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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N chemical shift backbone NMR assignment and secondary structure of the *Arabidopsis thaliana* PIN1At protein

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Biological context

Peptidyl prolyl *cis*/*trans* isomerases are enzymes that catalyze the *cis*/*trans* isomerization of the peptide bond preceding proline and are divided in three structurally distinct classes: cyclophilins, FK506 binding proteins and parvulins. The parvulin class comprises a number of eukaryotic enzymes that were shown to be essential for cell growth, like the PIN1 from human (Lu et al., 1996). These proteins contain two domains, a 100-residue C-terminal catalytic domain and a small N-terminal WW domain. The catalysis is more efficient for the bond following a phosphoserine or phosphothreonine (Ranganathan et al., 1997; Yaffe et al., 1997). The WW domain of human PIN1 also binds phosphorylated peptides through interaction with phosphoserine and phosphothreonine (Lu et al., 1999). Both site-specific catalytic activity and phosphoserine/phosphothreonine binding are essential for PIN1 biological activity (Lu et al., 1999).

We have initiated NMR studies of PIN1At from *Arabidopsis thaliana.* The 119 amino acid PIN1At is 53% identical to human PIN1 in the catalytic domain, but lacks the WW protein–protein interaction domain. We showed by 2D NMR exchange spectroscopy that PIN1At is a prolyl *cis*/*trans* isomerase with specificity for phosphoserine/proline bonds (Landrieu et al., 2000). The 3D solution structure of PIN1At will be used to better understand the interactions with a phosphorylated peptide substrate and the structural aspects of the lacking WW. Here, we present near complete ^{1}H, ^{13}C and ^{15}N assignment and the secondary structure of PIN1At in solution.

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Methods and results

Expression and purification of PIN1At were described previously (Landrieu et al., 2000). [^{15}N]- and [^{15}N,^{13}C]-labeled proteins were prepared by growing cells in M9 medium with [^{15}N] ammonium chloride (1 g/l) and [^{13}C] glucose (2 g/l) (Cambridge Isotopes, Cambridge, MA), as sole nitrogen and carbon sources.

NMR samples contained 0.6–1 mM [^{15}N]- or [^{13}C,^{15}N]-labeled PIN1At protein in 50 mM deuterated Tris-HCl pH 6.3 (Cambridge Isotopes), 100 mM NaCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride and 0.5 mM EDTA in 90% H_2O/10% D_2O or 100% D_2O. All NMR spectra were recorded at 20 °C using a DMX 600 MHz spectrometer (Bruker, Karlsruhe) with triple-resonance probe with actively shielded z-gradient. The sequence-specific backbone assignment was based on 3D CBCANH, 3D CBCA(CO)NH, 3D HNCA, 3D HN(CO)CA, HNCO and HN(CA)CO. Hα and Hβ were assigned using ^{15}N-edited TOCSY-HSQC, ^{15}N-edited NOESY-HSQC and

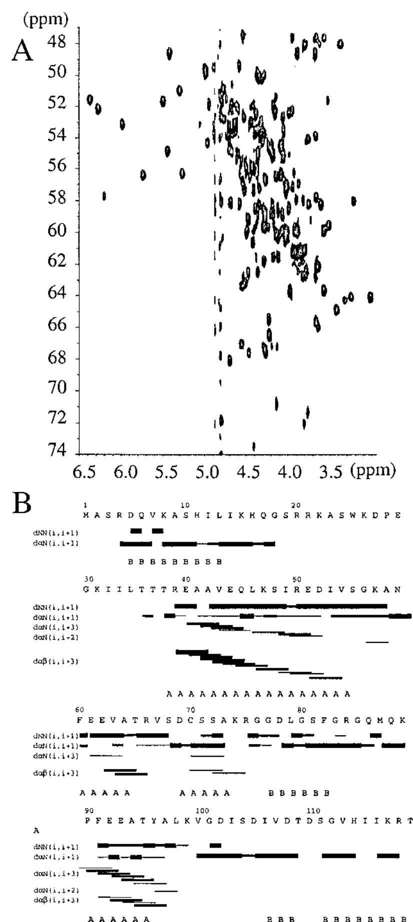

Figure 1. (A) 800 MHz ^{1}H-^{13}C HSQC spectrum of PIN1At in D_2O, showing the Hα-Cα region. (B) Amino acid sequence of PIN1At, short and medium range NOEs and secondary structure predicted from chemical shift indices (CSI) analysis based on ^{13}Cα, ^{13}CO and Hα chemical shifts. Positions of α and β secondary structures are shown at the bottom as letters A and B, respectively.

3D HBHA(CO)NH. Short and medium range NOE interactions were observed from a ^{15}N-resolved [^{1}H,^{1}H] NOESY experiment (τ_m = 150 ms) and 3D ^{13}C-resolved [^{1}H,^{1}H] NOESY spectrum (τ_m = 150 ms). The latter 3D ^{13}C-resolved [^{1}H,^{1}H] NOESY spectrum was also recorded at 800 MHz. Details of 3D pulse sequences can be found in Grzesiek et al. (1997). Chemical shifts were measured relative to TMSP for ^{1}H. Data were transformed and processed using SNARF (F. Van Hoesel, Groningen).

The secondary structure elements were identified by qualitative interpretation of the short and medium range NOEs and chemical shift differences from random coil values for ^{13}Cα, ^{13}CO and Hα (Wishart et al., 1994). A summary of the observed short and medium range NOEs and of the secondary structure elements identified in PIN1At is presented in Figure 1B. The characterized secondary structure of PIN1At is similar to the secondary structure observed in the crystal structure of human PIN1 (Ranganathan et al., 1997).

Extent of assignments and data deposition

Backbone sequence-specific assignment is completed for all ^{13}Cα, ^{1}Hα, ^{13}CO, ^{1}H^{N} and ^{15}N nuclei, except for the residues located between Ser20 and Thr35, that we suppose to be located on a flexibly disordered loop exposed to the solvent, and ^{13}CO resonances for residues Thr36, Ser71 and Thr95.

Full assignment of side chain resonances (except NH_2 and OH) was obtained for 76 residues and the majority of the resonances in the remaining residues.

A table of the ^{1}H, ^{15}N and ^{13}C chemical shifts has been deposited in the BioMagResBank under accession number BMRB-4670.

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References

Grzesiek, S., Bax, A., Hu, J.S., Kaufman, J., Palmer, I., Stahl, S.J., Tjandra, N. and Wingfield, P.T. (1997) *Protein Sci.*, **6**, 1248–1263.

Landrieu, I., De Veyder, L., Fruchart, J.-S., Casteels, P., Portetelle, D., Inzé, D. and Lippens, G. (2000) *J. Biol. Chem.*, **275**, 10577–10581.

Lu, K., Hanes, S. and Hunter, T. (1996) *Nature*, **380**, 544–547.

Lu, P., Zhou, X., Shen, M. and Lu, K. (1999) *Science*, **283**, 1325–1328.

Ranganathan, R., Lu, K., Hunter, T. and Noel, J. (1997) *Cell*, **89**, 875–886.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.

Yaffe, M., Schutkowski, M., Shen, M., Zhou, X., Stukenberg, P., Rahfeld, J., Xu, J., Kuang, J., Kirschner, M., Fischer, G., Cantley, L. and Lu, K. (1997) *Science*, **278**, 1957–1960.