Journal of Biomolecular NMR, **19:** 383–384, 2001.
KLUWER/ESCOM

Letter to the Editor: Backbone ^{1}H, ^{15}N, and ^{13}C resonance assignments of ARPP-19

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Received 12 December 2000; Accepted 22 December 2000

Key words: ARPP-16, ARPP-19, heteronuclear NMR

Biological context

ARPP-19 and ARPP-16 are two phosphoproteins of $Mr = 19\,000$ (112 a.a.) and 16 000, respectively, that are substrates for protein kinase A (Horiuchi et al., 1990). In intact cells, the phosphorylation of these two proteins is regulated by cAMP and by hormones and neurotransmitters such as vasoactive intestinal peptide and dopamine (Girault et al., 1988; Dulubova et al., 2001). Protein and cDNA sequence analyses have indicated that the coding region of ARPP-16 is completely included within the coding region of ARPP-19, suggesting that the mRNAs coding for these two proteins are probably produced by alternative splicing (Horiuchi et al., 1990). Specifically, with the exception of the presence of 16 additional amino acids at the N-terminus of ARPP-19, both protein sequences are identical. However, ARPP-19 and ARPP-16 have a distinct tissue distribution. ARPP-19 is a ubiquitous protein presented in all regions of brain and non-neuronal tissues, while ARPP-16 is enriched in the basal ganglia. In addition, during development ARPP-19 concentration is highest in the embryo and decreases during the pre- and postnatal periods. In contrast, ARPP-16 appears in ontogenesis, and increases during the postnatal period (Girault et al., 1990).

There is no information available concerning the structure of ARPP-19. 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 ARPP-19.

Methods and experiments

The cDNA coding for bovine ARPP-19 was subcloned into the pET-28a vector with a His-tag sequence added at the N-terminus of the protein and *E. coli* BL21(DE3) was transformed with the recombinant pET-28a. Bacteria were grown, and protein was expressed and labeled with ^{15}N or ^{15}N plus ^{13}C as described (Huang et al., 2000). Recombinant ARPP-19 was purified by affinity chromatography using a Ni^{2+} column as described by the manufacturer (Novagen). Fractions containing ARPP-19 were pooled and concentrated to 5 ml by ultrafiltration using a YM-10 membrane. Protein was further purified by FPLC gel filtration using a Hiprep 16/60 Superdex 200 column and was pure as judged by SDS-PAGE. The His-tag motif was removed by thrombin as described by the manufacturer (Amersham Pharmacia Biotech), leaving an additional sequence, Gly(−3)-Ser(−2)-His(−1), at the N-terminus of ARPP-19.

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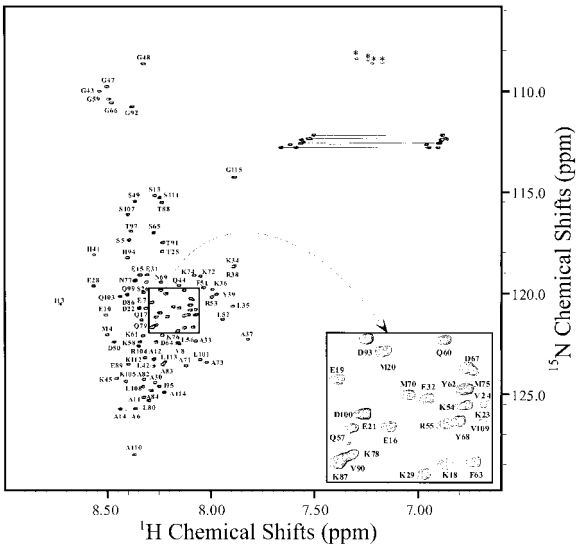

Figure 1. 2D ^{1}H-^{15}N-HSQC spectrum of 1.8 mM uniformly ^{15}N-enriched ARPP-19 in 50 mM sodium phosphate buffer, at pH 6.0, 296 K. Assignments of the backbone amide protons and ^{15}N cross peaks are indicated in the figure. The expanded region indicated by an arrow is for the purpose of clarity. The cross peaks marked by asterisks are the folded arginine side-chain N$^{\varepsilon}$H protons. The amide side-chain resonances of asparagine and glutamine residues are connected by horizontal lines.

Samples for NMR experiments contained 50 mM sodium phosphate buffer, pH 6.0, 0.02% NaN_3, 1.8 mM protein in 90% H_2O/10% D_2O. DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid) was used as an internal chemical shift standard (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 home-written Fortran program was used to facilitate the search of sequential connectivity.

Extent of assignments and data deposition

All of the ^{1}H and ^{15}N backbone resonances were assigned, except the Gly(−3) at the N-terminus. $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO resonances were assigned for all residues. All of the $^{1}H^{\alpha}$ resonances were assigned based on 3D HBHA(CBCACO)NH and ^{1}H-^{15}N-TOCSY-HSQC spectra. Using the completed 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 ARPP-19. The results of consensus CSI analysis suggest two short α-helices spanning the regions from residue 29 to 37 and from residue 69 to 73. 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 ARPP-19. Assignments of the backbone amide protons and ^{15}N cross peaks are labeled on the spectrum. The amide proton and ^{15}N cross peak of Ser(−1) is not observed in Figure 1, but it can be seen in 3D spectra. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4913.

Acknowledgements

This work was supported by the National Science Council of the Republic of China (NSC89-2311-B-075-001 and NSC88-2314-B-320-012), the Veterans General Hospital-Taipei, Taiwan, Republic of China, and USPHS grant DA10044 (A.C.N. and P.G.).

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