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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the 22 kDa LC1 light chain from *Chlamydomonas* outer arm dynein

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Biological context

Dyneins are molecular motors that transport their attached cargo towards the minus-end of microtubules. These enzymes are highly complex and contain up to 15 different polypeptides with a total mass of 1–2 MDa. Dyneins occur both in the cytoplasm where they are required for many intracellular transport activities (e.g. vesicular trafficking and mitosis), and in the flagellum where they provide the motive force for flagellar beating (see Holzbaur et al. (1994), Mitchell (1994) and Witman et al. (1994) for reviews). The outer arm from *Chlamydomonas* flagella is one of the best characterized dyneins and consists of three motor-containing heavy chains (HCs; ∼500 kDa each), two intermediate chains of the WD-repeat family (70–80 kDa; at least one of which is involved in cargo binding), a trimeric docking complex, and a series of light chain (LC1-8) components of 10–25 kDa that appear to function in both assembly and regulatory processes.

The 22 kDa LC1 protein shares partial similarity with the leucine-rich repeat protein family but nonhomologous segments indicate a unique structure (Patel-King et al., 1997; Kajava, 1998). This LC has been found to associate with the motor domain of the γ dynein HC and to directly interact with an ∼45 kDa axonemal protein (Benashski et al., 1998). To gain further insight into the role this protein plays in dynein motor function, we have initiated an NMR structural analysis and detail here the ^{1}H, ^{15}N and ^{13}C resonance assignments for this 198 residue polypeptide.

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Methods

The LC1 protein (1–198) was expressed from the pET-16b vector with an N-terminal His_{10} tag in BL21 cells grown in [U-^{13}C] glucose and $^{15}NH_4Cl$ supplemented with vitamins as described previously (Weber et al., 1992). The protein was purified on a Ni^{2+} column and digested with Factor Xa to separate the tag from the LC. Subsequently, the His_{10} tag was removed by a batch procedure using Ni^{2+}-charged resin. The purified LC1 was then dialyzed extensively against 20 mM Tris.Cl pH 8.0, 50 mM NaCl, 10 mM EDTA to remove any Ni^{2+}. This procedure resulted in a full length LC1 protein containing a single additional His residue at the N-terminus (designated as residue −1). The protein was exchanged into 2.5 mM Tris.Cl pH 6.76, 100 mM NaCl buffer made in 90% H_2O / 10% D_2O. Data were collected from ^{15}N- and ^{15}N,^{13}C-labeled samples at concentrations of ∼1 mM in 600 μl. The ^{15}N, ^{13}C-labeled sample was exchanged into >99% D_2O, concentrated to ∼250 μl using a Centricon 3 (Amicon) and used for the HCCH-TOCSY, ^{1}H-^{13}C HSQC and (HB)CBCACO(CA)HA experiments.

All NMR experiments were recorded at 25 °C on 4-channel Varian INOVA-600 or INOVA-500 spectrometers equipped with pulse field gradient triple resonance probes. The spectra were processed using Felix 95.0 (Biosym Technologies, San Diego, CA, U.S.A.) and analyzed with the program XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

Sequential assignments of HN, ^{15}N, $^{13}C_\alpha$ and $^{13}C_\beta$ for 55% of the residues were derived predominately from

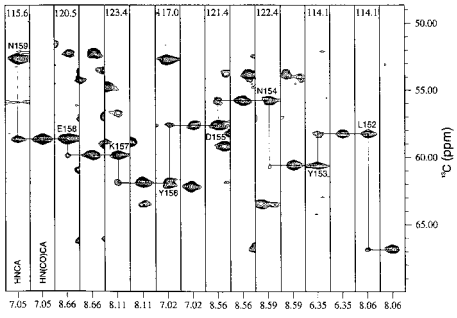

Figure 1. Sequential assignments for residues Leu152-Asn159 determined from through-bond connectivities using the 3D HNCA and HN(CO)CA experiments. Strips from these spectra along the amide ^{1}H and ^{13}C dimensions are shown for each ^{15}N amide chemical shift (indicated at the top of each panel).

the 3D HNCACB and CBCA(CO)NH experiments (Grzesiek and Bax, 1992; Wittekind and Mueller, 1993). For the remaining residues, the HNCA and HN(CO)CA spectra were used to identify several sequential candidate C_α resonances and their correlated amide proton resonances. The ^{1}H-^{15}N NOESY-HSQC spectrum was then used to identify the amide protons which showed sequential d_{NN} connectivities. These assignments were confirmed using the HNCACB and CBCA(CO)NH spectra. We assigned all C_α and C_β resonances except for Pro35, as there are two contiguous prolines (Pro35 and Pro36) in the sequence. There were no correlations for Glu29 in the HN(CO)CA and CBCA(CO)NH spectra; these chemical shifts were obtained from the HNCACB and HNCA experiments.

For illustrative purposes, the sequential assignment of residues Leu152-Asn159 is shown in Figure 1. Except for Val28, Ile34, Pro35, Asn150, Leu173, Met182, and Gly198, complete carbonyl carbon assignments were obtained from the HNCO experiment. The H_α chemical shifts for the nine Gly residues were identified from the ^{1}H-^{13}C HSQC spectrum; the remainder (except for Leu72, Leu95 and Pro35) were assigned in the ^{1}H-^{15}N TOCSY-HSQC, HCCH-TOCSY, HC(CO)NH-TOCSY or (HB)CBCACO(CA)HA spectra.

Complete side chain assignments were made for all Ala, Asn, Asp, Cys, Gln, Gly, Ser, Thr, and Val residues (except for the side chain amide protons and carbonyl ^{13}C of 6/16 Asn residues and the carbonyl ^{13}C of all Asp residues) using the HCCH-TOCSY, HC(CO)NH-TOCSY, HNCACB, CBCA(CO)NH and ^{1}H-^{15}N NOESY spectra. The β-proton and $^{13}C_\beta$ resonances were assigned for all Tyr and both Trp residues; the two Trp indole protons were also assigned. The side chain ^{1}H and ^{13}C resonances (excluding the guanidino group) were assigned for all Arg residues, however only 4/9 $^{1}H_\epsilon$-$^{15}N_\epsilon$ correlations could be identified. In addition, we assigned the $^{13}C_\beta$, β-proton and methyl proton resonances for 21/27 Leu and 11/16 Ile residues. Only the $^{13}C_\beta$ resonances were assigned for 7 Lys, 7 Glu and both His residues. All other Lys (11/18) and Glu (12/19) side chain resonances (except for the ζ-$^{15}NH_3^+$ of all Lys residues and the ^{13}C carbonyl of all Glu residues) were assigned. The $^{13}C_\beta$ resonances were assigned for all Met residues; the β-proton resonances were found for 3/6. Resonances within the ring for the five Pro residues were partially assigned. The remaining aliphatic side chain resonances could not be assigned due to a lack of connectivities and/or overcrowding in certain regions of the spectra. For example, many Leu ^{13}C methyl resonances are overlapping.

The NMR experimental data and the chemical shift assignments for LC1 are available as supplementary material and have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4265.

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