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Letter to the Editor: Backbone ^{1}H, ^{15}N, and ^{13}C resonance assignments of inhibitor-2 – a protein inhibitor of protein phosphatase-1

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Biological context

Protein phosphatase-1 (PP-1) is one of the serine/threonine protein phosphatases that regulates diverse cellular functions, including carbohydrate metabolism, muscle contraction, neuronal signaling, protein synthesis and the cell cycle (Shenolikar and Nairn, 1991). The catalytic subunit of PP-1 is regulated by several heat-stable proteins, including inhibitor-1, DARPP-32 and inhibitor-2. In the absence of phosphorylation, inhibitor-2 binds to PP-1 and inhibits enzyme activity. In turn, PP-1 and inhibitor-2 form an inactive complex, termed ATP-Mg-dependent phosphatase, that can subsequently be activated following phosphorylation of inhibitor-2 at Thr-72 by glycogen synthase kinase-3 (GSK-3) (Park and DePaoli-Roach, 1994). Mutagenesis studies indicated that residues 9–13 (PIKGI) of inhibitor-2 (204 total amino acids) were critical for binding of PP-1. Deletion of either Lys^{11} or Ile^{13} resulted in a significant decrease of PP-1 inhibition (Huang et al., 1999). While unnecessary for inhibition, the C-terminal half of inhibitor-2 has been suggested to play a key role in reactivation of the inactive ATP-Mg-dependent phosphatase following phosphorylation by GSK-3 (Park and DePaoli-Roach, 1994; Huang et al., 1999).

There is no information available concerning the structure of inhibitor-2. To help to elucidate the structure of inhibitor-2, and to clarify the molecular basis of the regulation of PP-1 activity, we have applied multidimensional heteronuclear NMR techniques to study residues 1–172 of inhibitor-2 (inhibitor-2[1–172]). Here we report the backbone ^{1}H, ^{15}N and ^{13}C resonance assignments of this truncated protein.

Methods and results

The cDNA coding for residues 1–172 of human inhibitor-2 was subcloned into the pET-3a vector and *E. coli* BL21(DE3) was transformed with the recombinant pET-3a plasmid. Bacteria were grown at 37 °C in M9 medium supplemented with $^{15}NH_4Cl$ and glucose or with $^{15}NH_4Cl$ and ^{13}C-glucose (Cambridge Isotope Laboratories, Inc., Andover, MA, U.S.A.). When the absorbance (at 600 nm) of the growing cells was between 0.6 and 1.0, 0.4 mM IPTG (final concentration) was added to induce expression of recombinant protein. After 5 h, cells were harvested by centrifugation and recombinant protein was purified from bacteria as described (Huang et al., 1999). Inhibitor-2[1–172] showed similar properties to wild-type inhibitor-2, with respect to the IC_{50} for inhibition of PP-1, the formation of an inactive complex with PP-1 and re-activation of PP-1 following phosphorylation by GSK-3 (data not shown). Specific amino acids of inhibitor-2[1–172] were labeled with ^{15}N as described (Lin et al., 1998).

Samples for NMR experiments contained 100 mM phosphate buffer, pH 6.0, 0.02% NaN_3, 1.6 mM

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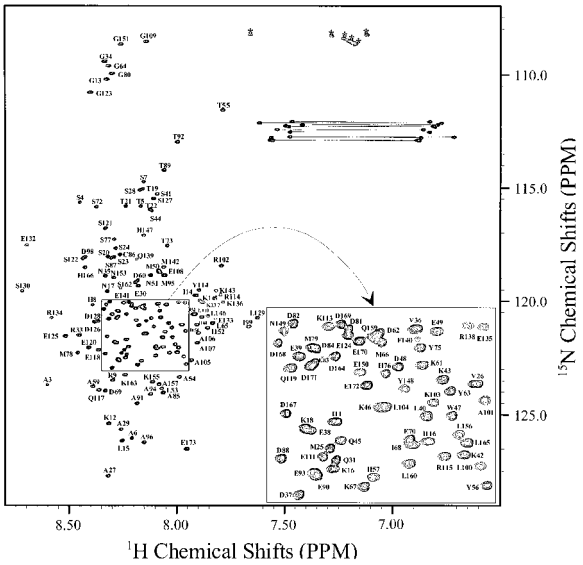

Figure 1. 2D ^{1}H-^{15}N-HSQC spectrum of 1.6 mM uniformly ^{15}N-enriched inhibitor-2[1–172] in 100 mM sodium phosphate buffer, at pH 6.0, 296 K. Assignments of the backbone amide protons and ^{15}N cross peaks are indicated in the figure. The expanded region indicated by an arrow is for the purpose of clarity. The cross peaks marked by asterisks are the folded arginine side-chain N$^{\epsilon}$H protons. The amide side-chain resonances of asparagine and glutamine residues are connected by horizontal lines.

protein in 90% H_2O/10% D_2O. DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid) was used as internal chemical shift standard (Wishart and Sykes, 1994; Wishart et al., 1995). The final protein sample solutions were transferred to 5 mm Shigemi NMR tubes (Shigemi Co., Tokyo, Japan) for recording NMR spectra. All NMR experiments were performed at 296 K on a Bruker AVANCE-500 spectrometer equipped with a 5 mm inverse triple resonance (^{1}H/^{13}C/BB), z-axis gradient probe. Experimental parameters and data processing are as described previously (Lin et al., 1998).

Backbone sequential assignments were obtained using the following heteronuclear 3D spectra: HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH. A Fortran program has been developed to search the sequential connectivity semiautomatically. 2D ^{1}H-^{15}N-HSQC spectra of four specifically ^{15}N-labeled ([^{15}N-Ala], [^{15}N-Lys], [^{15}N-Leu] and [^{15}N-Ser]) inhibitor-2[1–172] fragments were also obtained to facilitate and confirm the assignments.

The results of chemical shift index (CSI) analysis for $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO suggest a short α-helix-spanning region from residue 132 to 142. The rest of the molecule has a random coil conformation.

Extent of assignments and data deposition

All of the ^{1}H and ^{15}N backbone resonances were assigned, except Ala^{2}, Arg^{158} and the N-terminal Met^{1} residues. $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO resonances were assigned for all but the N-terminal Met^{1} residue. All of the $^{1}H^{\alpha}$ resonances, except for Pro^{112} and Met^{1}, were assigned based on 3D HBHA(CBCACO)NH and ^{1}H-^{15}N-TOCSY-HSQC spectra. Figure 1 shows the 2D ^{1}H-^{15}N-HSQC spectrum of uniformly ^{15}N/^{13}C-enriched inhibitor-2[1–172]. Assignments of the backbone amide proton and ^{15}N cross peaks are labeled on the spectrum. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4720.

Acknowledgements

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