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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C chemical shift assignments of the Pleckstrin Homology domain of the Human Protein Kinase B (PKB/Akt)

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Biological context

Protein kinase (PKB), also named Akt (Staal, 1987), is a 57–60 kDa serine/threonine protein kinase that has emerged as a crucial regulator of widely divergent cellular processes including apoptosis, proliferation, differentiation and metabolism (Nicholson and Anderson, 2002). PKB is activated in a pathway induced by the lipid products of the phosphoinositide 3-kinase (PI3K) that bind to the N-terminal Pleckstrin Homology (PH) domain of the enzyme, thus recruiting the kinase to the plasma membrane where it is phosphorylated. It was previously shown that the PH domain of PKB interacts also with the TCL1/MTCP1 family of proto-oncogene (the proteins $p14^{TCL1}$ and $p13^{MTCP1}$, respectively), mediating the formation of oligomeric TCL1/MTCP1-PKB high molecular weight protein complexes *in vivo* (Laine et al., 2000; Künstle et al., 2002; French et al., 2002). Within these complexes, PKB is preferentially phosphorylated and activated. The structures of $p14^{TCL1}$ (Hoh et al., 1998), of $p13^{MTCP1}$ (Yang et al., 1998), and recently, the crystal structure of the isoform PKBα-PH (Thomas et al., 1998) have been determined. Here we report ^{1}H, ^{15}N and ^{13}C resonance assignments for PKBβ-PH (73% homology) in the presence of Ins(1,4,5)P_3, a first step toward obtaining the solution structure as well as information on the interaction between PKB-PH and the TCL1/MTCP1 proto-oncogenes using chemical shift mapping studies.

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Methods and experiments

Protein expression and purification

The cDNA encoding for the 111 residues of the human PKBβ/Akt2 PH domain was sub-cloned into the *Eco*RI/*Bam*HI sites of the pGEX-2T plasmid (Amersham Biosciences). The construct was transformed for over-expression in *Escherichia coli* BL21 strain. Uniform ^{15}N and $^{15}N/^{13}C$ labelling was obtained by growing cells in ECPM1 medium containing $^{15}NH_4Cl$ and/or $^{13}C6$-glucose as the sole nitrogen and carbon sources. Protein expression was induced for 2 hours by addition of 0.5 mM IPTG. The cells were then harvested by centrifugation, and the pellets were sonicated in a lysis buffer. The supernatant was applied onto a Glutathione-Sepharose (Amersham Biosciences) column. After washing, the fusion protein was cleaved overnight at 4 °C by adding bovine thrombin (Sigma Aldrich) to the beads in the low salt buffer (50 mM Tris/HCl pH 7.4, 300 mM NaCl, 2.5 mM EDTA). The cleaved protein was further purified using size exclusion chromatography with an Sephadex-HR100 column (Amersham Biosciences).

NMR spectroscopy

NMR samples were 0.3–0.4 mM PKBβ-PH in a buffer containing 10 mM Tris/HCl pH 7.4, 300 mM NaCl, 0.1 mM EDTA, 0.1 mM benzamidine and 4 mM Ins (1,4,5)P_3 as stabilizing reagent (5% D_2O for the lock). All NMR experiments were performed at 13 °C on Bruker AVANCE 500 MHz and 600 MHz equipped with 5 mm *z*-gradient ^{1}H-^{13}C-^{15}N. ^{1}H chem-

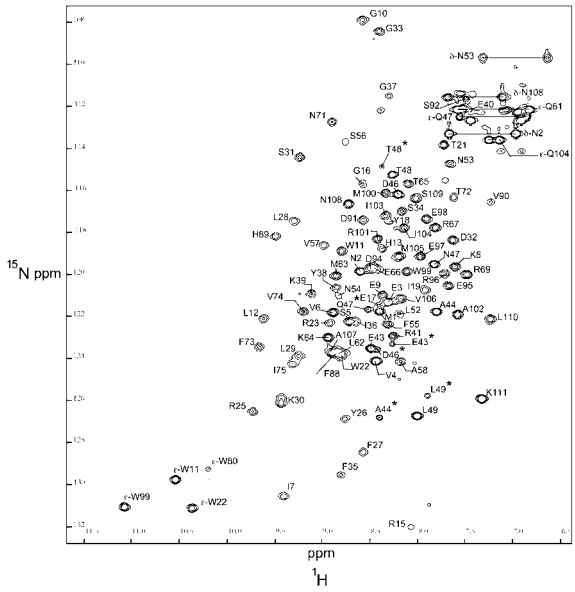

Figure 1. ^{1}H-^{15}N HSQC spectrum of the PKBβ PH domain recorded at 13 °C. The assignment of peaks is indicated with their one-letter amino acid and number. Peaks belonging to the 'minor form' are indicated with an asterisk.

ical shifts were directly referenced to the resonance of cryogenic or standard probe, respectively DSS, while ^{13}C and ^{15}N chemical shifts were indirectly referenced. The following spectra were used for the ^{1}H, ^{15}N, ^{13}Cα and ^{13}Cβ and ^{13}CO resonance assignments: ^{1}H-^{15}N HSQC, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, essentially used for sequential assignments, and H(CC)H-TOCSY, ^{15}N- and/or ^{13}C-edited NOESY and TOCSY experiments, essentially used for side-chains assignments. All NMR experiments were processed with Gifa (Pons et al., 1996).

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC spectrum of the PKBβ PH domain is shown in Figure 1. About 85% of the expected backbone resonances, and 77% of the side-chain resonances have been assigned. Sequential assignment failed for two segments (Glu^{59}-Gln^{61} and Arg^{76}-Thr^{87}: ≈ 15% of the backbone resonances) linked by a disulfide bond (Cys^{60}-Cys^{77}), probably due to local conformational exchange on an intermediate timescale arising from the bridge isomerization. In addition, the residues belonging to the segment Pro^{42}-Pro^{51} exhibited 'minor form' peaks, ranging in intensity from 15–25% of the 'major form' peak. Potential sources for the minor peaks presumably involve proline isomerization states (4 proline residues in this segment), and modification of aromatic ring current effects in the close neighbourhood of this peptidic segment. The chemical shifts of the PKBβ PH domain (major conformation) have been deposited in the BioMagResBank under the accession number BMRB-5778. A full 3D structure determination is in progress.

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