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# Letter to the Editor: Backbone and sidechain $^{1}H$, $^{13}C$ and $^{15}N$ resonance assignments of human cofilin

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*Abbreviations:* ADF: actin depolymerizing factor; A/C: ADF/cofilin; NaPi: sodium phosphate buffer

## Biological context

The actin depolymerizing factor (ADF)/cofilin (A/C) proteins play an essential role in the regulation of eukaryotic actin filament dynamics. These 15-20 kDa, actin-binding proteins are involved in a range of motile activities such as cell locomotion, cytokinesis, endocytosis and muscle development (for review see Bamburg (1999)). They increase actin filament turnover in vitro by severing filaments (Maciver et al., 1998) and accelerating the rate of dissociation of subunits from their pointed ends (Carlier et al., 1997). In addition, they modify the pitch of the F-actin helix (McGough et al., 1997) which weakens longitudinal actin-actin contacts (McGough and Chiu 1999) and inter-subunit interactions (Bobkov et al., 2002), thereby facilitating the biochemical effects observed above.

Human ADF and cofilin are highly homologous but display notable differences in their biological activities. Principally, ADF shows a much higher depolymerizing activity than cofilin. In addition, unlike other A/C proteins, ADF and cofilin depolymerize F-actin in a pH-dependent manner. To understand the origins of these differences, the three dimensional structure of cofilin is needed so that it can be closely compared with that of ADF (destrin) (Hatanaka et al., 1996). To this end, we have expressed and assigned the backbone and sidechain $^{1}H$, $^{13}C$ and $^{15}N$ resonances of human cofilin. This information will also be valuable for locating the G-actin binding site and identifying specific residues responsible for the pH sensitivity of the proteins' activity as well as for investigating cofilin binding to other ligands.

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## Methods and experiments

### *Expression and purification of human cofilin*

Expression and purification of human cofilin was essentially as described previously (Giuliano et al., 1988, Hawkins et al., 1993). Uniformly $^{15}N$-labelled (U-$^{15}N$) cofilin was expressed in BL21(DE3) cells containing the plasmid (human cofilin in pMW172 (McGough et al., 1997)) and grown in 500 ml M9 minimal medium (50 μg/ml ampicillin) containing 18 mM $^{15}NH_4Cl$ as the sole nitrogen source, 0.4% glucose, 80 mM MOPS, 8 mM Tricine pH 7.4, 50 mM NaCl, 1 mM $MgCl_2$, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$ plus micronutrients. Double labelled cofilin (U-$^{13}C$, $^{15}N$) was grown in a commercially prepared medium produced from bacterial hydrolysate (OD2 EC-CN, Silantes, Germany). Best yields were obtained after 36 h at 37 °C with shaking at ∼150 rpm. As the T7 promoter is not tightly regulated, no induction was necessary. Cells were treated with lysozyme (5 mg/l of original cell culture), DNase1 (150 μg/L of cells), 1 mM DTT, sonicated to maximise release of soluble ADF/cofilin and clarified. Pooled supernatants were loaded onto a 50 ml Whatman DE52 cellulose column. Cofilin eluted in the flow through, which was concentrated to 10 ml and loaded onto a 5 ml Green A column (Millipore, MA, U.S.A.). Cofilin was then eluted with a salt gradient (40:40 ml) to 0.4 M NaCl and dialysed against NMR buffer: 10 mM NaPi,

25 mM NaCl, 1 mM DTT, 0.2 mM EGTA, 1 mM $NaN_3$ (pH 6.0). Proteins were concentrated to between 200 μM and 1 mM for NMR experiments. Concentrations were determined by UV at 280 nm using $A_{280}$ of 1.0 $cm^{-1}$ = 74 μM. Protein MW was confirmed by mass spectrometry which showed that, as expected for *E. coli* expressed proteins, the N-terminal Met was not processed.

## NMR spectroscopy

NMR spectra were acquired at 300 K, using Bruker DRX 600 and DMX 750 spectrometers with triple resonance probes equipped with self-shielded triple axis gradient coils. Spectra for the resonance and NOE assignment were recorded essentially as described in the original references. A 1 mM $^{15}N$-labelled sample in 90% $H_2O$/10% $D_2O$ (NMR buffer; pH 6.0) was used for 3D $^{15}N$-edited TOCSY-HSQC, NOESY-HSQC, HNHB, HNHA, $^{15}N$ $T_1$ and $^{15}N$ $T_2$ relaxation, and heteronuclear $^{15}N$-$^{1}H$ NOE experiments. A 0.8 mM $^{13}C$,$^{15}N$-labelled sample in 90% $H_2O$/10% $D_2O$ (NMR buffer; pH 6.0) was used for 3D CBCA(CO)NNH, CBCANNH, CC(CO)NNH and H(CCCO)NNH and for two 3D $^{13}C$-separated NOESY spectra (aliphatic and aromatic centred; each acquired in $H_2O$ to allow observation of $H_C$-$H_N$ NOEs). Data were processed using the programs XWIN-NMR (version 1.3) of Bruker BioSpin (Rheinstetten, Germany) and AZARA (version 2.1) of W. Boucher (unpublished). Assignment of $^{13}C$, $^{15}N$ and $^{1}H$ resonances was carried out on Silicon Graphics O2 workstations and a P2-566 PC, using the interactive program ANSIG version 3.3 (Kraulis 1989).

## Extent of assignment and data deposition

The assigned $^{15}N$ HSQC spectrum of the 166 residue cofilin protein (Swissprot accession SP:P18282) is shown in Figure 1. With the exception of the extreme N-terminal residues A2 and S3 which were not detected in our experiments, all backbone $^{1}H$, $^{13}C_{\alpha}$ and $^{15}N$ resonances were assigned. The extreme overlap of the 25 lysines in the protein meant that 19 Lys Cδ, and 23 Cε, 7 Lys Hδ and 23 Hε resonances could not assigned unambiguously. Almost all remaining sidechain $^{13}C$ and $^{1}H$ resonances were assigned. All Asn and Gln

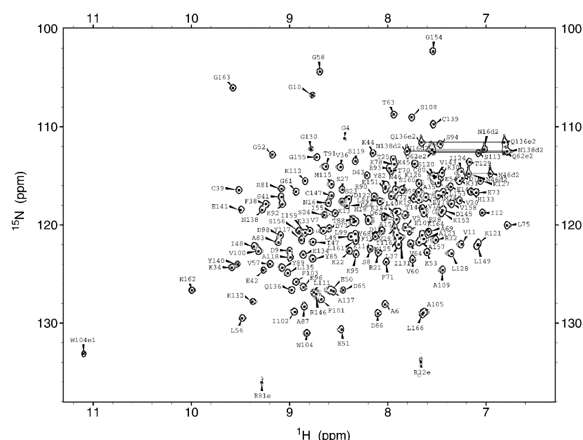


*Figure 1.* Assigned $^{1}H$-$^{15}N$-HSQC spectrum of human cofilin recorded at 300 K and 750 MHz.

sidechain $NH_2$ groups were assigned, as were the $^{15}N\varepsilon$ atoms of R32 and R81 (see Figure 1). Remaining Arg sidechains and the His $^{15}N\delta1$ and $^{15}N\varepsilon2$ were not detected. The assignments are deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession code BMRB-6004.


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