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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the first cadherin domain of Cadherin-related neuronal receptor (CNR)/protocadherin α

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Biological context

Cadherins form a large family of single-pass transmembrane proteins, which mediate Ca^{2+}-dependent cell–cell adhesion (Yagi and Takeichi, 2000). Cadherins regulate embryonic morphogenesis and also contribute to neurite outgrowth and synaptic modulation in the central nervous system (CNS) (Tepass et al., 2000). The cadherin family can be divided into several subgroups: the classical (type I) cadherins and highly related type II cadherins; the desmosomal cadherins; and the protocadherins. The classical cadherins have been most extensively studied, and their structural determinations have revealed the importance of the first extracellular cadherin (EC1) domain in cell adhesion (Patel et al., 2003). However, the classical cadherins account for only a fraction of the cadherin superfamily, which has multitude of diverse members. It is now found that protocadherins constitute the largest subgroup within the cadherin superfamily. Recently, a novel family of diverse synaptic protocadherin, designated the Cadherin-related neuronal receptor (CNR)/Protocadherin(Pcdh) family, was identified as a viable candidate for neural network organization (Kohmura et al., 1998; Wu and Maniatis, 1999). The proteins in this family are composed of six extracellular (EC1–6) domains, a transmembrane domain and an intracellular domain. The EC1 domain is especially well conserved in mouse CNR/Pcdhα family members, and has an RGD motif. Recently, the adhesive activity of CNR1EC1 via β1 integrin was observed using HEK293T cells (Mutoh et al., 2004). Nevertheless, current knowledge about the function of CNR/Pcdhα is still insufficient, compared to that of classical cadherins, and no protein structure of the protocadherin family is yet known.

Methods and experiments

CNR1–EC1, labeled uniformly with ^{15}N and ^{13}C stable isotopes, was expressed by culturing the bacteria, BL21 (DE3), in an M9 minimal medium containing 1.0 g/l [U−^{13}C]-D-glucose and 0.5 g/l $^{15}NH_4Cl$, as sole carbon and nitrogen sources, respectively. The protein was purified as described elsewhere. The solvent for the purified protein solution was exchanged with a 50 mM Tris-HCl buffer at pH 8.0 containing 80 mM NaCl, 1.5 mM $CaCl_2$, 0.02%(w/v) NaN_3, 0.3 mM PefaBloc, and 8% (v/v) D_2O, using a ultra-filtration (Amicon, Ultra-4).

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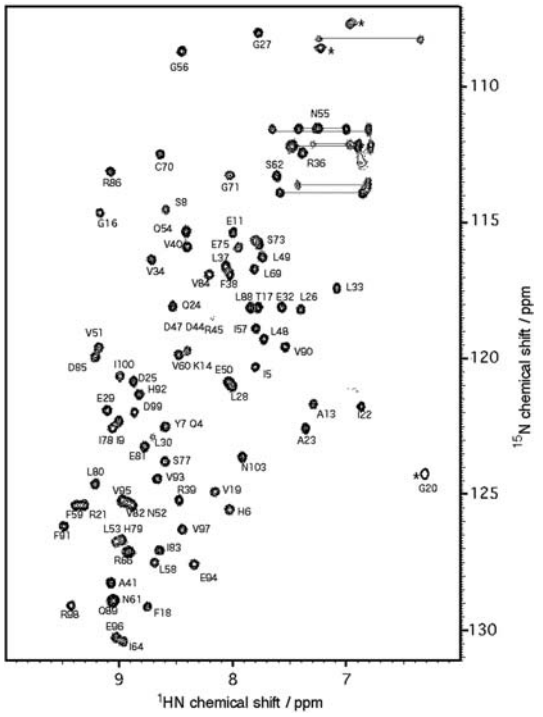

Figure 1. A two-dimensional ^{1}H–^{15}N-HSQC spectrum of uniformly ^{15}N-labeled CNR1/Pcdhα4 EC1. Asterisks (*) indicate the folded peaks, which would otherwise appear at higher ^{15}N fields outside the spectral region shown.

NMR spectra for the resonance assignment of the main-chain were acquired at 303 K with a Bruker DRX-800 NMR spectrometer equipped with a triple resonance (^{1}H, ^{15}N and ^{13}C) cryogenic probe with a *z*-axis gradient coil. Spectra for the side-chain assignments were acquired with a DRX-500 spectrometer equipped with a normal triple resonance probe with a self-shielded triple axis gradient coil.

For the assignments of the ^{1}H, ^{15}N and ^{13}C resonances, a series of two- (2D-) and three-dimensional (3D-) experiments were performed, which were ^{15}N–^{1}H-HSQC, ^{13}C–^{1}H-HSQC, ^{1}H–^{1}H-TOCSY with the mixing time of 57.6 ms, ^{15}N-edited TOCSY with the mixing time of 69 ms, HNCO, HN(CA)CO, CBCA(CO)NH, HNCACB, HBHA(CBCACO)NH, C(CO)NH, H(CCO)NH and HCCH-TOCSY with the mixing times of 20.2 ms (Cavanagh et al., 1996).

Extent of assignments and data deposition

Figure 1 shows a 2D-^{1}H–^{15}N-HSQC spectrum of CNR1/Pcdhα4 EC1 domain with well-dispersed peaks. The backbone assignment was carried out along with the sequential assignment procedure, and 82% of the backbone amide resonances were identified. All the amide resonances observable in ^{1}H–^{15}N-HSQC spectra were unambiguously assigned. Excluding the N-terminus (G1 and N2), the assigned atoms for ^{13}Cα, ^{13}Cβ, ^{13}C$_{O}$, ^{1}Hα, ^{1}Hβ and the other side-chain spins account for 99, 98, 88, 96, 95 and 60%, respectively, of the remaining 101 residues (3–103). The small extent of the side-chain assignments was due to the low signal intensity at the protein concentration as low as 0.1 mM. As for the stereospecific assignments of the methyl groups of 12 leucine and 11 valine residues, however, we have achieved 96% of them using 15% ^{13}C-labeled proteins. Based on the chemical shifts obtained, seven β-strands were identified at residues 4–9, 17–21, 39–42, 48–52, 57–61, 77–84, and 89–98, suggesting that CNR1/Pcdhα4 EC1 domain have a folding topology similar to already determined classical cadherin EC1 structures. The assignment results have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 6405.

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