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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances of the catalytic domain of the rat 2′,3′-cyclic nucleotide 3′-phosphodiesterase

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Biological context

2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) is one of the earliest myelin-related proteins expressed in differentiating oligodendrocytes and Schwann cells (Vogel and Thompson, 1988). CNP is abundant in the central nervous system and in oligodendrocytes. This protein is also found in mammalian photoreceptor cells, testis and lymphocytes (Tsukada and Kurihara, 1992; Weissbarth et al., 1981). Although the biological function of CNP is unknown, it is thought to play a significant role in the formation of the myelin sheath, where it comprises 4% of total protein. CNP selectively cleaves 2′,3′-cyclic nucleotides to produce 2′-nucleotides *in vitro* (Sprinkle, 1989; Tsukada and Kurihara, 1992). Although physiologically relevant substrates with 2′,3′-cyclic termini are still unknown, numerous cyclic phosphate-containing RNAs occur transiently within eukaryotic cells. Other known protein families capable of hydrolyzing 2′,3′-cyclic nucleotides include tRNA ligases and plant cyclic phosphodiesterases. The catalytic domains from all these proteins contain two tetra-peptide motifs H-X-T/S-X, where X is usually a hydrophobic residue. Mutation of either histidine in CNP abolished enzymatic activity (Lee et al., 2001).

Recently, the crystal structure of the cyclic phosphodiesterase (CPDase) from *Arabidopsis thaliana* has been determined and a mechanism of its enzymatic activity proposed (Hofmann et al., 2000). Since CNP lacks any sequence similarity to CPDase outside of the tetra-peptide motifs, we are not able to establish the structural and functional relatedness between these proteins. The structure of the catalytic domain of CNP will clarify the functional role of this protein and should help in the identification of natural substrates. Here, we report ^{1}H, ^{15}N and ^{13}C resonance assignments for the 24.2 kDa catalytic domain of rat 2′,3′-cyclic nucleotide 3′-phosphodiesterase.

Methods and experiments

The catalytic domain of CNP was subcloned into pET15b (Novagen, Inc., Madison, WI) and expressed in *E. coli* BL21 as a His-tagged fusion protein. The protein was purified by immobilized metal affinity chromatography on Ni^{2+}-loaded chelating sepharose (Amersham Pharmacia Biotech, Piscataway, NJ). Isotopically labeled CNP was prepared from cells grown on minimal M9 media containing ^{15}N ammonium chloride and/or ^{13}C glucose (Cambridge Isotopes Laboratory, Andover, MA). For the backbone assignments, the partially deuterated triple-labeled (^{2}H, ^{15}N, ^{13}C) CNP was produced by expressing the protein in 90% D_2O/10% H_2O minimal M9 media. The N-terminal His-tag was cleaved from CNP by treatment for 2 h with thrombin (Amersham Pharmacia Biotech) at 1 unit per mg fusion protein at room temperature. Benzamidine sepharose and Ni^{2+}-loaded chelating sepharose were used to remove thrombin and the His-tag peptide from CNP. The resulting 219 amino acid protein contains 4 residues from the His-tag and

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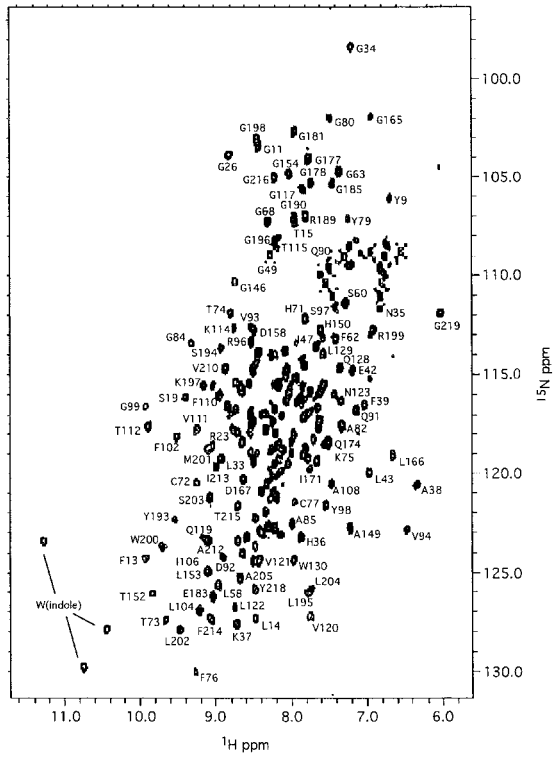

Figure 1. ^{1}H-^{15}N HSQC spectrum of the catalytic domain of CNP recorded at 310 K. The assignment of the peaks is indicated with their one-letter amino acid and number. Amino acid labels were omitted from the middle of the HSQC for clarity.

residues 184 to 398 from CNP. NMR samples were 1 to 2 mM in 50 mM sodium phosphate buffer, 0.15 M NaCl, 1 mM DTT and 0.1 mM sodium azide at pH 6.0. NMR experiments (Bax and Grzesiek, 1993) were performed at 310 K on Bruker DRX500 and Varian UNITYplus 800 MHz spectrometers.

Main-chain C^{α}, N, HN, CO and side-chain C^{β} resonances were assigned using HNCACB, HNCA, CBCA(CO)NH and HNCO experiments. The remaining backbone and side-chain signal assignments were obtained from ^{1}H-^{13}C HSQC, HCCH-TOCSY, ^{13}C-separated NOESY and verified by ^{15}N-separated NOESY. Chemical shifts were measured relative to internal DSS for ^{1}H and calculated assuming $\gamma^{15}N/\gamma^{1}H = 0.101329118$ and $\gamma^{13}C/\gamma^{1}H = 0.251449530$ (Wishart et al., 1995). NMR spectra were processed using GIFA (Pons et al., 1996) and analyzed with XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

All the ^{1}H and ^{15}N backbone resonances were assigned except for the first 2 residues of the CNP catalytic domain, 4 amino acids from the his-tag, and residues K16, K17 and V151. The H^{α}, H^{β}, C^{α}, C^{β} and CO resonances were assigned for all residues except 1–6, K16, P66 and P144. Nearly complete ^{1}H side chain assignments were obtained for non-proline residues. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of uniformly ^{15}N-enriched catalytic domain of CNP. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5202.

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