Journal of Biomolecular NMR, **17:** 89–90, 2000.
KLUWER/ESCOM
© 2000 *Kluwer Academic Publishers. Printed in the Netherlands.*

Letter to the Editor: Sequence-specific ^{1}H, ^{15}N and ^{13}C resonance assignments of the EEA1 FYVE domain

Tatiana G. Kutateladze & Michael Overduin*
Department of Pharmacology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262, U.S.A.

Received 10 February 2000; Accepted 20 March 2000

Key words: EEA1, FYVE domain, phosphatidylinositol 3-phosphate binding, resonance assignments, zinc binding

Biological context

The FYVE domain is a conserved signaling module that has been found in several mammalian and yeast proteins involved in intracellular membrane trafficking, protein sorting and endocytosis (reviewed in Wurmser et al., 1999). One such protein, the human early endosome antigen 1 (EEA1), is an important component of the vesicle trafficking machinery. It binds endosomal membranes through the interaction of its FYVE domain and phosphatidylinositol 3-phosphate (PtdIns(3)P) (Burd and Emr, 1998). The FYVE domain is approximately 65 amino acids in length and is named for the proteins Fab1, YGLO23, Vps27p and EEA1. The FYVE domain family members share ∼40% sequence identity and require two zinc ions coordinated by eight conserved cysteines. Although the FYVE domain has some structural similarity to the RING finger domain, it differs by the presence of a highly conserved RRHHCR motif, which is required for PtdIns(3)P interaction, and a conserved region N-terminal to the first cysteine. Like the solution structure of the EEA1 FYVE domain (Kutateladze et al., 1999), the crystal structure of the FYVE domain of Vps27p consists of a pair of double stranded antiparallel β-sheets and one C-terminal α-helix (Misra and Hurley, 1999). The FYVE domain forms functional homodimers at millimolar protein concentrations based on translational diffusion coefficients measured by NMR (Kutateladze et al., 1999). Here we report the first ^{1}H, ^{15}N and ^{13}C resonance assignments of a FYVE domain, that of the EEA1 protein.

*To whom correspondence should be addressed. E-mail: Michael.Overduin@UCHSC.edu

Methods and results

The FYVE domain of human EEA1 was expressed as a glutathione S-transferase (GST) fusion protein. A DNA fragment encoding amino acids 1325–1410 of the EEA1 was cloned into a modified pGEX vector (Amersham Pharmacia Biotech). The FYVE domain was expressed in the *E. coli* BL21 pLysS strain by isopropyl β-D-thiogalactopyranoside induction for 7 h. Unlabeled protein was purified from cells grown at 37 °C in Luria-Bertani broth supplemented with vitamins and 50 μM $ZnSO_4$. Uniformly ^{15}N and ^{13}C/^{15}N labeled FYVE was produced in M9 minimal media, also supplemented with $^{15}NH_4Cl$ and $^{13}C_6$-glucose. Harvested cells were disrupted by sonication. The GST fusion protein was immobilized on Glutathione Sepharose 4B (Amersham Pharmacia Biotech) and eluted by cleavage with thrombin (Sigma). The yield of unlabeled and ^{15}N labeled protein was 20 mg/L and that of ^{15}N/^{13}C labeled protein was 5 mg/L. The size of the purified FYVE domain was verified by SDS-PAGE and MALDI mass spectrometry. NMR samples contained 0.5–2 mM of the FYVE domain, 90% H_2O/10% ^2H_2O or 99.9% ^2H_2O, 20 mM Tris-d_{11} buffer (pH 6.8), 200 mM KCl, 20 mM perdeuterated dithiothreitol, 1 mM NaN_3 and 50 μM 4-amidinophenylmethane sulfonyl fluoride.

All NMR experiments were performed at 298 K on Varian INOVA 600 and 500 MHz spectrometers equipped with triple resonance shielded probes with z-axis pulse field gradients. Spectra were processed with the NMRPipe package (Delaglio et al., 1995) and analyzed using PIPP (Garret et al., 1991), NMRDraw, and in-house software programs on Sun Microsystems and Silicon Graphic workstations. Chemical shifts were assigned from homonuclear TOCSY, COSY,

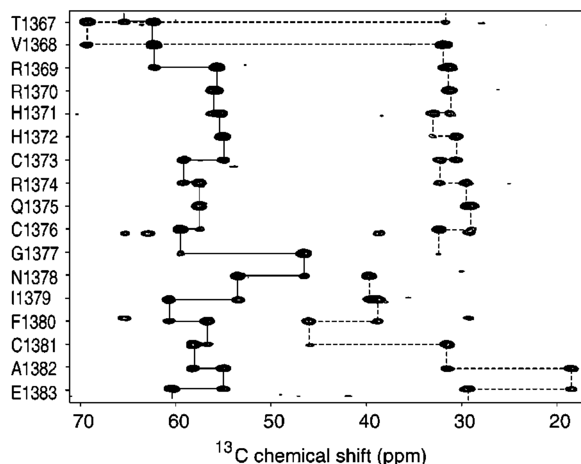

Figure 1. Representative strips from the HNCACB spectrum showing sequential connectivities for Cα (solid line) and Cβ (dotted line) of residues T1367–E1383 of the EEA1 FYVE domain.

heteronuclear ^{15}N-edited DIPSI-HSQC, ^{13}C-edited HCCH-TOCSY, and ^{15}N-edited and ^{13}C-edited HSQC (Kay et al., 1993). Aromatic side chain resonances were assigned using HBCBCGCDHD-TOCSY and HBCBCGCDCEHE-TOCSY experiments (Yamazaki et al., 1993). The HNCACB, CBCA(CO)NH, HNHA, HNCO, HCC-TOCSY and CCC-TOCSY experiments were employed for sequential assignment (Figure 1) (Grzesiek et al., 1993; Muhandiram et al., 1994). Asn and Gln side chain ^{1}H and ^{15}N resonances were assigned using 3D ^{15}N-edited NOESY and 3D CBCA(CO)NH spectra.

Extent of assignments and data deposition

The FYVE domain construct contains two N-terminal residues from the vector, Gly and Ser, and these residues are not included in the assignment statistics. The ^{1}H, ^{15}N and ^{13}C resonances of unstructured residues 1325–1345, N-terminal to the FYVE domain, are assigned. The $^{1}H_N$ and ^{15}N resonances of 83 of 84 possible backbone amides and ^{13}C resonances of 82 of 86 possible backbone carbonyls are assigned. The ^{1}H and ^{13}C resonance assignments for the aromatic side chain of all three phenylalanines, one tryptophan and three histidines and the ^{1}H and ^{15}N resonances for the NH_2 side chains of all 7 Asn and 7 Gln are completed at 100%, 81%, 96% and 100%, respectively. All the Hα, Hβ, Cα and Cβ resonances are assigned. No assignments were made for the labile hydroxyl Hγ of Ser and Thr, the guanidino moiety of Arg, the side chain NH_3^+ of Lys, and the side chain carboxyl group of Asp and Glu. Of the rest, 99% of Hγ, 100% of Hδ and Hϵ, 95% of Cγ and Cδ, and 90% of Cϵ, Cζ, and Cη are assigned. Chemical shift index analysis, together with the NOE patterns in 3D ^{15}N-edited NOESY and ^{13}C-edited NOESY, indicates the presence of two antiparallel double stranded β sheets and one C-terminal α-helix. The chemical shift values of the ^{1}H, ^{15}N and ^{13}C resonances have been deposited at the BioMagResBank under accession number 4579.

Acknowledgements

We thank T. de Beer and J. Enmon for discussions, C. Burd and S. Emr for providing cDNA of the EEA1 FYVE domain, and R. Muhandiram and L.E. Kay for NMR pulse sequences. The NMR Center is supported by the Howard Hughes Medical Institute. This research is funded by the American Cancer Society (ACS) and Pew Scholar's Program in the Biomedical Sciences (M.O.). T.K is the recipient of an ACS Fellowship Award.

References

Burd, C.G. and Emr, S.D. (1998) *Mol. Cell*, **2**, 157–162.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Garret, D.S., Powers, R., Gronenborn, A.M. and Clore, G.M. (1991) *J. Magn. Reson.*, **95**, 214–220.

Grzesiek, S., Anglister, J. and Bax, A. (1993) *J. Magn. Reson.*, **B101**, 114–119.

Kay, L.E., Xu, G.Y., Singer, A.U., Muhandiram, D.R. and Forman-Kay, J.D. (1993) *J. Magn. Reson.*, **B101**, 333–337.

Kutateladze, T.G., Ogburn, K.D., Watson, W.T., de Beer, T., Emr, S.D., Burd, C.G. and Overduin, M. (1999) *Mol. Cell*, **3**, 805–811.

Misra, S. and Hurley, J.H. (1999) *Cell*, **97**, 657–666.

Muhandiram, D.R. and Kay, L.E. (1994) *J. Magn. Reson.*, **B103**, 203–216.

Yamazaki, T., Forman-Kay, J.D. and Kay, L.E. (1993) *J. Am. Chem. Soc.*, **115**, 11054–11055.

Wurmser, A.E., Gary, J.D. and Emr, S.D. (1999) *J. Biol. Chem.*, **274**, 9129–9132.