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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C resonance assignments of low molecular weight human cytoplasmic protein tyrosine phosphatase-A (HCPTP-A)

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Biological context

Human cytoplasmic protein tyrosine phosphatase (HCPTP) is a low molecular weight ($M_r \sim 18$ kDa) protein tyrosine phosphatase (PTP). In cultured cells, HCPTP modulates signaling by insulin, platelet-derived growth factor (PDGF), ephrins and vascular endothelial growth factor suggesting a role for this phosphatase in multiple biological processes including glucose metabolism, wound healing and angiogenesis (Huang et al., 1999). HCPTP exists in two isoforms: HCPTP-A and HCPTP-B, that are derived from a single gene by an alternative splicing of the primary RNA transcript, an event which may dictate substrate specificities. A bovine form of the low molecular weight PTPase, BPTP (Bovine Heart Protein Tyrosine Phosphatase), has 81% homology with HCPTP-A and 94% homology with HCPTP-B suggesting that this is the bovine form of the HCPTP-B splice variant. Van Etten's group determined the solution (Logan et al., 1994) and crystal (Zhang et al., 1994) structures of BPTP in 1994. Subsequently, the crystal structure of HCPTP-A was solved at 2.2 Å resolution by the same group (Zhang et al., 1998).

In order to understand the conformational changes that occur upon ligand binding, we have undertaken NMR studies of HCPTP-A in solution. The published crystal structure of HCPTP-A, together with the backbone resonance assignments presented here and the ligand induced changes in backbone chemical shifts, provides a sound basis for the design and development of a suitable inhibitor of PTPs. This precludes the need for complete side-chain resonance assignment. We are also pursuing protein dynamics studies on HCPTP-A with and without the inhibitor, which would be useful for understanding substrate specificity of the PTPs and for optimization of PTP inhibitors. Finally, the 3D structure of the protein-inhibitor complex determined by utilizing residual dipolar coupling (RDC) constraints would provide deeper understanding of the interplay between the protein-ligand interactions coupled with the protein loop dynamics.

Here, we present the backbone assignments and the secondary structure of HCPTP-A in the presence of phosphate.

Methods, NMR experiments, and results

Uniformly $^{13}C^{15}N$-labeled HCPTP-A was overexpressed in the *Escherichia coli* BL21 (DE3) strain grown in a minimal media containing ^{15}N-NH_4Cl (1 g l^{-1}) and $^{13}C_6$-glucose (2 g l^{-1}) as the sole nitrogen and carbon sources, respectively. A triple labeled sample was obtained by growing the bacteria in 100% $^{2}H_2O$. Details of the expression and purification steps have been reported earlier (Wo et al., 1992). NMR samples contained ~1.0 mM HCPTP-A in 50 mM phosphate buffer, pH 5.1. NMR experiments were recorded at 298 K on a Varian INOVA 600 MHz spectrometer and a Varian INOVA 800 MHz spectrometer equipped with a triple resonance z-gradient probe. Some of the NMR experiments were recorded at 308 K to resolve the spectral overlaps. Spectra were

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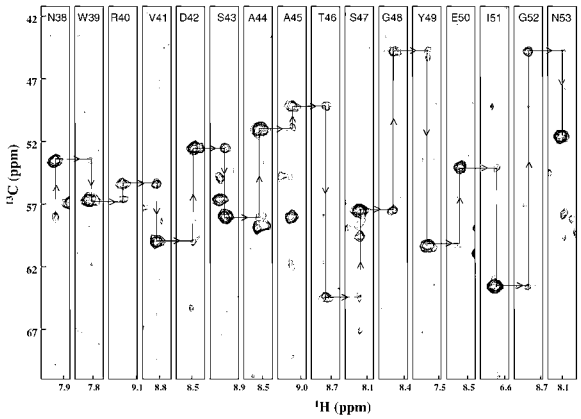

Figure 1. Illustrative sequential connectivity pathway in HNCA experiment in HCPTP-A. 2D-strips shown are for residues 38 through 53. Each NH showed cross-peaks to the C_α and $C_{\alpha-1}$ (weaker intensity) of both its own as well as the preceding residue.

processed and analyzed with nmrPipe (Delaglio et al., 1995) and NMRview (Johnson and Blevins, 1994). All experiments made use of pulsed field gradients for coherence selection and artifact suppression (Bax and Pochapsky, 1992) and utilized gradient sensitivity enhancement schemes wherever appropriate (Kay et al., 1992). NMR experimental conditions were optimized using the ^{15}N^{1}H-HSQC.

Sequence-specific assignments of backbone (^{1}H, ^{15}N, ^{13}C) resonances were obtained by making use of a series of double and triple resonance experiments: ^{15}N^{1}H-HSQC, CT-^{13}C^{1}H-HSQC, HNCO, HNCA, HN(CO)CA, HNCACB, CBCACONH, ^{15}N-TOCSYHSQC, ^{15}N-NOESYHSQC (Cavanagh et al., 1996). An example of the assignments of ^{13}C-resonances using 2D-strips from HNCA experiment is shown in Figure 1.

HCPTP-A aggregated at concentrations greater than 1.0 mM leading to broad resonances. The NMR resonances sharpened and several new ones appeared upon serial dilution. We also observed severe exchange broadening of the ^{15}N-resonances of several residues belonging to the loops (12–18, 46–55, 67–80, 120–134). Aggregation as well as significant loop dynamics were the major bottlenecks in carrying out the sequential assignment. Comparison of $^{13}C_\alpha$, $^{13}C_\beta$, and ^{13}CO chemical shifts of HCPTP-A residues with respect to those in random coil conformation obtained by CSI calculation (Wishart and Sykes, 1994) indicated the presence of 4 β-strands and 5 α-helices with two βαβ motifs, consistent with the crystal structure of HCPTP-A.

Despite very high structural and sequence similarities (∼80%), the two low molecular weight PTP forms (human-A and bovine) possess subtle differences in their dynamics properties and in the backbone chemical shifts of the two forms. The major differences are observed in the loop regions as well as in the variable sequence region (40–73).

Extent of assignments and data deposition

Over 90% of the backbone amide-nitrogens, amide-protons, α-protons, α-carbons, β-carbons, and carbonyl-carbons have been assigned. The chemical shifts of α-carbons, β-carbons, and carbonyl-carbons were used for the secondary structure determination with the Chemical Shift Index (Wishart and Sykes, 1994).

The chemical shift values have been deposited in BioMagResBank database (http://www.bmrb.wisc.edu) under the accession number BMRB-5350.

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