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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of the N-terminal microtubule-binding domain of human doublecortin

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Biological context

Doublecortin (DCX) is a 40 kDa protein required for normal migration of neurons and formation of cerebral cortex during development (des Portes et al., 1998; Gleeson et al., 1998). Mutations in DCX gene result in females in the X-linked double cortex syndrome, and in males in lissencephaly (smooth brain). Doublecortin interacts with microtubules and stimulates tubulin polymerization and bundling (Gleeson et al., 1999; Horesh et al., 1999). Patient mutations in doublecortin cluster in two regions defining a tandem of homologous stretches, referred to as DCX domains (Sapir et al., 2000; Taylor et al., 2000). Doublecortin does not exhibit any similarity to known microtubule associated proteins (MAPs) and interestingly, may interact with tubulin in a novel manner. Sequence analysis shows no similarity to proteins with known structures, but the DCX domain was predicted to have a β-grasp fold (Taylor et al., 2000). On the basis of previous results showing that the isolated N-terminal DCX domain is required for interaction with tubulin (Taylor et al., 2000) we undertook a structure determination of N-DC (45–150) by NMR spectroscopy. Here in, we report chemical shift assignments and a description of the secondary structure elements of this N-terminal domain of DCX.

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Methods and experiments

Uniformly ^{15}N- as well as $^{15}N/^{13}C$-labeled DCX 45-150 with seven additional residues attached at the N-terminus (113 residues) was overexpressed in *E. coli* as a GST fusion. The protein was purified by GST affinity chromatography, released from GST using rTEV protease, and final purification was achieved by size-exclusion chromatography.

Samples for NMR contained 1 mM protein in 50 mM sodium phosphate buffer with 5 mM DTT, pH 6.0 in 90%H_2O/10% D_2O in Shigemi tubes. NMR spectra were measured at 25 °C on a Varian Inova 500 MHz spectrometer. Backbone assignments were obtained from 3D HNCACB, CBCA(CO)NH, and HNCO spectra. Side chain chemical shifts were assigned from CC(CO)NH-TOCSY, HC(CO)NH-TOCSY and HCCH-TOCSY recorded with 8 and 24 ms mixing times. $^{3}J_{HNH\alpha}$ coupling constants were measured for 98 non-glycine residues using HNHA. All NMR spectra were processed and analyzed using NMRPipe (Delaglio et al., 1995) and Sparky (Goddard and Kneller).

Analysis of chemical shifts using TALOS (Cornilescu et al., 1999) and backbone-backbone NOEs (from ^{15}N-edited NOESY) clearly indicates the presence of five β-strands and one α-helix arranged in a β-β-α-β-β-β fold. The β-strands comprise residues: 53-60, 68-72, 103-106, 111-113, 123-128 while the α-helix is formed by residues 80-89. Figure 1 shows the arrangement of the β-strands obtained from analysis of characteristic inter-strand NOEs. The arrangement of

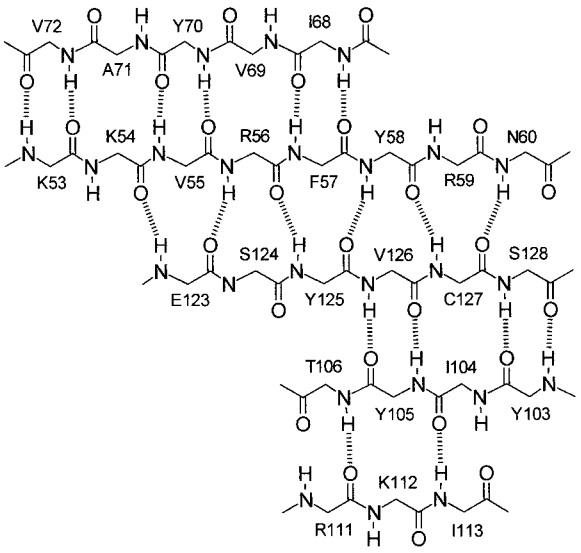

Figure 1. Arrangement of β-strands in DCX 45-150 judged from chemical shift analysis using TALOS and qualitative examination of backbone NOEs from the ^{15}N-edited NOESY spectrum.

the secondary structure elements strongly suggests the presence of a ubiquitin-like fold.

Extent of assignment and data deposition

The backbone of DCX 45-150 was fully assigned with the exception of three backbone $^{1}H^{N}$ and ^{15}N resonances (G67, S74 and N131). The following proton chemical shifts were not assigned due to resonance overlap: H^{ζ} of F77 and F132; H^{γ} of L91; H^{ε} of R76, R78 and H^{δ} of N48 and N149). We were able to unambiguously assign the slowly exchanging side chain $H^{\gamma 1}$ protons of T88 (5.19 ppm) and T106 (4.50 ppm). Interestingly, we also assigned the side chain H^{δ} proton of C127 (1.55 ppm) according to cross-peak analysis in ^{15}N-edited TOCSY. The presence of this rarely observed proton (11 cases in BMRB database) suggests that C127 is buried in the hydrophobic core. Chemical shifts were deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5482.

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