Clontech) into the expression plasmid pGEX-4T-2 (Pharmacia). The domain was expressed as glutathione S-transferase (GST)-fusion protein, induced at A_{600} of 0.5–1.0 using 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) and incubated for a further 4 h before harvesting. Cells were lysed by sonication and the desired protein purified on a glutathione Sepharose 4B column. The GST moiety was cleaved using thrombin protease (Pharmacia), and separated after thrombin inactivation from the protein by a further chromatography step on glutathione Sepharose 4B followed by gelfiltration on a 120 ml Superdex

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75 column (Pharmacia). Samples were then concentrated to ∼2.5 mM in 20 mM KPi (pH 6.0), 50 mM KCl, 0.2 mM NaN_3 using Centriplus-10 concentrators (Amicon). Protein molecular masses and homogeneity of the preparations were determined by mass spectrometry.

Uniformly ^{15}N- and ^{13}C/^{15}N-labelled samples of the Spred2 EVH1 domain were prepared by growing cells either in a MOPS minimal medium (Neidhardt et al., 1974) containing 9.5 mM $^{15}NH_4Cl$ or in ^{13}C,^{15}N-labelled complete medium 'E. coli OD CN' (Silantes, München). The samples were purified as described above and concentrated to ∼2.5 mM in the same buffer.

For all spectra, samples were prepared in aqueous buffer containing 20 mM NaPi, 50 mM NaCl and 0.1% NaN_3 at pH 6.0, containing 10% D_2O for the ^{2}H lock. All spectra were recorded at 300.0 K. Spectra were recorded on a Bruker DRX 600 spectrometer equipped with a cryoprobe, except for the ^{13}C-NOESY-HMQC, which was recorded on Bruker DMX 750, equipped with triple axis self shielded gradient coils.

A CBCACONNH and CBCANNH pair of spectra was recorded for backbone assignment. The assignment of the backbone was further facilitated by several amino acid type selective experiments. These were a G(i+1), G(i,i+1), P(i−1), P(i−1,np), P(i+1,np), P(i+1), DNG(i+1) and DNG(i,i+1). For details of these spectra, refer to the literature (Schubert et al., 1999, 2000, 2001a, b). Phi angles were experimentally determined via $^3J_{HnH\alpha}$ experiments.

A ^{15}N-HSQC and ^{15}N-NOESY-HSQC (40 ms mixing time) were recorded for identification of NH-spinsystems, a TOCSY-HSQC (35 ms), HNHA and HNHB were recorded for identification of intraresidue peaks in the ^{15}N-NOESY-HSQC. A ^{13}C-NOESY-HSQC in water and a ^{13}C-NOESY-HMQC in D_2O were recorded. For facilitation of sidechain assignments a CCCONNH and HCCCONNH experiment were recorded. To guide the assignment of aromatic spin systems a 2D-NOESY, 2D-TOCSY and 2D-COSY in 100% D_2O were recorded.

All spectra were processed using Bruker XWIN-NMR and the programs Azara (W. Boucher) and ANSIG (P. Kraulis) (available via anonymous ftp from ftp://www.bio.cam.ac.uk/pub/).

Extent of assignments and data deposition

The construct for the Spred2 EVH1 domain contains the first 124 amino acids of human Spred2 (SwissProt Q924S7), with an additional GS at the N-terminus

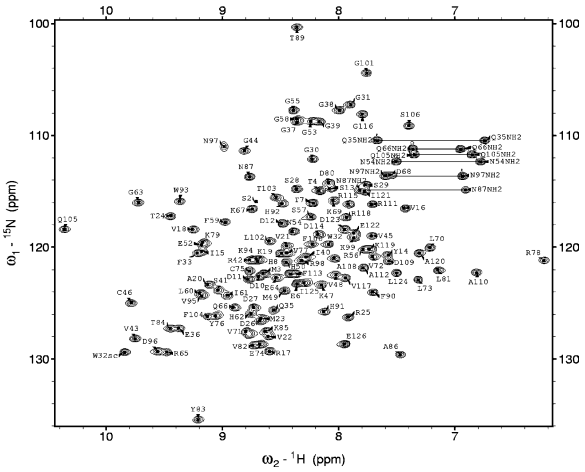

Figure 1. ^{1}H-^{15}N-HSQC spectrum of the human Spred2 EVH1 domain (residue 1-124), recorded at 300 K on a Bruker DRX 600 spectrometer. The total protein concentration was 2.5 mM.

from the thrombin cleavage site. With the exception of residue 1, which is invisible due to broadening, and residue 5, which is overlapped with residue 6 in the HSQC-based experiments, all backbone non-proline residues have been assigned, as depicted in Figure 1. Assignments for Asn and Gln sidechains are also given in the figure. The sidechain assignment is 95% complete. The resonance assignment has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession code 5939.

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