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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments and secondary structure of the human protein tyrosine phosphatase, PRL-2

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Biological context

Phosphatases of regenerating liver (PRLs) represent a distinct class of protein tyrosine phosphatases (PTPs) with a C-terminal prenylation site (Tamanoi et al., 2001). Although the PRLs possess the PTP active site signature sequence HCX_5R, their primary sequence more closely resembles the dual specificity (serine/threonine and tyrosine) phosphatases Cdc14p and PTEN than the classical cytosolic PTPs (Zeng et al., 1998). While the details of the biological functions of the PRLs remain to be determined, accumulating evidence indicates that they play important roles in cell growth, development and differentiation (Kong et al., 2000). PRL-1 is an immediate early gene expressed in regenerating liver tissue (Diamond et al., 1994) and is required for normal mitosis, while over-expression of PRL-2 is associated with prostate cancer (Wang et al., 2002). Recent studies have also established that PRL-3 is important in colorectal cancer metastasis where it is expressed at elevated levels in metastases but at lower levels in nonmetastatic tumors and normal colorectal epithelium (Saha et al., 2001). These results suggest that PRLs could be valuable drug targets. The high level of primary sequence identity between the family members (PRL-1/PRL-2 86%, PRL-1/PRL-3 78%, PRL-2/PRL-3 75%) suggests that accurate structural studies are needed to guide drug design specificity, both between different members of the PRL family and other PTPs in general. *In vitro* studies show that pentamidine equally inhibits each of the PRLs and inactivates ectopically expressed PRL-2 in WM9 human melanoma tumors in nude mice (Pathak et al., 2002). There are no known 3D structures of any member of the PRL family. NMR assignments of PRL-3 have recently been reported (Kozlov et al., 2002), and likewise here we report backbone and sidechain assignments for PRL-2, as well as a secondary structure (Supplementary information available from the author), as a first step in a full 3D structural study aimed at identifying inhibitor binding and optimization.

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Methods and experiments

PRL-2 was amplified by PCR from a human placenta c-DNA library, subcloned into the NheI/XhoI multiple cloning site of a modified pGEX-4T plasmid and transformed into BL21 cells for overexpression. This process adds an additional GlySerAlaSer to the N-terminus, and appends a LeuGlu to the C-terminus of the 2–167 residue protein construct. Isotopically-enriched PRL-2 was prepared by growing cells in M9 minimal media with ^{15}N-ammonium chloride as the sole nitrogen source with or without ^{13}C-glucose as the carbon source. The N-terminal GST-fusion protein was isolated by affinity chromatography on a glutathione-agarose column and then cleaved by treatment overnight with thrombin. Final purification steps used ion-exchange chromatography on a Hi-Trap Blue column followed by size exclusion with a Superdex 75. Final yields of 5–10 mg per liter of culture were attained. NMR samples were prepared at 0.6–1.4 mM protein in 50 mM sodium phosphate buffer at pH 7.0, 10 mM DTT-d10, 1 mM EDTA-d16, 0.01% sodium azide in Shigemi tubes purged with argon, and sealed with parafilm to minimize sulfhydryl oxidation.

All NMR data were collected at 30 °C on a Bruker Avance 600 MHz spectrometer equipped with either a 3-axis TXI probe or a Z-axis cryoprobe typically using crushing gradients. All data were processed

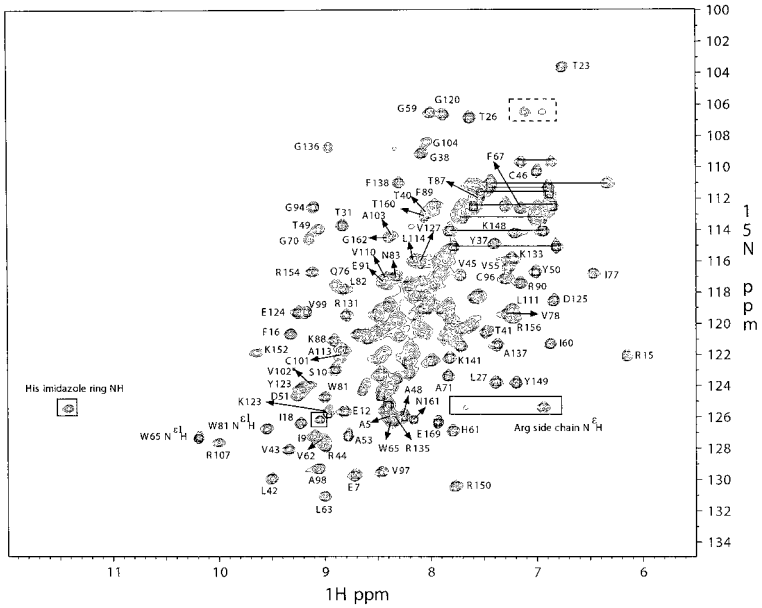

Figure 1. ^{1}H-^{15}N HSQC spectrum with assignments of PRL-2 in 50 mM $NaPO_4$ buffer at pH 7.0, with 10 mM DTT-d10, 1 mM EDTA-d16, and 0.01% sodium azide at 30 °C. Aliased peaks are enclosed by solid boxes. A dashed box encloses two weak, broad peaks for which no sequential or NOE correlations can be found. Sidechain NH_2 peaks from Asn and Gln residues are connected by horizontal, straight lines. Sequence numbering starts with −2 at the N-terminus and ends at 169 at the C-terminus.

with nmrPipe or Azara software and analyzed with the program ANSIG4OpenGL on Linux workstations. Backbone amide ^{1}H and ^{15}N, C_α, C=O and sidechain C_β resonances were assigned using ^{1}H-^{15}N HSQC, HNCO, HNCACB and CBCA(CO)NH experiments. Sidechain assignments were extended with 3D HCC(CO)NH-TOCSY, HCCH-TOCSY, and HCCH-COSY and 4D HCNH NOESY experiments. Secondary structure determinations are based on C_α, C_β and C=O chemical shift indices (Wishart and Sykes, 1994).

Extent of assignments and data deposition

Over 90% of backbone NH (excluding 11 prolines) and CO, C_α and C_β nuclei are assigned. There are no significant peaks left unassigned in the ^{1}H-^{15}N HSQC and the HNCO spectrum. Sidechain ^{1}H and ^{13}C assignments are over 85% complete. Resonances from aromatic rings, including the two Trp indole rings, are partially assigned. No assignment of any nucleus can be made for Gly(-2)-Ser1, Asn24, Ala108, His163 and Cys164. Regions near these sites exhibit weakened and broadened signals in all spectra collected. Reduced intensities of crosspeaks near the active site H100-CVAGLG-R107 are attributed to intermediate exchange broadening. Two sets of resonances were observed for residues in the C-terminal region from Asp 159 to Val 166 that encompasses the prenylation site. Figure 1 shows a ^{1}H-^{15}N correlation spectrum partially labeled with assignments. Assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number XXXX.

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