Journal of Biomolecular NMR, **25:** 253–254, 2003.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the kinase-interacting FHA domain of *Arabidopsis thaliana* kinase-associated protein phosphatase

Gui-in Lee[a], Jia Li[b,**], John C. Walker[b] & Steven R. Van Doren[a,*]
[a]*Department of Biochemistry, 117 Schweitzer Hall, University of Missouri, Columbia, MO 65211, U.S.A.;* [b]*Division of Biological Sciences, 105 Tucker Hall, University of Missouri, Columbia, MO 65211, U.S.A.*

Received 1 November 2002; Accepted 3 December 2002

Key words: FHA domain, kinase-associated protein phosphatase, NMR peak assignments, receptor-like kinase

Biological context

Kinase-associated protein phosphatase (KAPP) is a ubiquitous downstream regulator of receptor-like protein kinase (RLK) signaling pathways in plants (Stone et al., 1994). RLKs play pivotal roles in many developmental processes in plants such as meristem differentiation, hormone perception and abscission (Li et al., 2000). KAPP is composed of three functional domains: An N-terminal membrane anchor, a kinase interaction (KI) domain, and a C-terminal type 2C protein phosphatase (PP2C) (Stone et al., 1994). Activated RLKs autophosphorylate or transphosphorylate serine and threonine residues of their kinase domains. KAPP uses its kinase interaction domain to bind to the phosphorylated kinase domain of a subset of the RLKs. The KI domain of KAPP contains an FHA sequence motif with highly conserved glycine, arginine, serine, histidine and aspargine residues in three short conserved blocks (Hofman, 1995; Li et al., 1999). These residues of KAPP are essential to binding of phosphorylated RLKs (Li et al., 1999). Deletion mutagenesis showed the minimal functional KI domain comprises 119 residues, though a 134 amino acid construct appears to have slightly higher affinity for RLKs (Li et al., 1999). The minimal kinase-interacting FHA domain of Arabidopsis KAPP is here designated KI-FHA. Interest in the molecular mechanism of protein-protein interactions in RLK-dependent signal transduction in plants demands the atomic-resolution structure of the novel KI-FHA domain. Tertiary structures of FHA domains from yeast and humans have been determined by NMR and crystallography (Stavridi, 2002; Zhou, 2000). Very low overall sequence homology, however, precludes homology modeling of KI-FHA from those FHA domain structures. Here, we report expression, purification and the ^{1}H, ^{13}C and ^{15}N resonance assignments of the 134-residue construct of KI-FHA as the first step in studying its solution structure, dynamics and phosphoprotein interactions.

Methods and results

The KI-FHA-encoding sequence from residue 180 to 313 of Arabidopsis KAPP was amplified by PCR using the 5′ primer TCTGGATCC(BamHI)$_{180}$AGTTGG CTGTTCCTGGAAGT and the 3′ primer TCTGAATT C(EcoRI)**TTA**(stop codon)$_{313}$AGCCATGGGATCTG AGGCCAC. The PCR product was subcloned into the plasmid pGEX-6P-1 (Amersham-Pharmacia) at the BamHI and EcoRI restriction sites. The fusion protein was expressed in ^{15}N/^{13}C –labeled M9 minimal medium supplemented with 6% (v/v) ^{15}N/^{13}C labeled Celtone (Spectra Stable Isotopes) at 30 °C for 3–4 h in the *E. coli* DH5α strain after induction with 100–200 μM IPTG. After breakage with a French press, the soluble supernatant was incubated with glutathione agarose beads (Sigma). The N-terminal Glutathione-S-transferase (GST) domain of the GST-KI-FHA fusion protein was cleaved using GST-tagged PreScission™ protease (Amersham-Pharmacia) while the GST-KI-FHA was still bound to the beads. KI-FHA was washed off with a buffer of 50 mM HEPES (pH 7.4) with 150 mM NaCl, 10 mM EDTA, 1 mM DTT and 20 μM PMSF. After cleavage, a five-residue linker of Gly-Pro-Leu-Ser-Ser from the GST tag remained at the N terminus of KI-FHA. Purified KI-FHA was dialyzed and concentrated to 0.6 mM in 20 mM sodium phosphate pH 6.3 with 120 mM NaCl and 7% D_2O.

The NMR experiments were performed at 22 °C with a Bruker DRX-500 NMR spectrometer equipped

*To whom correspondence should be addressed. E-mail: vandorens@missouri.edu
**Current address: Department of Botany and Microbiology, George Lynn Cross Hall, 770 Van Vleet Oval, University of Oklahoma, Norman, Oklahoma, 73019, U.S.A.

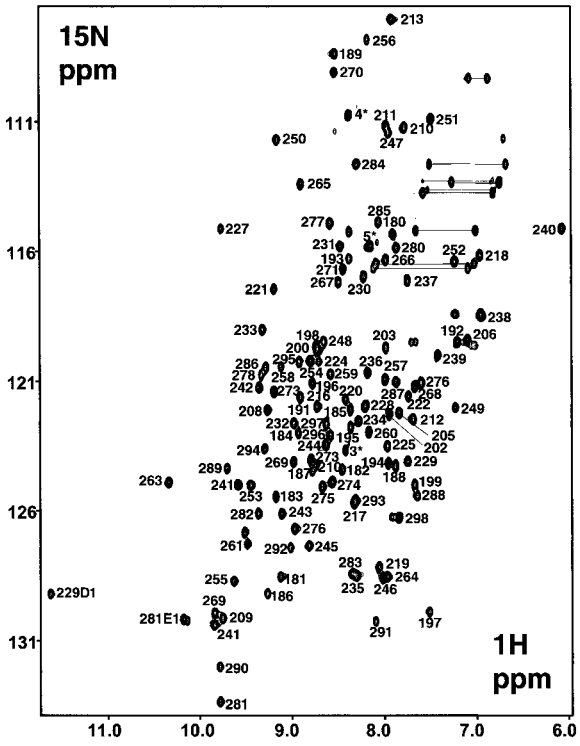

Figure 1. The 600 MHz ^{1}H-^{15}N HSQC spectrum of KI-FHA at 295 K. Peak assignments are annotated with the residue numbering used for KAPP. Peaks labeled with 3* through 5* are from the linker of five foreign residues at the N-terminus of KI-FHA.

with an 8 mm triple resonance probe with shielded z-gradient coil (Nalorac) or with a Varian INOVA 600 with 5 mm triple resonance probe with shielded xyz gradient coils. Quadrature detection in the indirect dimension was obtained using States-TPPI. SYBYL TRIAD (Tripos) was used for processing and interpreting NMR spectra. The backbone assignments are obtained based on the triple resonance spectra including 3D HNCA, (HA)CA(CO)NH, HN(CA)HA, HA(CACO)NH, HNCO, and HACACO(Bax and Grzesiek, 1993), along with ^{15}N-sperated NOESY-HSQC. Aliphatic side chain assignments were achieved using (H)CCA(CO)NH, CBCA(CO)NH, HCCH-TOCSY (Kay et al., 1993). Aromatic assignments were derived from HCCH-TOCSY combined with ^{13}C-separated NOESY-HSMQC (Van Doren and Zuiderweg, 1994). Stereospecific valine and leucine methyl assignments were obtained as described (Neri et al., 1989).

Extent of assignments and data deposition

Backbone resonances were 98.6% assigned and resonances of protonatable side chain groups were 96.3% assigned for the structured region of KI-FHA from residue 180 to 298. The assignments missing were NH of Ser204, Ser214, NH of Ser226, δ1 of Trp241, δ1 and ε2 of His196, as well as δ1, δ2 and ε2 of both His257 and His261. The 15 residues beyond Phe299 appear to be an unstructured linker region, between KI-FHA and PP2C domains of KAPP, with most backbone amide peaks missing, though the side chains of Gln300, Ile301, Lys304, Gly307, and Val308 were completely assigned. The N-terminal linker of five foreign residues was completely assigned except for Gly1. Considering all 134 residues of KI-FHA plus the N-terminal linker of five foreign residues, 90.9% of the backbone resonances and 91.2% of the resonances of protonatable side chain groups were obtained. The resonance assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5564.

Acknowledgements

This research was supported by NSF grants MCB0111589 to S.R. V.D. and MCB98098848 and MCB0112278 to J.C. W. The Varian Inova 600 and Bruker DRX-500 spectrometers were funded in part by NSF grants DBI0070359 and CHE8908304, respectively.

References

Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 132–138.

Hofman, K. and Bucher, P. (1995) *Trends Biochem. Sci.*, **20**, 347–349.

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

Li, J., Lee, G., Van Doren, S.R. and Walker, J.C. (2000) *J. Cell Sci.*, **113**, 4143–4149.

Li, J., Smith, G.P. and Walker, J.C. (1999) *Proc. Natl. Acad. Sci., USA*, **96**, 7821–7826.

Neri, D., Szyperski, T., Otting, G., Senn, H. and Wüthrich, K. (1989) *Biochemistry*, **28**, 7510–7516.

Stavridi, E.S., Huyen, Y., Loreto, I.R., Scolnick, D.M., Halazonetis, T.D., Pavletich, N.P., and Jeffrey, P.D. (2002) *Structure*, **10**, 891–899.

Stone, J.M., Colinge, M.A., Smith, R.D., Horn, M.A. and Walker, J.C. (1994) *Science*, **266**, 793–795.

Van Doren, S.R. and Zuiderweg, E.R.P. (1994) *J. Magn. Reson.*, **B104**, 193–198.

Zhou, M.-M. (2000) *Nat. Struct. Biol.*, **7**, 1085–1087.