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Letter to the Editor: ^{1}H, ^{15}N, ^{13}C resonance assignments of the human protein tyrosine phosphatase PRL-1

Jennifer S. Laurence[a], Klaas Hallenga[b] & Cynthia V. Stauffacher[a,*]
[a]*Purdue University, Department of Biological Sciences, West Lafayette, IN 47907, U.S.A.;* [b]*University of Wisconsin-Madison, Department of Biochemistry, Madison, WI 53706, U.S.A.*

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Biological context

PRL enzymes have become highly sought after targets for drug design because of their involvement in metastatic cancer. Elevated levels of PRL-1 and PRL-3 have been shown to trigger metastasis, and PRL-1 and PRL-2 promote cellular transformation and tumorigenesis (Zeng et al., 2003; Saha et al., 2001; Cates et al., 1996). PRL-1 was originally identified as an early response gene involved in liver regeneration following partial hepatectomy in rats (Diamond et al., 1994). PRL enzymes are found only in multicellular eukaryotic organisms. Three isoforms exist in mammals, and C. elegans and Drosophila each appear to have a single homolog. A distinguishing feature of the PRLs is their C-terminal CaaX motif, which directs farnesylation of the Cys (Zeng et al., 2000). The modified protein can be altered further by proteolysis of the two aliphatic residues (aa) and additional C-terminal residue (X), and by α-carboxyl methylation of the new C-terminal farnesyl-Cys (Clarke, 1992). In addition, the PRL phosphatases encode a highly basic region near the C-terminus, which may act as a nuclear localization signal or secondary membrane binding motif.

These 21 kDa proteins belong to the protein tyrosine phosphatase (PTPase) family and contain the canonical CX_5R active-site motif. Nucleophilic attack on phosphorylated substrates is carried out by the Cys, while the Arg and several backbone amides from intervening residues in the P-loop coordinate the phosphate moiety. PRLs have little sequence similarity to other PTPases outside of the active site region and appear to constitute a unique subgroup. Although a three-dimensional structure has not been published for any member of this family, the resonance assignments for PRL-2 and PRL-3 are available (Zhou et al., 2003; Kozlov et al., 2002).

*To whom correspondence should be addressed. E-mail: cyndy@gauguin.bio.purdue.edu

Methods and experiments

The human PRL-1 gene, provided by P. Crowell, was sub-cloned into the pET-30 Xa/LIC vector using ligation independent cloning. The DNA of the modified plasmid was sequenced to confirm the fidelity of the PRL-1 gene. The construct was transformed into BL21(DE3) competent cells and selected based on Kanamycin resistance. Cells were grown on minimal media containing $^{15}NH_4Cl$ and ^{12}C- or ^{13}C-glucose. Affinity chromatography of the poly-His containing protein was performed on a HiTrap metal chelation column to purify PRL-1. Factor Xa was used to cleave the affinity purification tags from the PRL-1 protein. Factor Xa was removed by incubation with Xarrest resin (Novagen) and the tags removed by passage over the chelation column. Samples were concentrated to 1.5–2.0 mM protein in 50 mM sodium phosphate, pH 6.5 using Millipore BioMax-10 concentrators.

NMR samples contained 5% D_2O and were analyzed in Shegimi tubes. 2D HSQCs, 3D versions of the CBCA(CO)NH, HNCACB, C(CO)NH, HC(CO)NH and HNCO experiments were carried out on a Varian Unity Inova 600 MHz NMR spectrometer equipped with a triple resonance probe capable of z-gradient pulses (Cavanagh et al., 1996). The 3D HCCH-TOCSY, collected with a mixing time of 12 ms, was acquired on the 800 MHz Varian Unity Inova at NMRFAM, University of Wisconsin, Madison. All data

Figure 1. HSQC of 2 mM PRL-1 in 50 mM sodium phosphate, pH 6.5 and 37 °C, taken on a Varian 600 MHz Unity Inova spectrometer.

were collected at 37 °C and referenced relative to DSS (Wishart et al., 1995). Spectra were processed using NMRpipe and analyzed with Sparky (Goddard and Kneller).

Extent of assignments and data deposition

Complete sequential backbone assignments were achieved for residues P7-Q135 with the exception of two small regions, the active site phosphate binding loop (P-loop) and residues 25–29. Peaks corresponding to the residues in these regions are not observed in the spectra. Overall 85% of the backbone resonance assignments for PRL-1 were assigned using the HNCACB, CBCACONH and HNCO spectra, with the majority of unassigned residues confined to the N- and C-termini. No assignments could be made for residues 1-6, and only partial assignment of residues in the C-terminal region beyond Q135 could be made. Sidechain assignment was carried out using the CCONH,HCCONH and HCCH-TOCSY spectra. Approximately 70% of the sidechain atoms could be assigned. Due to extensive overlap in the aliphatic regions, complete sidechain assignments for Leu, Ile, Lys and Arg residues were not possible. Figure 1 shows the assigned ^{15}N HSQC data for PRL-1. Resonance assignments for PRL-1 have been deposited with the BioMagResBank under the accession number 6080 (http://www.bmrb.wisc.edu).

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