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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignment of the peptidyl-prolyl cis-trans isomerase Pin1

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Abbreviations: WW, Trp-Trp domain; SH, Src homology; FKBP, FK506-binding protein; NOE, nuclear Overhauser effect, NOESY, nuclear Overhauser effect spectroscopy; pSer/pThr, phosphoserine/phosphothreonine.

Biological context

The peptidyl-prolyl cis-trans isomerase Pin1 regulates entry into the mitotic cell cycle and is involved in interactions with numerous mitosis-specific phosphoproteins, including for example the kinases Cdc2, Cdk2, Wee1 and Myt1 as well as the phosphatases Cdc25 and PP2A (Yaffe et al., 1997; Wintjens et al., 2001). Inhibition of Pin1 induces mitotic arrest and apoptosis, hence Pin1 is a potential target for cancer therapy (Göthel and Marahiel, 1998).

The 18.4 kDa (163-residue) monomeric protein consists of two domains connected by a flexible linker. The N-terminal WW-domain ($Pin1_{WW}$) is a small (40-residue) conserved module with properties similar to SH2 and SH3 domains. These modules are known to bind specific sequences in target proteins and recruit proteins into signaling complexes. $Pin1_{WW}$ binds preferentially to proteins containing Ser/Thr-Pro motifs in which the serine or the threonine residues are phosphorylated (Lu et al., 1999).

Structurally, the C-terminal catalytic PPIases domain ($Pin1_{CAT}$) belongs to the parvulin family of PPIases. $Pin1_{CAT}$ catalyzes phosphorylation dependent prolyl-peptide bond isomerization in pSer-Pro segments such as those found in the phosphorylated C-terminal domain of RNA polymerase II.

The crystal structures of Pin1 (Ranganathan et al., 1997; Verdecia et al., 2000) and the solution structure of $Pin1_{WW}$ (Wintjens et al., 2001; Kowalski et al., 2002) reveal global similarities of $Pin1_{CAT}$ with the FKBP-like class of PPIase and of $Pin1_{WW}$ with other WW domains.

Although the structural and functional properties of Pin1 and its two domains are already well characterized, little is known about the interplay between these two domains. Herein, we report the backbone and C^{β} resonance assignment of Pin1, as a step towards a better understanding of Pin1's functional role as a mitotic regulator.

Methods and experiments

Expression and purification of Pin1

Recombinant human Pin1 and its domains were expressed in *Escherichia Coli* strain BL21(DE 3) Codon Plus (RIL) (Novagen) transformed with the plasmid pKM-Pin1 (residues 1–163), pKM-$Pin1_{WW}$ (residues 1–54), and pKM-$Pin1_{CAT}$ (residues 44–163). These constructs contain the wild type or deletion mutant Pin1 gene preceded by a 6-histidine tag, a glutathione *S*-transferase protein (GST) and a Tobacco etch virus (TEV) cleavage site. The expression plasmids were constructed by ligation of the vector pKM263 (Melcher, 2000) with different regions/domains of the amplified Pin1 gene.

To produce ^{13}C/^{15}N-labelled protein, cells were grown in minimal M9 media using $^{15}NH_4Cl$ (Cambridge Isotope Labs, Andover, MA) and uniform ^{13}C-glucose (2 g l^{-1}). Bacterial cultures were grown up to OD_{600} = 0.6, then supplemented with IPTG,

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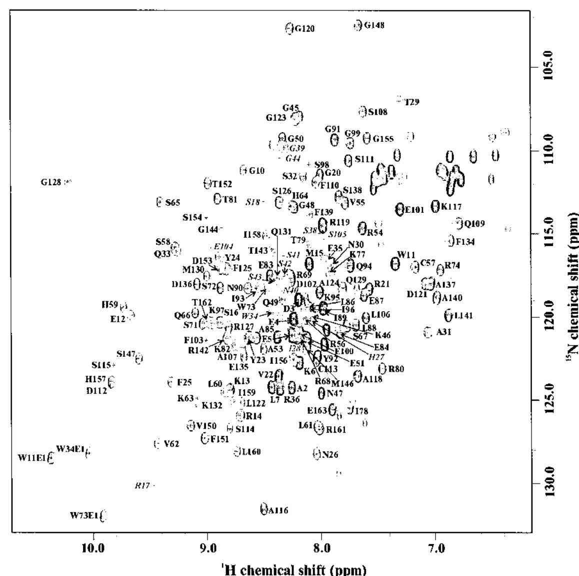

Figure 1. ^{1}H-^{15}N HSQC spectrum at 25 °C and at 800 MHz of 1.5 mM Pin1, pH 6.6. Unlabelled cross peaks correspond either to side-chain amides (Gln, Asn) or to the histidine tag. Correlation peaks that were assigned using spectra recorded on either Pin1$_{WW}$ or Pin1$_{CAT}$ domains are italicized.

and subsequently incubated at 25 °C for 4 h. Purification and cleavage of the GST fusion proteins were done as published by Melcher (2000) with minor modifications.

NMR spectroscopy

The backbone of Pin1 was assigned using three different constructs, namely Pin1, Pin1$_{WW}$ and Pin1$_{CAT}$. Each sample contained 1.5 mM [^{13}C, ^{15}N] protein in phosphate buffer pH 6.6 with 100 mM Na_2SO_4, 1 mM DTT, 5 mM EDTA, 0.03% NaN_3 and 10 % D_2O. Spectra were acquired at 298 K on Bruker DRX-600 and DRX-800 spectrometers. All chemical shifts were referenced to internal DSS, either directly (^{1}H) or indirectly (^{13}C, ^{15}N) (Markley et al., 1998). Backbone assignments rely mainly on the combination of data obtained from ^{1}H-^{15}N HSQC (Figure 1), CBCA(CO)NH, HNCACB, HNCO, HN(CA)CO, and HCC(CO)NH (Sattler et al., 1999). The final backbone resonance assignment was confirmed using a 3D ^{15}N-edited NOESY-HSQC spectrum (mixing time: 100 ms). Secondary structure elements were identified by backbone NOE contacts and the deviation of C^{α} and C^{β} chemical shift values from their random coil values. In agreement with the crystal structures, we find seven strands and four helices: Trp11-Met15 (β1), Val22-Asn26 (β2), Ser32-Gln33 (β3), Val55-Val62 (β4), Lys82-Ser98 (α1) Phe103-Phe110 (α2), Ser114-Ala118(α3), Asp121-Phe125(β5), Lys132-Ala140(α4), Val150-Phe151 (β6), Ile156-Glu163 (β7). The interstrand NOE's are constistent with two antiparallel β-sheets organized as β1β2β3 and β5β4β7β6.

Extent of assignments and data deposition

All amide backbone resonances of Pin1 were assigned, except for the histidine tag and for the residues Ser 19, Gln 75, Glu 76 and Glu 145. The amide resonances of Arg 17, Ser 18, His 27, Ile 28, Trp 34, Glu 104 and Ser 105 were absent in all spectra recorded on Pin1. However, these residues could be assigned from spectra recorded either on Pin1$_{WW}$ or on Pin$_{CAT}$. Resonances originating from residues Ser 38-Gly 44 of the linker sequence are broadened in the Pin1 spectra. Fortunately, these residues could be assigned using the spectra of Pin1$_{WW}$. Hence, a 97% complete resonance assignment of the H^N, N, C^{α}, H^{α} C^{β}, C′ shifts was obtained and has been deposited in the BMRB (http://www.bmrb.wisc.edu) database under accession number BMRB 5305.

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