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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C resonance assignment of the PH domain from *C. elegans* UNC-89

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Biological context

Pleckstrin Homology (PH) domains form one of the largest families of signaling domains. Originally identified as an internal repeat in pleckstrin, PH domains have now been found in proteins with widespread functions such as phospholipases, GTPase regulating proteins, protein kinases, and cytoskeletal proteins. A number of PH domains bind to negatively charged phosphatidyl inositol phosphates and to the corresponding soluble headgroups, inositol phosphates, serving as dynamically regulated membrane-anchoring modules (Bottomley et al., 1998). There are also reports on binding to non-lipid ligands, indicating that, although phospholipid binding indisputably is the function for the majority of the studied PH domains, it might not be the whole story (Bottomley et al., 1998).

The known PH domain structures (see Bottomley et al. (1998) for a recent review) show that PH domains are commonly characterized by a strong polarization of their electrostatic potential with a prominent positively charged pole. It has been suggested that electrostatic interactions play an important role in correctly orienting the PH domains on membranes, where the positive pole would interact with negatively charged phospholipids. In a modeling study of the PH domain family we identified a number of PH domains with strong negative overall potential, and we concluded that binding of negatively charged phospholipids to these domains is highly unlikely (Blomberg and Nilges, 1997).

Interestingly, most of these negatively charged PH domains are found in proteins belonging to the Dbl oncogene family. A hallmark of this family is the Dbl homology domain, a specific exchange factor for the Rho class of small GTPases, in tandem with a PH domain (Cerione and Zheng, 1996). The DH-PH domain is also found in a number of cytoskeletal proteins and in UNC-89, a giant protein (732 kDa) associated with the M-line of body wall muscle in *C. elegans*. The protein is mainly composed of Ig-domains but has an N-terminal SH3 domain followed by a DH-PH pair. The presence of these signaling domains suggests a link between cell signaling and muscle organisation and led to the suggestion that UNC-89 functions in muscle assembly by responding to cues laid out in the membrane (Benian et al., 1996).

A homology model of the PH domain of UNC-89 exhibits a strong negative potential, and the domain does not bind to inositol phosphate (Blomberg and Nilges, unpublished data). We are determining the structure of this PH domain in order to get additional insight into possible ligands for this class of uncharacterised negatively charged PH domains. Here, we present the resonance and secondary structure assignment for this PH domain. The secondary structure assignment allowed us to correct the sequence alignment of this domain, where the first strand was misaligned by six residues. Thus, the data presented in this paper allowed us to improve the structural model of this protein and analyse the electrostatic properties in more detail. The increased availability of structural homologues for any given sequence from structural genomics projects opens up new possibilities for the use of NMR spectroscopy in combination with molecular modeling techniques to approach biological problems.

Methods and results

The PH domain of *C. elegans* UNC-89 (residues 341–458) was produced in *E. coli* from the pBAT4 expression vector (Peränen et al., 1996) by induction of

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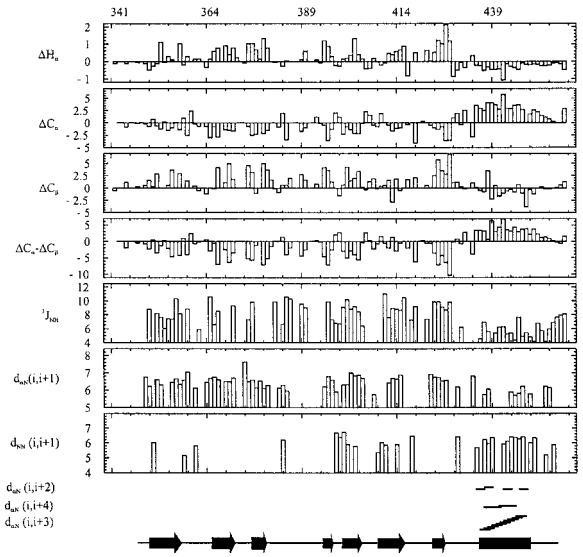

Figure 1. Summary of NMR data for secondary structure determination. From top to bottom: Deviations from random coil chemical shifts for H_α, C_α, and C_β resonances, $^3J_{HNH\alpha}$ coupling constants, and intensities of sequential alpha-amide and amide-amide proton NOEs (peak volume, arbitrary logarithmic scale). Helix-type NOEs are summarized at the bottom, together with a cartoon of the secondary structure elements.

BL21 cells in mid-log phase ($OD_{600} \approx 0.5-1.0$) with 1.0 mM IPTG. Cells were harvested after 4 h. The protein was purified by anion exchange chromatography (Q-Sepharose FF) at pH 8.0 and subsequent size-exclusion chromatography (Superdex-75) into NMR buffer (sodium phosphate, 5 mM, pH 6.8, 50 mM NaCl). NMR samples were 600 to 800 μM protein in 95 % H_2O / 5% D_2O in volumes of 300 μl (labelled) or 500 μl (unlabelled). The samples had a tendency to aggregate and precipitate and it was found that addition of 10–15% deuterated DMSO was critical for long term stability. Addition of 15% DMSO produced only minor changes in chemical shift and it is therefore unlikely that the structure is affected. U-^{15}N and U-^{13}C, ^{15}N samples were produced in standard M9 media supplemented with ^{15}N ammonium chloride and ^{13}C glucose (Martek Biosciences). The U-^{13}C, ^{15}N sample was exchanged into $>$ 99% D_2O by lyophilisation.

All NMR experiments were recorded at 303 K on Bruker DRX600 or DRX500 spectrometers equipped with pulsed field gradient triple resonance probes. Backbone assignments were obtained by a combination of sensitivity-enhanced CBCA(CO)NH/CBCANH, HNCA/HN(CO)CA, HBHA(CO)NH and ^{15}N-HSQC NOESY experiments (Sattler et al., 1999) using water flip-back pulses where appropriate. Side chains were assigned from HCCH-TOCSY and ^{13}C/^{15}N heteronuclear NOESY experiments (Sattler et al., 1999). Aromatic side chains were mainly assigned from homonuclear NOESY experiments acquired in D_2O, since heteronuclear NOESYs optimized for the detection of aromatic ^{13}C/^{1}H signals suffered from poor signal-to-noise. Spectra were processed with NMRPipe (Delaglio et al., 1995) and analysed with XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

The assignment is essentially complete for residues 9–119 of our construct (corresponding to residues 348–458 of UNC-89). Amide proton resonances from residues 363, 373–74, 387–88, 391–92 are not visible and therefore missing from the assignment. Residues P420 and E433 have less than four resonances assigned. Residues 386 and 389 have small R1/R2 ratios from relaxation experiments, and the other missing residues are located in loops. Secondary structure assignment has been made based on NOE patterns and secondary chemical shifts for C_α and C_β resonances (Wishart et al., 1994). The ^{1}H, ^{13}C, and ^{15}N chemical shifts of the UNC-89 PH domain have been deposited at BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4373.

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