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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C resonance assignments of DARPP-32 (dopamine and cAMP-regulated phosphoprotein, *Mr.* 32,000) – a protein inhibitor of protein phosphatase-1

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Biological context

DARPP-32 is a thermostable protein. Phosphorylation of DARRP-32 at Thr-34 by cAMP-dependent protein kinase (PKA) converts the protein into a potent inhibitor of the catalytic subunit of protein phosphatase-1 (PP1) (Shenolikar and Nairn, 1991). Several lines of evidence have indicated that two subdomains are essential for inhibition of PP1 by DARPP-32. Subdomain 1 is defined as the region, ranging from residue-29 to -35, that contains the phosphorylated Thr-34. Subdomain 2 is defined as a short motif from residue-7 to -11 (KKIQF). This consensus basic/hydrophobic motif is often observed in PP1-binding proteins, providing the structural basis for their interactions with PP1 in a mutually exclusive manner (Kwon et al., 1997; Huang et al., 1999; Egloff et al., 1997). DARPP-32 is also an inhibitor of PKA when Thr-75 is phosphorylated by cyclin-dependent kinase 5 (cdk5) (Bibb et al., 1999). Thus, DARPP-32 can regulate both an important protein kinase and an important phosphatase through phosphorylation of distinct sites. This dual function is believed to be important for the integration of the effects of various neurotransmitters on signaling pathways.

There is no information available concerning the structure of DARPP-32. In order to understand the structural properties of DARPP-32, and to gain more insight into the interactions between DARPP-32 and PP-1 from structural point of view, we have applied multidimensional heteronuclear NMR techniques to study this protein. We report here the ^{1}H, ^{15}N and ^{13}C resonance assignments of DARPP-32[1–118].

Methods and experiments

The cDNA coding for residues 1–118 of rat DARPP-32 was subcloned into the pET-3a vector and *E. coli* BL21(DE3) was transformed with the recombinant pET-3a plasmid. Bacterial growth, stable isotope labeling (^{15}N or ^{13}C,^{15}N) of the recombinant DARPP-32 mutant, DARPP-32[1–118], and protein expression followed methods as described (Huang et al., 2000). Recombinant DARPP-32[1–118] was purified as described (Huang et al., 1999). DARPP-32[1-118] showed similar properties to wild-type DARPP-32, with respect to the IC_{50} for inhibition of PP1 (data not shown).

Samples for NMR experiments contained 100 mM sodium phosphate buffers, pH 5.5, 0.02% NaN_3, 1.0 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). The final protein sample solutions were transferred to 5 mm Shigemi NMR tubes (Shigemi Co.) for re-

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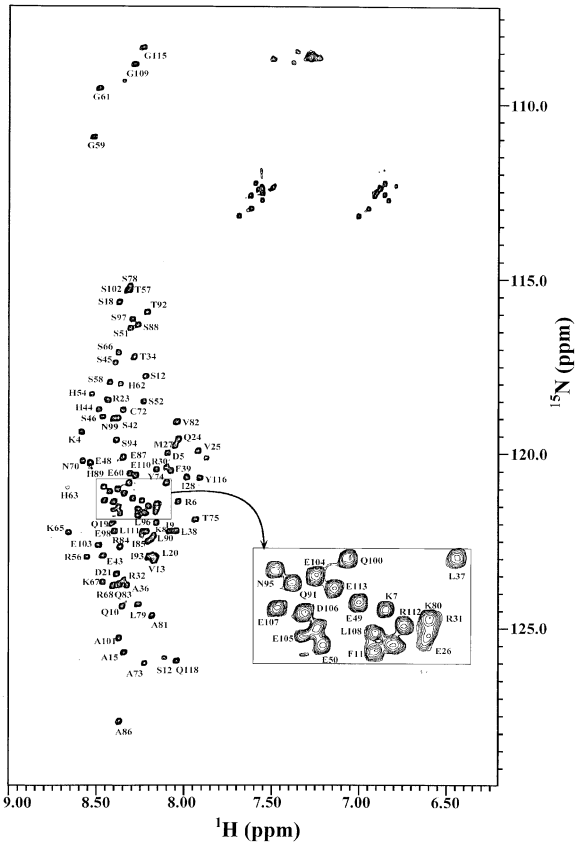

Figure 1. 2D ^{1}H-^{15}N-HSQC spectrum of 1.0 mM uniformly ^{15}N-enriched DARPP-32[1–118] in 100 mM sodium phosphate buffer, at pH 5.5, 296 K. Assignments of the backbone amide proton and ^{15}N cross peaks are indicated in the figure. The boxed region of the spectrum is expanded for the purpose of clarity.

cording NMR spectra. All NMR experiments were performed at 296 K on Bruker AVANCE-500 spectrometer equipped with a 5 mm inverse triple resonance (^{1}H/^{13}C/BB), Z-axis gradient probe. All spectra were processed using the program XWIN-NMR and analyzed using AURELIA (Bruker) on an SGI workstation. Linear prediction was used in the indirectlydetected dimensions to improve the digital resolution. ^{1}H Chemical shifts were referenced to the ^{1}H frequency of the methyl resonances of DSS at 0 ppm. The ^{15}N and ^{13}C chemical shifts were indirectly referenced using the following consensus Ξ ratios of the zero-point frequencies: 0.101329118 for ^{15}N/^{1}H and 0.251449530 for ^{13}C/^{1}H (Wishart et al., 1995). Backbone sequential assignments were accomplished by using the following heteronuclear 3D spectra: HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH.

Extent of assignments and data deposition

All of the amide proton and ^{15}N backbone resonances were assigned except Arg^{40}, Leu^{114} and the N-terminal Met^{1} residues. ^{13}C$^{\alpha}$ and ^{13}C$^{\beta}$ resonances were assigned for all residues except Pro^{16} and Pro^{76} residues. For ^{13}CO resonances, the missing assignments were Asp^{2}, Pro^{16} and Pro^{76} residues. All of the ^{1}H$^{\alpha}$ and ^{1}H$^{\beta}$ resonances were assigned based on 3D HBHA(CBCACO)NH and ^{1}H-^{15}N-TOCSY-HSQC spectra except Pro^{16} and Pro^{76} residues. Aliphatic side-chain proton and carbon chemical shifts were extracted from C(CO)NH, H(CCO)NH, HBHA(CBCACO)NH and ^{1}H-^{15}N-TOCSY-HSQC spectra. About 80% of the aliphatic side-chain proton resonances and 85% of the aliphatic side-chain carbon resonances were assigned. Assignments of the aromatic ring carbon and proton resonances have not been made. 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 analyze the secondary structure of DARPP-32[1-118]. The results of consensus chemical shift index analysis suggest four short helical regions corresponding to residues 23–27, 78–81, 85–88 and 109–113. 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 DARPP-32[1–118]. 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 5906.

Acknowledgements

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