Journal of Biomolecular NMR **27:** 189–190, 2003.

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

Martin Vogtherr, Susanne Grimme, Barbara Pescatore & Harald Schwalbe*
Institut für Organische Chemie und Chemische Biologie, Zentrum für Biomolekulare Magnetische Resonanz, Johann Wolfgang Goethe-Universität, Marie-Curie-Strasse 11, D-60439 Frankfurt/M., Germany

Received 1 April 2003; Accepted 14 May 2003

Key words: NMR assignment, NMR spectroscopy, structural biology

Biological context

VASP (vasodilator-stimulated phosphoprotein) plays an important role in linking signal transduction pathways and actin polymerization. It influences the localized synthesis of actin fibrils in response to protein kinases that activate VASP and is thus indispensable for the coordinated synthesis of the cytoskeleton. This synthesis is a prerequisite for many cellular processes. VASP and its homologues such as the *Drosophila* Ena protein or the mammalian Mena protein share an architecture consisting of three domains. These three domains contain the two homologous C- and N-terminal Ena-VASP homology domains 1 and 2 (EVH1 and EVH2) that are separated by a proline-rich central domain. The proline-rich sequence is a binding motif for SH3 and WW domains essential for targeting kinases that interact with VASP, while the EVH2 domain is responsible for oligomerization. The N-terminal EVH1 domain consists of 115 amino acids. It is a proline-binding peptide recognition module like WW and SH3 domains. It binds to the (E)FPPPPX(D/E)(D/E) sequence (Ball et al., 2000). This epitope occurs in proteins such as vinculin or zyxin, or in the bacterial ActA surface protein (Niebuhr et al., 1997). Its structure has been elucidated both by X-ray crystallography (Fedorov et al., 1999; Prehoda et al., 1999) and by NMR (Ball et al., 2000). The emerging fold, which consists of an incomplete seven-stranded β-barrel and a single α-helix, resembles the fold found in PH, PTB or RanBD domains (Fedorov et al., 1999; Prehoda et al., 1999). Epitope recognition occurs both via the distinct fold of the polyproline helix and electrostatic interactions at the C-terminus of the peptide (Prehoda et al., 1999; Carl et al., 1999; Ball et al., 2000). Although VASP EVH1 has been thoroughly characterized both functionally and structurally, no NMR resonance assignments have been published as a basis for further work on this interesting protein. Here, we report the backbone and C_β assignment of VASP EVH1.

*To whom correspondence should be addressed. E-mail: schwalbe@nmr.uni-frankfurt.de

Methods and experiments

Expression and purification of VASP EVH1

DNA sequence of VASP(1–115) was cloned into pJC40 vector which adds an N-terminal His-tag to the inserted sequence. VASP EVH1 domain was expressed in *E. coli* BL21 (DE3) pLysS, induced at $A_{600} = 0.5$ with 0.4 mM IPTG and incubated for another 4 h before harvesting. The cells were resuspended in buffer A (50 mM sodium phosphate, 140 mm NaCl, 10 mM β-mercaptoethanol, 5 mM Imidazol pH 8.0), lysed by sonication and the supernatant was applied onto a nickel-column (Qiagen). The bound protein was eluted with 500 mM Imidazol in buffer A. The protein containing fractions were pooled, dialysed against 20 mM potassium phosphate, 50 mM KCl, 10 mM DTT pH 6.0 and run on a gelfiltration column (Superdex 75 HiLoad26/60) as a final purification step. For isotope labeling with ^{13}C/^{15}N, cells were grown in 2 l of M9 minimal medium containing 1 g $^{15}NH_4Cl$/l and 2 g ^{13}C-glucose/l.

NMR spectroscopy

VASP EVH1 was assigned using 1 mM [^{13}C, ^{15}N] protein in 20 mM potassium phosphate buffer pH 6.5,

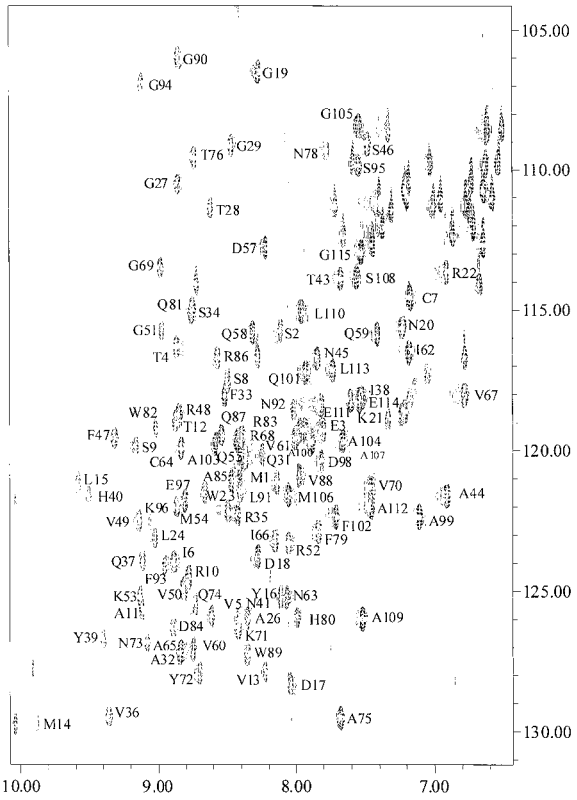

Figure 1. 800 MHz ^{1}H-^{15}N HSQC spectrum of 1 mm VASP EVH1 without histidine tag at 25 °C . Unlabelled cross peaks belong to Gln and Asn side chain amides.

50 mM KCl, 10 mM DTT, 10% D_2O. The construct used for assignment included an N-terminal His-tag (sequence MGHHHHHHASENLYFEGH). However, the HSQC spectra showed identical peaks as compared to a ^{15}N sample lacking the His-tag. All spectra were acquired at 298 K on a Bruker DRX-800 spectrometer. All proton resonances were referenced to internal TSP. Backbone assignments are based on a combination of CBCA(CO)NH, HNCACB, HNCO, HN(CA)CO, and H(CC)(CO)NH (Sattler et al., 1999). Secondary structure elements were identified with a TALOS (Cornilescu et al., 1999) chemical shift prediction using CA, CB, N and HA chemical shifts. The predicted values agree well with the values emerging from the structure (Ball et al., 2000), with 80% of the experimental values within less than 30° deviation from the predicted values. In agreement with the available structures, we find a high degree of β-sheet structure and the C-terminal α-helix. pH-titrations show strong pH-dependent shifts clustered around the semi-conserved basic amino acids His40 and Arg46. Remarkably, these residues are distant from the basic patch around His80 that has been identified as prerequisite for epitope binding (Carl et al., 1999; Ball et al., 2000).

Extent of assignments and data deposition

All amide backbone resonances and all C_α and C_β signals including the prolines of VASP EVH1 were assigned, excluding the histidine tag. Most α and β protons were assigned, excluding the N-terminal proline neighbors, residues Thr12 and Ser34 and the C-terminal Gly115. Ser2, Thr4, Asn20, Thr28, Met54, Asn73, Asn78 α/β protons are only partially assigned, resulting in a 90% assignment of α/β protons. This assignment has been deposited in the BMRB (http://www.bmrb.wisc.edu) database under accession number BMRB-5754.

References

Ball, L.J., Kühne, R., Hoffmann, B., Häfner, A., Schmieder, P., Volkmer-Engert, R., Hof, M., Wahl, M., Schneider-Mergener, J., Walter, U., Oschkinat, H. and Jarchau, T. (2000) *EMBO J.*, **19**, 4903–4914.

Carl, U.D., Pollmann, M., Orr, E., Gertler, F.B., Chakraborty, T. and Wehland, J. (1999) *Curr. Biol.*, **9**, 715–718.

Cornilescu, G., Delaglio, F. and Bax, A. (1999) *J. Biomol. NMR,* **13**, 289–302.

Fedorov, A.A., Fedorov, E., Gertler, F. and Almo, S.C. (1999) *Nat. Strct. Biol.*, **6**, 661–665.

Niebuhr, K., Ebel, F., Frank, R., Reinhard, M., Domann, E., Carl, U.D., Walter, U., Gertler, F.B., Wehland, J. and Chakraborty, T. (1997) *EMBO J.*, **16**, 5433–5444.

Prehoda, K.E., Lee, D.J. and Lim, W.A. (1999) *Cell*, **97**, 471–480.

Sattler, M., Schleucher, J. and Griesinger, C. (1999) *Prog. NMR Spectrosc.*, **34**, 93–158.