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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of Ca^{2+}-free DdCAD-1: A Ca^{2+}-dependent cell-cell adhesion molecule

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Biological context

Cell-cell adhesion plays an important role in the regulation of cell proliferation, motility, differentiation, and morphogenesis. The cellular slime mold *Dictyostelium discoideum* provides an excellent model for the study of cell-cell interactions. During development, *D. dictyostelium* cells express several adhesion systems that allow cells to adhere to each other as they migrate to form multicellular aggregates (Fontana, 1995; Siu et al., 1997). Early studies distinguished two major classes of cell adhesion sites (Gerisch, 1980). One class is sensitive to low concentrations of EDTA, while the other is stable in EDTA up to a concentration of 15 mM (Beug et al., 1973). The EDTA-sensitive cell adhesion sites can be divided into two subtypes, the EDTA/EGTA-sensitive adhesion sites and the EDTA-sensitive/EGTA-resistant adhesion sites (Fontana, 1993). The EDTA/EGTA-sensitive sites are mediated by the cell adhesion molecule DdCAD-1, which is encoded by the *cadA* gene and appears soon after the initiation of development (Brar and Siu, 1993; Yang et al., 1997). DdCAD-1 is a unique cell adhesion molecule because it does not contain a signal peptide or a transmembrane domain and shows limited sequence similarities with classical cadherins (Wong et al., 1996). Similar to cadherins, DdCAD-1 is a Ca^{2+}-binding protein and its adhesive activity is dependent on Ca^{2+} (Brar et al., 1993; Wong et al., 1996). The results of disruption of the *cadA* gene indicate that, in addition to cell-cell adhesion, DdCAD-1 plays a role in cell type proportioning and pattern formation (Estella Wong et al., 2002).

Here, we report a nearly complete assignments of backbone and non-aromatic sidechain resonances for the full-length 24 kDa DdCAD in Ca^{2+}-free state as a step towards a better understanding of cell-cell adhesion mediated by DdCAD-1.

Methods and experiments

The gene coding cDNA of DdCAD-1 was subcloned into pET-M over-expression vector and over-expressed in ^{15}N- or ^{15}N, ^{13}C-labeled form in *E. coli BL21 (DE3)* growing in M9-minimal medium containing only ^{15}N-labeled NH_4Cl or/and ^{13}C-labeled glucose as the sole nitrogen and carbon source. The protein was purified by immobilized metal affinity chromatography on Ni-NTA. The N-terminal His-tag was cleaved by thrombin. Ni-NTA and pAminoBenzamidine-Agarose (Sigma) were used to remove His-tag and thrombin, respectively. Finally, EGTA was used to remove Ca^{2+} and gel filtration was applied to obtain monomeric DdCAD-1 in the the Ca^{2+}-free state. The DdCAD-1 protein contains two additional residues (Gly-Ser) coming from the expression vector and lacks a Met at the N-terminus. NMR samples containing ~0.8 mM protein were prepared in 10 mM PIPES buffer, 1 mM EGTA, 1 mM DTT and 50 μM sodium azide at pH 6.2.

NMR experiments were performed at 30 °C on a Bruker Avance 500 MHz spectrometer equipped with pulse field gradient units and an actively shielded cryoprobe. Sequential backbone resonance assignments were obtained using HNCACB, CBCA(CO)NH,

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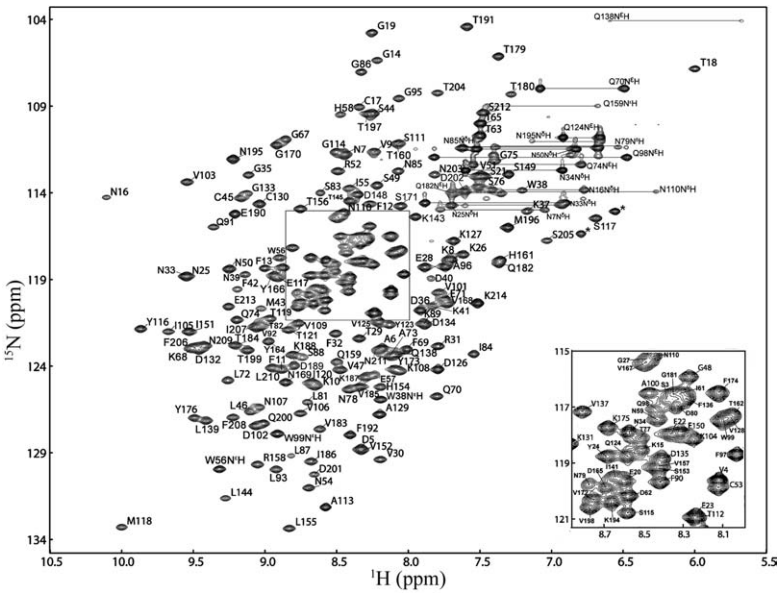

Figure 1. ^{1}H-^{15}N HSQC spectrum of DdCAD-1 acquired at 500 MHz and 30 °C on a sample of 0.8 mM protein, 10 mM PIPES, 1 mM EGTA, 1 mM DTT and 50 μM sodium azide, and pH 6.2. Gln/Asn sidechain cross peaks are denoted with horizontal lines. Unassigned sidechain resonances are indicated by asterisks (*).

HNCO, HN(CA)CO and ^{1}H-^{15}N HSQC experiments. Side chain resonances were assigned based on HCC(CO)NH, CC(CO)NH, HCCH-TOCSY, 3D ^{15}N-edited NOESY, 4D H-^{13}C-^{15}N-H NOESY and 2D ^{1}H-^{13}C HSQC experiments. All NMR data were processed with nmrPipe software and analyzed with nmrView software respectively.

Extent of assignments and data deposition

All the ^{1}H and ^{15}N backbone resonances were assigned except for the first 2 amino acids from the expression vector and residue E60, for which signals could not be detected on the ^{1}H-^{15}N HSQC spectrum. About 95% of the sidechain ^{1}H and ^{13}C resonances were assigned. Sidechain ^{1}H-^{15}N$^{\epsilon}$ peaks from the three Trps were designated, while sidechain NH_2 moieties from Gln and Asn were partially assigned. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of DdCAD-1 monomer in Ca^{2+}-free state. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number BMRB-6159.

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