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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N resonance assignments of human microtubule-associated protein light chain-3

Takahide Kouno[a], Mineyuki Mizuguchi[a], Isei Tanida[b], Takashi Ueno[b], Eiki Kominami[b] & Keiichi Kawano[a,*]

[a]*Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan;* [b]*Department of Biochemistry, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

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Biological context

Microtubule-associated protein (MAP) light chain-3 (LC3) is a protein that has been co-purified in both fractions of MAP1A and MAP1B (Kuznetsov and Gelfand, 1987). Although it has been revealed that these proteins are localized in the brain and are capable of binding to microtubules (Bloom et al., 1985), their detailed physiological functions remain to be elucidated.

Recent study has shown that MAP-LC3 is a homologue of yeast Apg8p/Aut7p involved in autophagy and the Cvt pathway, and is essential for the formation of autophagosomes (Kabeya et al., 2000). Apg8p/Aut7p is cleaved by Apg4p, a cysteine protease of yeast, to expose the C-terminal glycine to solvent (Kirisako et al., 2000). In addition, the cleaved Apg8p/Aut7p is covalently attached to phosphatidylethanolamine at the C-terminal glycine residue by Apg3p and Apg7p, other members of the Apg family (Ichimura et al., 2000). The modified Apg8p/Aut7p thereby interacts directly with the autophagosome membranes. Previous study has shown that the precursor of MAP-LC3 is located in the cytosolic fraction, and is converted to MAP-LC3-I and MAP-LC3-II by post-translational processing in the cells (Tanida et al., 2003). Interestingly, only MAP-LC3-II is fractionated to the membrane compartment. MAP-LC3 is, therefore, also cleaved and modified as in the case of Apg8p/Aut7p.

*To whom correspondence should be addressed. E-mail: kawano@ms.toyama-mpu.ac.jp

We prepared non-labeled, ^{15}N-labeled and ^{13}C-/^{15}N-labeled recombinant proteins of human MAP-LC3-I in order to study the solution structure by means of NMR spectroscopy. Here we report the ^{1}H, ^{13}C and ^{15}N resonance assignments of MAP-LC3-I on the basis of 2D- and 3D-NMR experiments.

Methods and results

The recombinant MAP-LC3-I was expressed as glutathione S-transferase (GST)-fusion proteins using pGEX vector in *E. coli* strain BL21. Bacterial extract was applied to a glutathione-immobilized column in order to purify the GST-fusion protein. The GST-MAP-LC3-I fusion protein was digested by protease into GST and MAP-LC3-I. After the digestion, MAP-LC3-I was additionally purified by cation-exchange chromatography. The ^{15}N-labeled and ^{13}C-/^{15}N-labeled proteins were prepared by growing the bacteria in minimal medium containing $^{15}NH_4Cl$ and ^{13}C-glucose/$^{15}NH_4Cl$, respectively. All of the NMR samples contained 0.8-1.0 mM MAP-LC3-I, 25 mM sodium phosphate (pH 7.0), 100 mM NaCl, 0.02 mM NaN_3, and 10% or 100% D_2O.

NMR experiments were performed at 25 °C on a Bruker DMX-500 spectrometer. The following spectra were used for the ^{1}H, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$ resonance assignments: ^{1}H-^{15}N HSQC, HNCA, CBCANH, CBCA(CO)NH, HNCO, HN(CA)CO, H(CCO)NH, C(CO)NH, HBHA(CBCA)NH, HBHA (CBCACO)NH, HCCH-COSY, HCCH-TOCSY (18.3 ms mixing time), ^{15}N-edited TOCSY (75.9 ms), and ^{15}N-edited NOESY (85 ms).

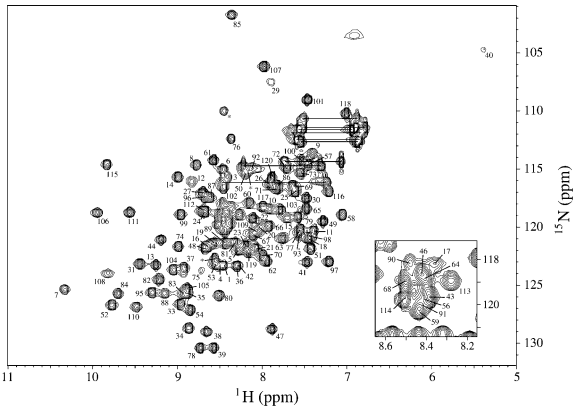

Figure 1. ^{1}H,^{15}N-HSQC spectrum of MAP-LC3-I. The assignments are presented alongside the corresponding signals. Several signals connected by horizontal lines correspond to the amide groups of the side chains of Asn and Gln, and the signals with asterisks are obtained from the tag sequence attached to the N-terminus of MAP-LC3-I.

^{1}H chemical shifts were directly referenced to the resonance of 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS), while ^{13}C and ^{15}N chemical shifts were indirectly referenced using internal DSS with the absolute frequency ratios Ξ (^{13}C/^{1}H) = 0.251449530 and Ξ (^{15}N/^{1}H) = 0.101329118 (Wishart et al., 1995). All of the NMR data were processed using NMRPipe (Delaglio et al., 1995) and analyzed by means of PIPP (Garrett et al., 1991) software on a Linux workstation.

Extent of assignments and data deposition

The ^{1}H,^{15}N-HSQC spectrum of MAP-LC3-I gives well-separated signals (Figure 1) in the condition described above. In the case of the resonances overlapping on ^{15}N-edited spectra, the assignments were performed with a set of spectra obtained from ^{13}C-edited NMR experiments. The ^{1}H, ^{15}N, and ^{13}C backbone resonances of MAP-LC3-I composed of 120 amino acids were almost completely assigned except for the residue P28. The NMR signals assigned to T29, K42, and A75 were very weak on not only the HSQC spectrum but also the ^{13}C-edited spectra. The residue T29 follows P28 in the amino acid sequence, whereas K42 and A75 are located in loop regions predicted from the chemical shift index of MAP-LC3-I (data not shown). These residues, therefore, may have some degree of fluctuation.

The data of the ^{1}H, ^{13}C, and ^{15}N chemical shifts of MAP-LC3-I have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5958.

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