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NMR assignments of actin depolymerizing factor (ADF) like UNC-60A and cofilin like UNC-60B proteins of *Caenorhabditis elegans*

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Abstract

The actin filament dynamics in nematode, *Caenorhabditis elegans*, is regulated by differential activity of two proteins UNC-60A and UNC-60B. UNC-60A exhibits strong pointed end depolymerization on *C. elegans* actin (Ce-actin), strong inhibition of polymerization, strong monomer sequestering activity, weak severing activity, and low affinity for F-actin binding, while UNC-60B exhibits strong pointed end depolymerization on rabbit muscle actin, strong severing activity, and high affinity for F-actin binding. Structural characterization of these proteins will help to understand (1) molecular mechanism of actin dynamics regulation and (2) the differential activity of these proteins. Here, we report ¹H, ¹³C, and ¹⁵N chemical shift assignments of these two proteins as determined by heteronuclear NMR experiments (at pH 6.5 and temperature 298 K).

Keywords

NMR; Resonance assignment; ADF like protein; UNC-60A; UNC-60B

Biological context

Actin filament plays several important roles in the eukaryotic organisms including movement of cell, cytokinesis etc. The dynamics of Actin filament is controlled by various actin binding proteins. The ADF/cofilin family proteins form an important family of actin binding proteins in regulation of actin filament dynamics (Ono 2007). The proteins of this family bind to monomeric actin (G-actin) as well as filamentous actin (F-actin). In nematode *Caenorhabditis elegans*, two ADF/cofilin family proteins, UNC-60A and UNC-60B, are expressed from the *unc60* gene by alternative splicing (Ono and Benian 1998). Mutation in

the *unc60* gene results in slow movement of or paralyzed nematode, because *unc60* gene is responsible for proper positioning and correct number of thin filaments (Ono and Benian 1998).

UNC-60A and UNC-60B proteins are 165 and 152 amino acids long, respectively, and these proteins show low sequence similarity with ADF/cofilins from other organisms, and are at an equal distance away from vertebrate ADFs and cofilins (Ono and Benian 1998). UNC-60A is expressed in the various tissues and is required for early embryogenesis, whereas UNC-60B is specially expressed in the body-wall muscle and is essential for myofibril assembly (Ono et al. 1999). The two proteins differentially regulate the actin filament dynamics and, therefore, slightly differ in their activities. On the basis of the characteristic activities of ADFs and cofilins, UNC-60A is classified into the ADF subgroup, while UNC-60B is classified in the cofilin subgroup (Yamashiro et al. 2005). Sequence alignment of UNC-60A and UNC-60B shows the insertion of four amino acids from K35 to V38 and insertion of eight amino acids from Ile50 to Asp57 in the UNC-60A in comparison to UNC-60B, out of which four residues are acidic. UNC-60A maintains high concentration of monomeric actin (G-actin) by monomeric sequestering activity, strong inhibition of polymerization and strong pointed end depolymerization of F-actin. UNC-60A does not co-sediment with F-actin filament, and has weak severing activity whereas UNC-60B co-sediments with actin filament and shows greater severing activity (Yamashiro et al. 2005). Severing activity of ADF/cofilin is responsible for maintaining the filament turnover; therefore, UNC-60B shows greater impact on actin filament dynamics. Both of the isoforms indicate low pH sensitivity in comparison to other ADF/cofilin isoforms of vertebrates. UNC-60A inhibits actin polymerization in a concentration-dependent manner. Both, UNC-60A and UNC-60B, bind to the monomeric form of actin (G-actin), and decrease the rate of nucleotide exchange in a dose dependent manner (Yamashiro et al. 2005).

The differences in the activities of the UNC-60A and UNC-60B proteins are likely to result from their structural differences. Determination of solution structure of UNC-60A and UNC-60B proteins would allow us to probe the role of structure and dynamics as a determining factor for specific activities of these proteins. Therefore, we have undertaken to determine the structures of UNC-60A and UNC-60B by using NMR spectroscopy.

Methods and experiments

Clones of UNC-60A and UNC-60B were obtained from Dr. Shoichiro Ono in expression vector pET-3d (Ono and Benian 1998). The *C. elegans unc60a* and *unc60b* genes were subcloned into pETNH6 vector using restriction site *NcoI* and *BamHI*. The clones were over-expressed in BL21 (λ DE3) strain of *E. coli*. The cloning procedure added extra residues at N-terminal, including a hexa-histidine tag and TEV-protease site. Conditions for optimal over-expression and purification were standardized. The yields of purified proteins were 30 and 40 mg/L of culture medium, for UNC-60A and UNC-60B, respectively. The expressed protein was digested with TEV-protease and was re-purified. This procedure added two additional residues (G and A) at the N-terminals of both these proteins. For isotopic labeling, over-expression of UNC-60A and UNC-60B were standardized in minimal

media containing ^{15}N -ammonium sulfate and ^{13}C -glucose (CIL, MA, USA) as the sole nitrogen and carbon sources, respectively.

NMR samples of $^{13}\text{C}/^{15}\text{N}$ -labeled UNC-60A and UNC-60B were prepared at concentration of approximately 0.65 and 1.00 mM in NMR buffer (20 mM sodium phosphate pH 6.5, 50 mM NaCl, and 0.1 % NaN_3) containing 93 % $\text{H}_2\text{O}/7$ % $^2\text{H}_2\text{O}$. For backbone and side-chain resonance assignments, the following experiments were acquired: two-dimensional ^{15}N - ^1H -HSQC, ^{13}C - ^1H -HSQC (aliphatic), ^{13}C - ^1H -HSQC (aromatic), 2D-HB(CBCGCE)HE, 2D-HB(CBCGCD)HD and three-dimensional HNCACB, CBCA(CO)NH, ^1H NCOA, HN(CA)CO, H(CCO)NH-TOCSY, (H)C(CO)NH-TOCSY, ^1H CCH-TOCSY, and ^{15}N -edited NOESY-HSQC ($\tau_{\text{mix}}=150$ ms), $^{13}\text{C}_{\text{ali}}$ -edited NOESY-HSQC ($\tau_{\text{mix}}=160$ ms), and $^{13}\text{C}_{\text{aro}}$ -edited NOESY-HSQC ($\tau_{\text{mix}}=160$ ms). All spectra were collected at 298 K on either Varian Inova 600 MHz or Bruker (AVANCE III) 800 MHz spectrometer equipped with actively shielded Z-gradient triple resonance Cold probe. Spectra were processed by using the software NMRPipe (Delaglio et al. 1995) and analyzed by using CARA (Keller 2004). The NMR data was referenced for ^1H chemical shifts by using 2, 2-dimethyl-2-silapentane-5-sulphonic acid (DSS) at 298 K as a standard. The ^{13}C and ^{15}N chemical shifts were referenced indirectly.

Assignments and data deposition

Chemical shift (backbone as well as side chain) assignments were made for 162 of the 163 possible and 147 of the 150 possible non-proline residue $\text{H}_\text{N}/\text{N}$ crosspeaks in the ^{15}N - ^1H HSQC spectrum for UNC-60A and UNC-60B, respectively. The ^{15}N - ^1H HSQC spectra of ^{13}C , ^{15}N labeled UNC-60A and UNC-60B, with assignments of residues name and number, are shown in Figs. 1 and 2, respectively. Main chain amide proton assignments were not made for the residue Ser3 of UNC-60A and K33, K46, N47 of UNC-60B. For UNC-60A, assignments of 99.04 % of C^α , C^β , and C' , and 99.43 % of non-aromatic and non-carbonyl side-chain carbons, 63.54 % of aromatic side chain carbons, and 100 % of H_α , were completed. For UNC-60B, assignments of 98.6 % of C^α , C^β , and C' , and 93.84 % of non-aromatic and non-carbonyl side-chain carbons, 60.87 % of aromatic side chain carbon, 98.00 % of H_α , were completed. The secondary structures of UNC-60A and UNC-60B were predicted on the basis of the characteristic NOE connectivities obtained from ^{15}N -edited NOESY-HSQC, the predictions from the program TALOS+ (Cornilescu et al. 1999; Shen et al. 2009) software, and from the weighted consensus values (Alfano et al. 2003). The solution structure of UNC-60A is composed of six stranded β -sheet region corresponding to residues M6-V7 (β_1), Y27-I32 (β_2), K36-V43 (β_3), R81-R93 (β_4), D102-I109 (β_5), and I138-V143 (β_6) and seven helical regions corresponding to residues D10-L18 (α_1), Q45-L48 (α_2), D54-D56 (α_3), S59-R73 (α_4), I116-L133 (α_5), D145-D148 (α_6), and H151-K161 (α_7) (Fig. 3a). The solution structure of UNC-60B is composed of six stranded β -sheet region corresponding to residues K6-V7 (β_1), Y26-I31 (β_2), T36-E45 (β_3), R68-V78 (β_4), T88-C98 (β_5), and Q128-S132 (β_6) and five helical regions corresponding to residues P9-H19 (α_1), Y50-V61 (α_2), V104-L121 (α_3), M134-D136 (α_4), and E139-S148 (α_5) (Fig. 3b). Unusual chemical shifts have not been seen for any of the residues of UNC-60A and UNC-60B. To evaluate the line broadening, the normalized intensities of backbone NH cross peaks were plotted as a function of residue number as per the assignment established.

In UNC-60A, the residues undergoing significant line-broadening are G4, R26, N35, T51, D54, T78, D86-T90, G95, G97, and G136 and most of these residues correspond to the unstructured loops or linker regions between the secondary structure elements (Fig. 2a). In UNC-60B, line broadening is not observed for any residue.

The chemical shifts of UNC-60A and UNC-60B have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 18356 and 18701, respectively.

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Fig. 1. ^{15}N - ^1H HSQC spectrum of 0.65 mM ^{13}C , ^{15}N labeled UNC-60A recorded at 298 K at 600 MHz in 95 % H_2O /5 % $2\text{H}_2\text{O}$, 20 mM sodium phosphate buffer, pH 6.5, containing 50 mM NaCl and 0.1 % NaN_3 . The cross peaks are labeled with *single letter* amino acid residue name and *number* in the sequence. Side chain amide protons of Asn and Gln are joined by a *line* and side chain peaks are labeled as SC and unassigned peaks are labeled as UA

Fig. 2. ^{15}N - ^1H HSQC spectrum of 1.0 mM ^{13}C , ^{15}N labeled UNC-60B recorded at 298 K at 600 MHz in 95 % H_2O /5 % $2\text{H}_2\text{O}$, 20 mM sodium phosphate buffer, pH 6.5, containing 50 mM NaCl and 0.1 % NaN_3 . The cross peaks are labeled with *single letter* amino acid residue name and *number* in the sequence. Side chain amide protons of Asn and Gln are joined by a *line* and side chain peaks are labeled as SC

Fig. 3. Prediction of secondary structures from Talos+ **a** Secondary chemical shifts of UNC-60A as a function of sequence **b** Secondary chemical shifts of UNC-60B as a function of sequence