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

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Biological context

Protein phosphatase-1 (PP-1) is one of the mammalian serine/threonine protein phosphatases that modulates a variety of cellular functions (Shenolikar and Nairn, 1991). The catalytic subunit of PP-1 is specifically inhibited by the thermostable proteins, including inhibitor-1, DARPP-32 and inhibitor-2. When either Thr-35 of inhibitor-1 or Thr-34 of DARPP-32 is phosphorylated by cAMP-dependent protein kinase (PKA), each protein will be converted into a potent inhibitor of PP-1. Inhibitor-1 and DARPP-32 share a remarkable identity in the sequence of NH_2-terminal region between residues 6 and 38. This region contains two inhibitory subdomains that are required in inhibition of PP-1. In the case of phospho-inhibitor-1, subdomain 1 is defined as the residues close to and including the phosphorylated-Thr-35. Subdomain 2 is defined as a short motif between residues 8 and 12 (RKIQF). This consensus basic/hydrophobic motif is often observed in PP-1 binding proteins, providing a structural basis for their interaction with PP-1 in a mutually exclusive manner (Kwon et al., 1997; Huang et al., 1999; Egloff et al., 1997).

There is no information available concerning the structure of inhibitor-1. To help to elucidate the structure of inhibitor-1, and to gain more insight into the interactions between inhibitor-1 and PP-1 from structural point of view, we have applied multidimensional heteronuclear NMR techniques to study this protein. Here we report the backbone ^{1}H, ^{15}N and ^{13}C resonance assignments of inhibitor-1(I29T).

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Methods and results

The recombinant pET28a vector subcloned with the cDNA of human inhibitor-1(I29T) was transformed into *E. coli* BL21(DE3). Bacterial growth, stable isotope labeling (^{15}N or ^{13}C,^{15}N) on the recombinant inhibitor-1(I29T), and the protein expression followed the methods as described (Huang et al., 2000). The recombinant inhibitor-1(I29T) was purified by a series of steps, including heat-treatment, anion exchange chromatography using Q Sepharose, FPLC using a HiPrep 16/60 Sephacryl S-200 High Resolution column, followed by the Mono-Q chromatography. The purified protein was a single band on SDS-PAGE, stained with Coomassie Brilliant Blue. After phosphorylation by PKA, the IC_{50} of the phospho-inhibitor-1(I29T) in inhibition of PP-1 was 2.65 $\pm$ 0.05 nM (data not shown). Specific amino acids of inhibitor-1(I29T) were labeled with ^{15}N as described (Lin et al., 1998).

Samples for NMR experiments contained 25 mM sodium phosphate buffers, pH 5.5, 0.02% NaN_3, 1.6 mM 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 et al., 1995).

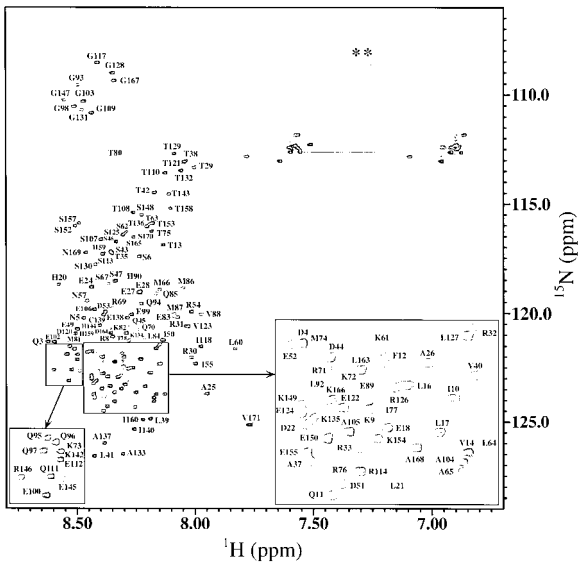

Figure 1. 2D ^{1}H-^{15}N-HSQC spectrum of 1.6 mM uniformly ^{15}N-enriched inhibitor-1(I29T) in 25 mM sodium phosphate buffer, at pH 5.5, 293 K. Assignments of the backbone amide proton and ^{15}N cross peaks are indicated in the figure. The expanded regions indicated by arrow are for the purpose of clarity. The cross peaks marked by asterisks are the folded arginine side-chain N$^{\varepsilon}$H protons. The amide side-chain resonances of asparagine and glutamine residues are connected by horizontal lines.

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 293 K on 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 home-built Fortran program has been used to search the sequential connectivity semi-automatically. 2D ^{1}H-^{15}N-HSQC spectra of specifically ^{15}N-labeled (^{15}N-Lys) inhibitor-1(I29T) were also obtained to facilitate and confirm the assignments.

Extent of assignments and data deposition

All of the ^{1}H and ^{15}N backbone resonances were assigned, except Glu^{2}, His^{91} and the N-terminal Met^{1} residues. ^{13}C$^{\alpha}$, ^{13}C$^{\beta}$ and ^{13}CO resonances were assigned for all residues except Met^{1}, Pro^{115} and Pro^{161} residues. All of the ^{1}H$^{\alpha}$ resonances were assigned based on 3D HBHA(CBCACO)NH and ^{1}H-^{15}N-TOCSY-HSQC spectra except Met^{1}, Pro^{115} and Pro^{161} residues. Using the assigned chemical shift of ^{1}H$^{\alpha}$, ^{13}C$^{\alpha}$, ^{13}C$^{\beta}$ and ^{13}CO, we have employed the consensus chemical shift index (CSI) (Wishart and Sykes, 1994) to identify the secondary structure of inhibitor-1(I29T). The results of consensus chemical shift index analysis suggest four short helical regions corresponding to residues 24–28, 81–86, 103–109 and 127–131. The rest of the molecule has a random coil conformation. Figure 1 shows the 2D ^{1}H-^{15}N-HSQC spectrum of uniformly ^{15}N-enriched inhibitor-1(I29T). 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 5040.

Acknowledgements

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