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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the Tctex1 dynein light chain from *Chlamydomonas* flagella

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Biological context

Dyneins are microtubule-based molecular motors that are found in both cilia/flagella and the cytoplasm. The cytoplasmic isozymes perform essential intracellular transport activities, whereas axonemal dyneins provide the motive force for ciliary and flagellar motility and are required for generation of left–right asymmetry in the developing mammalian embryo. Tctex1 is a component of both cytoplasmic dynein and axonemal inner dynein arm I1 (King et al., 1996; Harrison et al., 1998). Tctex1 from *Chlamydomonas* flagella contains 114 residues and exists as a 26-kDa dimer in solution (Harrison et al., 1998; DiBella et al., 2000). This protein is located at the base of the dynein particle in association with the intermediate chains. Members of the Tctex1/Tctex2 light chain family are essential for assembly of the dynein motor complex in vivo (Pazour et al., 1999). In cytoplasm, Tctex1 is involved in the attachment of specific cellular cargoes, such as rhodopsin, Fyn kinase and Doc-2, to dynein (Tai et al., 1999). Furthermore, Tctex1 has been identified in mouse testis as a candidate for one of the distorter proteins involved in the non-Mendelian transmission of variant forms of chromosome 17 known as the *t* haplotypes (Lader et al., 1989). This process involves defects in spermiogenesis that may be mediated by the action of a sperm motility kinase on various dynein components (Harrison et al., 1998; Herrmann et al., 1999; Pazour et al., 1999). To gain further insight into the role played by Tctex1 in dynein function, we have initiated NMR structural studies of the Tctex1 dimer from the inner dynein arm of *Chlamydomonas* flagella.

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Methods and experiments

Tctex1 from *Chlamydomonas* inner dynein arm I1 was cloned into the pET23d vector following addition of an N-terminal His_{10} tag and linker containing a Factor Xa cleavage site. Protein was expressed in BL-21 cells grown in ^{15}N,^{13}C Bioexpress-1000 complete medium (Cambridge Isotopes, Andover, MA) and purified by Ni^{2+} affinity chromatography. Following digestion with Factor Xa, the His_{10} tag was removed by batch chromatography on Ni^{2+} resin. Purified Tctex1 was dialyzed extensively against buffer containing 10 mM EDTA to remove any Ni^{2+}. This procedure resulted in purified Tctex1 containing a single additional His residue (designated as residue −1) at the N-terminus. The protein was exchanged into 20 mM Na phosphate pH 6.7, 100 mM NaCl, 20 mM DTT, 0.2 mM AEBSF in 90% H_2O/10% D_2O. For data collection, ^{15}N,^{13}C-labeled samples were concentrated to ∼0.7 mM in a volume of 250 μl. The protein sample was exchanged into 100% D_2O for the HCCH-TOCSY experiment.

All NMR experiments were recorded at 25 °C using four-channel Varian INOVA-600 or INOVA-500 spectrometers equipped with pulse field gradient triple resonance probes. All spectra were processed using FELIX 97.0 (Biosym Technologies, San Diego, CA, U.S.A.) and analyzed with the program XEASY (Bartels et al., 1995).

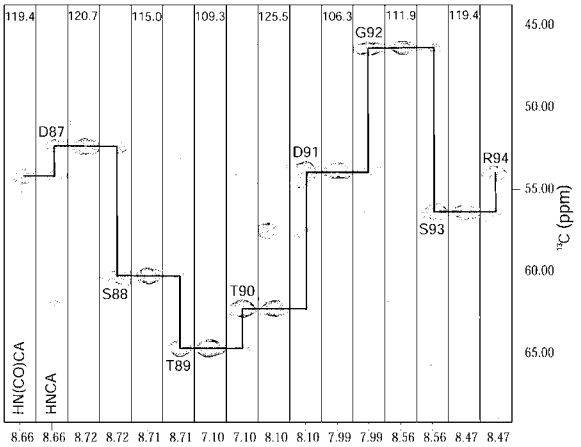

Figure 1. Strips from the 3D HNCA and HN(CO)CA spectra along the amide ^{1}H and ^{13}C dimensions showing sequential $^{13}C_{\alpha}$ resonance assignments for the segment of Tctex1 from Asp87 to Arg94. The ^{15}N chemical shift for each amide is indicated at the top of each panel.

Extent of assignments and data deposition

Sequential assignments of HN, ^{15}N, $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ resonances were made using the HNCACB and CBCA(CO)NH experiments in combination with the HNCA and HN(CO)CA experiments (Grzesiek and Bax, 1992; Wittekind and Mueller, 1993). The sequential connectivities obtained from the HNCA and HN(CO)CA spectra that were used in assigning HN, ^{15}N, and $^{13}C_{\alpha}$ resonances for residues Asp87 to Arg94 are shown in Figure 1. Combined with data from the C(CO)NH spectrum, all $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ resonances were assigned except for the $^{13}C_{\beta}$ of Asp16, Ser19, Ser25, and Ser45. Using the C(CO)NH spectrum, $^{13}C_{\gamma}$, $^{13}C_{\delta}$ and $^{13}C_{\varepsilon}$ resonances were assigned for 47 of 59, 18 of 26 and 5 of 7 residues, respectively. Due to apparent relaxation effects in this 26-kDa dimer, the ^{13}C-^{13}C TOCSY transfer was not efficient and resulted in a lack of connectivities. Amide ^{15}N resonances were identified for all residues except Met1, Pro6, Asp16 and Pro61. The HN resonances for Met1 and Asp16 were not assigned. All carbonyl ^{13}C assignments were obtained from the HNCO spectrum, except for Ala15 and Ile114 which derived from the (HACA)CO(CA)NH spectrum.

All H_{α} chemical shifts derived from the HNHA, HC(CO)NH and ^{15}N-edited NOESY experiments; no H_{α} resonance was assigned for Pro61 or Ser93. Side-chain proton assignments were made using the HC(CO)NH and HCCH-TOCSY spectra. The H_{β} for 56 of 109 residues and the H_{γ} of 24 of 59 residues were assigned. As in the C(CO)NH spectrum, connectivities in the HC(CO)NH experiment were lacking due to the line widths in this 26 kDa dimer. H_{δ} resonances were identified for Pro6, Leu48, Leu58, Ile69, Ile70, Lys101 and Ile106; the chemical shifts for H_{ε} of Lys53 and Lys101 also were obtained. The remaining side chain resonances were not assigned due to a lack of connectivities.

The chemical shift assignments for *Chlamydomonas* Tctex1 and the NMR experimental details are available as supplementary material from the authors and have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4929.

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References

Bartels, C., Xia, T., Billeter, M., Guntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **6,** 1–10.

DiBella, L.M., Patel-King, R.S., Salata, M.W. and King, S.M. (2000) *Mol. Biol. Cell*, **11**, 196a.

Grzesiek, S. and Bax, A. (1992) *J. Am. Chem. Soc.*, **114**, 6291–6293

Harrison, A., Olds-Clarke, P., and King, S.M. (1998) *J. Cell Biol.*, **140**, 1137–1147.

Herrmann, B.G., Koschorz, B., Wertz, K., McLaughlin, J. and Kispert, A. (1999) *Nature*, **40**, 141–146.

King, S.M., Dillman, J.F., Benashski, S.E., Lye, R.J., Patel-King, R.S. and Pfister, K.K. (1996) *J. Biol. Chem.*, **271**, 32281–32287.

Lader, E., Ha, H.S., O'Neill, M., Artzt, K. and Bennett, D. (1989) *Cell*, **58**, 969–979.

Pazour, G.J., Koutoulis, A., Benashski, S.E., Dickert, B.L., Sheng, H., Patel-King, R.S., King, S.M. and Witman, G.B. (1999) *Mol. Biol. Cell*, **10**, 3507–3520.

Tai, A.W., Chuang, J.Z., Bode, C., Wolfrum, U. and Sung, C.H. (1999) *Cell*, **97,** 877–887.

Wittekind, M. and Mueller, L. (1993) *J. Magn. Reson.*, **B101**, 201–205.