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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of the N-terminal region of calponin

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Biological context

The N-terminal region of the smooth muscle protein calponin contains a motif of ∼110 residues that has been identified in a large number of cytoskeletal and signal-transducing proteins (reviewed in Stradal et al., 1998) and has been termed the calponin homology (CH) domain. This motif occurs in proteins either as a single domain, or as tandem pairs of CH domains that together form a complete actin-binding domain (ABD) in these molecules. A phylogenetic analysis of all known CH domain-containing proteins has identified five distinct sub-groups of the CH domain, four of which comprise CH domains forming ABDs (i.e. from proteins containing pairs of CH domains) and one which comprises the CH domains from all the proteins that contain a single copy of this motif (Stradal et al., 1998). The CH domain of calponin belongs to this last group and, like several other proteins containing single CH domains, calponin also binds to actin, although the involvement of the single CH domain in this function remains equivocal.

There are published crystal structures of several CH domains from ABDs (Keep et al., 1999, and references therein); however, there are, as yet, no reported structures of CH domains from proteins containing only a single CH domain and there are no reported solution structures of any CH domain. Here we present the ^{1}H, ^{15}N and ^{13}C resonance assignments for the archetypal CH domain from chicken calponin, the first report of a structural investigation of a CH domain from this family of proteins.

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Methods and experiments

A modified pET vector (see Keep et al., 1999) encoding residues 27–134 of chicken gizzard calponin was transformed into *Escherichia coli* BL21(DE3). Overexpression of unlabelled protein was achieved by growth in 2× YT medium (37 °C, 100 mg L^{-1} ampicillin) to an OD_{600} of 0.6–0.8 followed by induction with isopropyl-β-D-thiogalactopyranoside (IPTG) (0.5 mM, 2 h). ^{15}N-, and ^{15}N-/^{13}C-labelled proteins were expressed with IPTG induction in M9 minimal medium modified with appropriately labelled ammonium sulphate ($(^{15}NH_4)_2SO_4$, >98% ^{15}N) and glucose (^{13}C-glucose, >99% ^{13}C). Following harvesting by centrifugation (5000 g, 15 min, 4 °C), inclusion bodies were isolated from the cell pellet, the protein was extracted by denaturation (6M guanidine hydrochloride) and purified, whilst denatured, using immobilised metal-ion affinity chromatography (Cu^{2+} immobilised on Chelating Sepharose Fast Flow, Pharmacia Biotech), reverse-phase chromatography (Source 15RPC, Pharmacia Biotech) and gel filtration (Superdex75, Pharmacia Biotech). The purified protein was refolded by gradual removal of the GdHCl by dialysis at 4 °C and concentrated for the NMR studies in the final buffer (1 mM sodium phosphate, pH 7.0). The resulting NMR samples contained 0.5–1.0 mM protein in 95% H_2O/5% $^{2}H_2O$. The size of the purified CH domain was verified by SDS-PAGE and electrospray mass spectrometry; the conditions for producing and maintaining the protein in a stable and folded form were determined by one-dimensional ^{1}H NMR spectroscopy. To prevent sample precipitation, the protein was kept in a salt-free solution at pH 7.0 and relatively low temperatures (18 °C for the

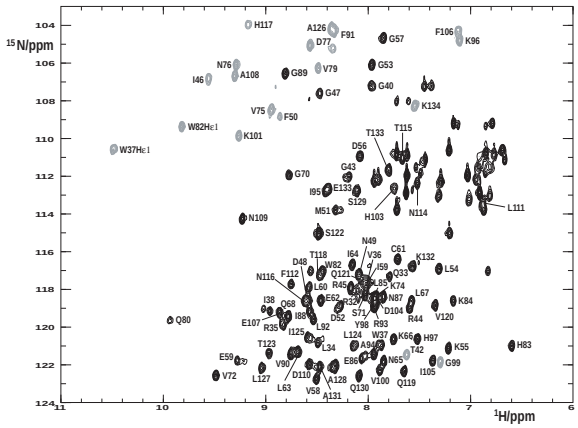

Figure 1. Two-dimensional ^{1}H,^{15}N-HSQC spectrum of the CH domain from chicken calponin (1 mM, pH 7.0, 18 °C) acquired at 600 MHz; cross peaks folded in the ^{15}N dimension are shown in dotted lines. The residue-specific assignment of the cross peaks arising from the backbone amide groups and the tryptophan side-chains is indicated; the remaining cross peaks correspond to the amide groups of asparagines and glutamines.

NMR experiments). NMR spectra were acquired on a Varian INOVA 600 MHz spectrometer using a 5 mm triple resonance probe equipped with triaxial gradients. All data were processed using the Azara suite of programmes provided by W. Boucher and the Department of Biochemistry, University of Cambridge, U.K. (the code may be obtained via anonymous ftp to www.bio.cam.ac.uk in the directory ~ftp/pub/azara) and analysed with ANSIG (Kraulis et al., 1989).

The majority of the resonance assignments for the backbone atoms were obtained using ^{1}H,^{15}N-HSQC (Figure 1) in parallel with the following 3D experiments that share the same ^{1}H and ^{15}N dimensions: HNCACB and CBCA(CO)NH (Muhandiram and Kay, 1994), HBHA(CBCA)NH (Wang et al., 1994), H(C)(CO)NH-TOCSY (Grzesiek and Bax, 1992) and HNCO (Kay et al., 1994). The assignment of the aliphatic side-chain resonances was achieved using (H)C(CO)NH-TOCSY (Grzesiek et al., 1993), H(C)(CO)NH-TOCSY and HCCH-TOCSY (Kay et al., 1993), and the aromatic resonances for each histidine $H_{\delta 2}$, tryptophan $H_{\delta 1}$, tyrosine H_{δ} and H_{ε}, and phenylalanine H_{δ} and H_{ε}, were assigned using the 2D (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HE experiments (Yamazaki et al., 1993). The recently reported ^{13}C,^{15}N HSQC-NOESY-CH_3NH experiment (Uhrín et al., in press) was used to confirm and complete the assignments.

Extent of assignments and data deposition

The $^{1}H_N$ and ^{15}N resonances for 99 out of 104 possible (108 less 4 prolines) backbone amides were assigned (95%) and the ^{13}CO resonances of the 99 corresponding 'i – 1' residues were assigned using the HNCO; 3 of the 5 missing amide resonances are from N-terminal residues. A complete set of chemical shift values for all of the side-chain atoms (^{1}H, ^{15}N and ^{13}C) has been obtained except for those of the labile groups and for 3 of the 4 histidine $H_{\varepsilon 1}$ and $C_{\varepsilon 1}$, where only that of H83 could be specifically assigned. The amide $^{15}NH_2$ and ^{13}CO resonances have been assigned for all the asparagines and glutamines; however, no resonances arising from the lysine amino, arginine guanido or histidine $N_{\delta 1}/H_{\delta 1}$ and $N_{\varepsilon 2}/H_{\varepsilon 2}$ groups are observed. The $^{1}H_{\gamma 1}$'s of T42 and T123 and the $^{1}H_{\gamma}$ of S71 have been assigned; however, no resonances are observed for the remaining hydroxyls and for the thiol of C61.

The chemical shifts of ^{1}H, ^{15}N and ^{13}C have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 4880.

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