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Letter to the Editor: Complete sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of the human PTK6 SH2 domain

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Biological context

Protein tyrosine kinases (PTKs) play an important role as regulators of cellular proliferation and differentiation, as well as apoptosis. Hyperactivation or over-expression of PTKs often occurs in the tumor development process by mutation, rearrangement, or gene amplification. The human PTK6 (also known as Brk) is a non-receptor PTK which was first identified from a partial cDNA clone during an extensive survey of PTK mRNAs expressed in human melanocytes (Lee et al., 1993). It was found to be overexpressed in a high percentage of breast carcinomas and colon tumors (Llor et al., 1999). Expression of PTK6 in human mammary epithelial cells potentiates mitogenic response of the cells to epidermal growth factor (Kamalati et al., 1996), implying its role in tumor development. Analysis of the primary structure of PTK6 revealed that it is composed of an SH3 domain, an SH2 domain, and a catalytic domain. PTK6 is the most closely related to the Src family members; however, it differs in several respects: lack of the N-terminal myristoylation site, lower homologies between PTK6 and Src family members than those among Src family members, and evolutionary divergence for genomic structure (Lee et al., 1998).

Methods and experiments

The cDNA segment of the encoding PTK6 SH2 domain (residues 75–174) was amplified using a primer pair by PCR from the full-length PTK6 cDNA (Lee et al., 1998) and cloned into the *Bam*HI-*Eco*RI sites of pGEX 4T-3 (Novagen), an *E. coli* expression vector. In addition to SH2 domain, a Gly residue is attached to the N-terminus for the thrombin cleavage. The intact SH2 domain contained a glutathione-S-transferase (GST) tag in its N-terminus coded from the vector sequence. This vector was used to transform the *E. coli* strain BL21(DE3)pLysS for fusion protein expression. Uniformly $^{13}C/^{15}N$- or ^{15}N-isotopically labeled protein samples were prepared by growing cells in the M9 minimal media containing $^{15}NH_4Cl$, either with or without $^{13}C_6$-D-glucose as the sole source of nitrogen and carbon. The cell pellets were suspended in phosphate-buffered saline (pH 6.5) that contained 1% Triton X-100, EDTA, 0.1 mM PMSF, 1% β-mercaptoethanol, and sonicated. The PTK6 SH2 domain fused to GST was purified with Glutathione Sepharose 4B (Amersham Pharmacia Biotech) and was then eluted with a buffer containing 10 mM of reduced glutathione. The purified fusion protein was subjected to digestion of GST with bovine thrombin (Amersham Pharmacia Biotech) for 17 h. Further purification of the PTK6 SH2 domain was accomplished using fast-protein liquid chromatography (FPLC) with a Superdex 75 HR 10/30 column in 2 mM DTT, 50 mM potassium phosphate at pH 6.5. The NMR samples were approximately 1 mM in concentration, placed in a 5 mm symmetrical micro cell (Shigemi).

All NMR experiments were performed at 298 K on either a Bruker DRX-500 MHz or a Varian Unity INOVA 500 MHz spectrometer equipped with a triple resonance probe with x, y, z-gradients. All spectra were processed using NMRPipe/NMRDraw software

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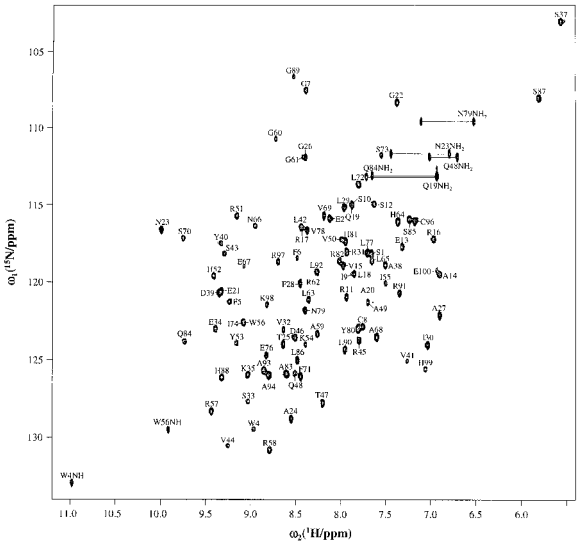

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of a uniformly ^{15}N-labeled PTK6 SH2 domain recorded on a Bruker DRX 500 MHz spectrometer at 298 K. The side chain NH of Trp and NH_2 of Asn and Gln residues are also labeled.

(Delaglio et al., 1996) and analyzed using the program XEASY (Bartels et al., 1995). Triple and double resonance experiments were recorded to obtain HN, ^{15}N, H_α, $^{13}C_\alpha$, $^{13}C_\beta$ and ^{13}CO resonances for all residues and to connect the resonances of residue i with those of residue i+1. The assignment was started by picking cross peaks in a two-dimensional ^{1}H-^{15}N HSQC spectrum, to obtain HN(i) and ^{15}N(i) resonance frequencies. The backbone assignments were accomplished using the HNCA and the HN(CO)CA spectra. The few ambiguities encountered were resolved by the HNCACB and the CBCA(CO)NH spectra (Muhandiram and Kay, 1994). Several ambiguities in resonance assignment due to resonance overlap were finally resolved using HNCO and HCA(CO)N spectra. Backbone carbonyl assignments were mainly derived from HNCO and confirmed by the HCACO data. The sequential resonance assignment was further confirmed by $d_{NN(i,i+1)}$ and $d_{\alpha N(i,i+1)}$ NOEs in ^{15}N-edited NOESY-HSQC and TOCSY-HSQC spectra (Cavanagh et al., 1996). For the complete side chain assignment, HCCH-TOCSY and ^{3}C-edited NOESY data were used (Kay et al., 1993).

Extent of assignments and data deposition

Figure 1 shows the 2D ^{1}H-^{15}N HSQC spectrum for the human PTK6 SH2 domain labeled with assignments. All resonances except the side chain amide of N66 have been assigned on this spectrum, which accounts for all the backbone and side chain amides of PTK6 SH2 domain. Complete side chain H and ^{13}C assignments were made for all Ala, Asp, Cys, Gln, Glu, His, Ile, Leu, Pro, Ser, Thr and Val residues. The ^{1}H and ^{13}C resonances of side chains (excluding the guanidine group) were assigned for all Arg residues, except for residue Arg62. The side chain resonances of Lys and Asn, except for the ζ-$^{15}NH_3^+$ of Lys residues and the amide NH_2 of Asn66, were assigned. We have determined the well-defined secondary structure composed of two α-helices and six β-strands based on NMR data. The chemical shift indices (CSI) were also used to confirm the secondary structure (Wishart and Sykes, 1994). The complete list of ^{1}H, ^{13}C and ^{15}N chemical shifts has been deposited at the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4902.

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