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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of coactosin, a cytoskeletal regulatory protein

Maarit Hellman[a,*], Ville Paavilainen[b], Arto Annila[a], Pekka Lappalainen[b] & Perttu Permi[a]
Programs in [a]Structural Biology and Biophysics and [b]Cellular Biotechnology, Institute of Biotechnology, P.O. Box 65, University of Helsinki, FIN-00014, Helsinki, Finland

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Biological context

The actin cytoskeleton participates in a wide variety of biological processes in cells, such as endocytosis, exocytosis, cell movement and division. Actin dynamics are regulated by a large number of actin-binding proteins that interact either with monomeric actin (G-actin) or with actin filaments (F-actin) or with both. Coactosin is a ∼145-residue actin filament-binding protein. It was first isolated from actin-myosin complexes from *Dictyostelium discoideum* (de Hostos et al., 1993). More recently, coactosin homologues have been identified from *Homo sapiens* and *Mus musculus* (Doucet et al., 2002), suggesting that it is an evolutionarily conserved regulator of the actin cytoskeleton. The exact mechanism by which coactosin regulates actin dynamics is not known, although it has been reported that coactosin may be an inhibitor of actin filament barbed-end capping (Röhrig et al., 1995).

Comparison of coactosin sequence to those in the database suggests that coactosin contains an actin depolymerizing factor homology (ADF-H) domain (Lappalainen et al., 1998). Members of this protein family are categorized as ADF/cofilins, Abp1/debrins and twinfilins. They all are actin-binding proteins with distinct biochemical functions. ADF/cofilins interact with both monomeric and filamentous actin and promote rapid actin dynamics in cells by increasing the depolymerization rate of actin filaments. Twinfilins are actin monomer-binding proteins that consist of two ADF-H domains. Abp1/drebrins are actin filament-binding proteins that contain an ADF-H domain in their N-terminal region. ADF/cofilins and twinfilin's N-teminal ADF-H domain share similar overall folds, however, atomic resolution structures provided an explanation for their biochemical differences (Paavilainen et al., 2002). To reveal coactosin's function and its evolutionary locus in the ADF-H family, we have now taken first steps towards the structure elucidation of coactosin.

*To whom correspondence should be addressed. E-mail: Maarit.Hellman@helsinki.fi

Methods and experiments

Expression and purification

Recombinant coactosin, cloned from *Mus musculus*, (GenBank Accession No AI325867) was overexpressed in the pRat5 expression vector (Peränen et al., 1996) in *E. coli* strain BL21(DE3). Uniformly ^{15}N- or ^{15}N,^{13}C-labeled proteins were prepared by growing cells in M9 minimal media with $^{15}NH_4Cl$ (1 g/l) and [^{13}C]-glucose (2 g/l) as the sole nitrogen and carbon sources, respectively. The coactosin protein was subsequently purified using anion exchange chromatography (Q-Sepharose Fast-Flow, Amersham Pharmacia), hydroxyl-apatite chromatography (Macro-prep CHT II, Bio-Rad) and size exclusion chromatography (Superdex75 16/60, Amersham Pharmacia). The degree of isotope labeling and protein composition were analyzed by mass spectrometry and shown to be >95% in all cases. The yield of purified protein was 55 mg per liter, which was comparable to the yield obtained from expression in rich medium. For NMR studies samples of 1–1.2 mM coactosin in 10 mM Bis-Tris (pH 6.0), 50 mM NaCl, 1 mM DTT and H_2O/D_2O (9:1) were prepared.

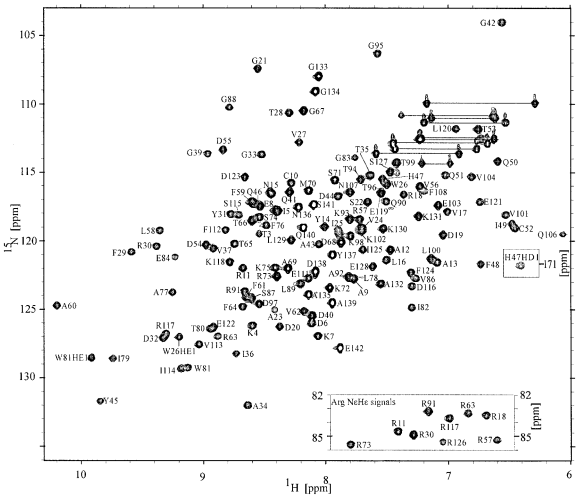

Figure 1. A sensitivity enhanced $^{1}H^{N}$-^{15}N HSQC spectrum of 1.2 mM ^{15}N-labeled coactosin sample acquired at 25 °C on a 800 MHz spectrometer in 15 min. Signals connected by horizontal lines, correspond to the side-chain amide groups of glutamines and asparagines.

NMR spectroscopy, data processing and analysis

All spectra were acquired at 25 °C using Varian UNITY Inova 600 and 800 MHz spectrometers equipped with triple-resonance z-axis and 3-axis gradient probes, respectively. The double- and triple-resonance experiments performed for the sequence-specific backbone and side-chain assignments included 2D ^{15}N-HSQC, ^{13}C-HSQC, ^{13}C-(CT)-HSQC, 3D HNCA, HN(CO)CA, iHNCA (Permi, 2002), HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, HC(C)H-COSY, H(C)CH-TOCSY, (H)C(CO)NH, H(C)(CO)NH, NOESY-^{15}N-HSQC, NOESY-^{13}C-HSQC. Spectra were processed using VNMR and converted to Sparky (Goddard and Kneller, 2002) for spectral analysis. The main chain assignment was facilitated using AutoAssign software package (Zimmermann et al., 1997).

Extent of assignments and data deposition

The coactosin displays well-dispersed signals with uniform intensities (Figure 1). The AutoAssign program was able to derive 85 % of the backbone $^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$ and $^{13}C'$ and $^{13}C^{\beta}$ assignments. Subsequently the assignments were finalized manually to completeness. In $^{1}H^{N}-^{15}N$ HSQC spectrum, 138 backbone HN resonances out of 140 expected non-proline residues were observable. Only signals of N-terminal Met1 were completely missing, whereas backbone C^{α}/H^{α} and C' resonances for C-terminal E142 remained unidentified. All side-chain $^{1}H/^{13}C$ chemical shifts for a total of 15 aromatic residues were assigned. Side-chain carboxyamide ^{1}H, ^{13}C and ^{15}N chemical shifts for asparagines and glutamines, as well as $N^{\varepsilon}/H^{\varepsilon}$ resonances of arginines were completely assigned. All, but three C^{ε} and one $C^{\varepsilon}/H^{\varepsilon}$ resonances of 11 lysines, were distinguishable.

The chemical shifts of $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{13}C'$ in comparison with random coil shifts (Wishart and Sykes, 1994) reveal that coactosin comprises of helices and β-sheets in accordance with the known structures of ADF-H family. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 6032.

Acknowledgements

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