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Letter to the Editor: Backbone and side-chain ^{1}H, ^{15}N, and ^{13}C assignments for chick cofilin

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Biological context

Cofilin is an actin-binding protein that is present in virtually all eukaryotic cells. It belongs to the actin depolymerizing factor (ADF) family of proteins that are considered to be the major regulators of actin microfilament assembly (Bamburg, 1999). They achieve this function by accelerating the rate of dissociation from the so-called 'pointed' ends of actin filaments, thereby increasing the concentration of actin monomers that can be reassembled at the 'barbed' end. The activity of cofilin is regulated by pH, phosphorylation, and the binding of various ligands including phosphatidylinositides (Bamburg, 1999).

The structure of four ADFs have been described. X-ray diffraction was used to resolve the atomic structure of yeast cofilin (Fedorov et al., 1997), *Acanthamoeba* actophorin (Leonard et al., 1997), and *Arabidopsis thaliana* ADF1 (Bowman et al., 2000), and an NMR structure of porcine destrin (Hatanaka et al., 1996) has been determined. However, unlike several other actin-binding proteins, no structures are available of cofilin in complex with actin or other relevant ligands. Putative phosphatidylinositide and actin-binding sites have been proposed but the exact location of these sites is unclear and it is not known whether ligand binding induces a conformational change in cofilin. In order to address these questions, we have obtained complete ^{1}H, ^{15}N, and ^{13}C chemical shift assignments for chick cofilin (166 residues, $M_W = 18\,663$ Da).

Methods and results

Chick embryonic skeletal muscle cofilin (Abe et al., 1990) was expressed in *Escherichia coli* XLI-Blue cells as a translational fusion to the C-terminus of glutathione *S*-transferase (GST), with an intervening thrombin recognition site. The overexpression strain was a gift from Dr Takashi Obinata (Chiba, Japan). GST-cofilin was purified from the soluble cell fraction using glutathione affinity chromatography and cleaved on-column with thrombin as described previously (Tedford et al., 2001). Recombinant cofilin was then eluted and further purified using cation exchange chromatography. NMR samples were prepared by dialyzing cofilin against NMR buffer (50 mM KCl, 10 mM Pipes, 0.02% NaN_3, pH 6.8) and concentrating the protein to ~1 mM. Expression of cofilin as a thrombin-cleavable GST fusion protein results in the purified protein having two non-native residues (Gly–Ser) appended to the N-terminus; the recombinant cofilin thus contains 168 residues ($M_w = 18\,807$ Da).

NMR experiments were performed at 298 K on Varian INOVA 500 or 600 MHz spectrometers. The data were processed with nmrPipe (Delaglio et al., 1995) using our web-based script generator (http://sbtools.uchc.edu/nmr/). Spectra were analyzed using XEASY (Bartels et al., 1995). $^{1}H_N$, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$, and $^{13}C'$ resonance assignments were made using HNCACB, CBCA(CO)NH, and HNCO spectra. Most side-chain ^{1}H, ^{15}N, and ^{13}C assignments were obtained from HC(CO)NH-TOCSY and C(CO)NH-TOCSY experiments. Additional side-chain assignments were obtained from analysis of HCCH-TOCSY spectra, and ambiguities were resolved using an HCCH-COSY spectrum. Aromatic side-chain ^{1}H assignments were obtained using a 2D (HB)CB(CGCD)HD experiment as well as ^{15}N- and ^{13}C-edited 3D NOESY-HSQC spectra. The HCCH-COSY, HCCH-TOCSY, and NOESY data were ac-

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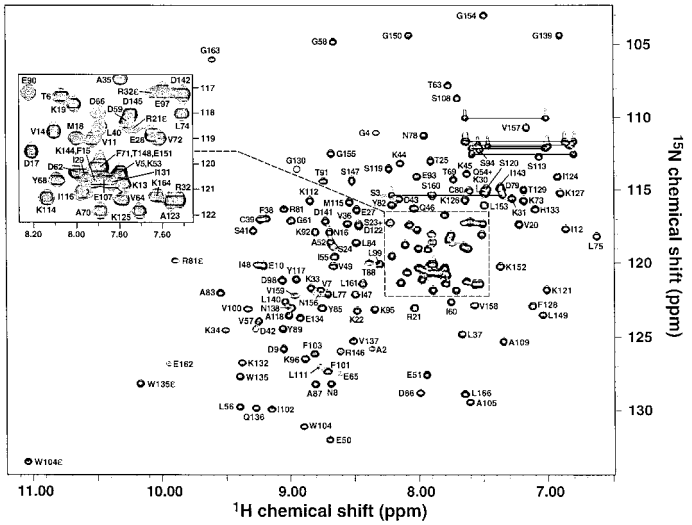

Figure 1. The fully assigned ^{1}H-^{15}N HSQC spectrum of cofilin at 298 K, pH 6.8. The insert shows an expansion of the most-crowded region. Gln/Asn sidechain cross peaks are connected by horizontal lines but are not labeled for the sake of clarity. The cross peak for Gly 130 is visible at lower contour levels at the position indicated by the open circle.

quired at 600 MHz; all other data were acquired at 500 MHz.

Extent of assignments and data deposition

Complete backbone $^{1}H_N$, ^{15}N, and $^{13}C_\alpha$ assignments were obtained for all 166 residues. $^{13}C'$ assignments were obtained for all residues except G150 and the six residues preceding a proline. Overall, the backbone assignments are 99% complete. The 23 lysine residues presented a particular challenge and the HCCH-COSY proved essential for obtaining unambiguous side-chain assignments. Excluding the highly labile Lys/Arg NH_2 protons, which are not visible in any of the spectra at this relatively high pH (6.8), the side-chain ^{1}H assignments are >99% complete. Side-chain ^{15}N nuclei were completely assigned with the exception of the $^{15}N_\varepsilon$ of R146 and the $^{15}N_{\delta 1}$ and $^{15}N_{\varepsilon 2}$ nuclei of H133. If the Glu/Asp carboxyl and aromatic ring nuclei are excluded, the side-chain ^{13}C assignments are 98% complete; only $^{13}C_\varepsilon$ of M1 and K34 are not assigned.

During the assignment process we detected a minor error in the reported sequence of chick cofilin. Residues 53–54, reported to be Thr-Arg (Swiss-Prot P21566), are in fact Lys-Gln. The NMR result was confirmed by re-sequencing chick cofilin cDNA (H. Abe and T. Obinata, personal communication). The revised sequence is more in accordance with the seven other currently available vertebrate cofilin sequences in which residue 53 is *always* Lys and residue 54 is *always* Glu or Gln.

In summary, we have obtained almost complete ^{1}H, ^{15}N, and ^{13}C assignments for chick cofilin; these chemical shift assignments have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BRMB accession number 5177. The 2D HSQC spectrum of chick cofilin (Figure 1) is well resolved and it provides a platform for studying the interactions of cofilin with various ligands as well as a facile means for monitoring structural changes in cofilin induced by pH, ligand binding, and phosphorylation.

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