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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of GABARAP, $GABA_A$ receptor associated protein

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Biological context

$GABA_A$ receptor associated protein (GABARAP) is a novel protein found in inhibitory nerves of humans, which acts as a binding partner with $GABA_A$ receptors ($GABA_A$Rs) (Wang et al., 1999). GABARAP interacts with both $GABA_A$R γ2 subunit and gephyrin, the latter of which is an essential scaffold protein for localization of $GABA_A$Rs (Kneussel et al., 2000). However, direct interactions between $GABA_A$R and gephyrin have been not reported. Therefore, it was thought that GABARAP plays a mediatory role in the localization to a specific cell membrane. Some biochemical studies have shown that GABARAP is also capable of binding to tubulin which is known to be involved in intracellular trafficking (Wang and Olsen, 2000). These reports suggest that GABARAP may participate in the transport of $GABA_A$Rs to the cell membrane, and it may be one of the most potent proteins used to elucidate the intracellular localization and the transport mechanism of $GABA_A$Rs. We prepared non-labeled, ^{15}N-labeled and ^{13}C-/^{15}N-labeled recombinant proteins of GABARAP in order to analyze its three-dimensional structure and intramolecular motions by NMR spectroscopy. We report here on the ^{1}H, ^{13}C and ^{15}N resonance assignments of GABARAP on the basis of 2D- and 3D-NMR experiments.

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Methods and results

Recombinant GABARAP 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 to isolate the GST-GABARAP. The GST-GABARAP fusion protein was digested by thrombin into GST and GABARAP. After the digestion, GABARAP was purified by cation-exchange chromatography. Although the GABARAP obtained by the above protocol was modified with the Gly-Ser sequence at the N-terminus, it was confirmed that this additional sequence did not prevent its binding activity to the γ2 subunit of $GABA_A$R. ^{15}N-labeled and ^{13}C-/^{15}N-labeled proteins were obtained by the same expression system described above in minimal medium containing $^{15}NH_4Cl$ and ^{13}C-glucose/$^{15}NH_4Cl$, respectively. All of the NMR samples contained 1 mM GABARAP protein, 20 mM sodium phosphate (pH 6.5), 50 mM NaCl, 0.02 mM NaN_3 and 10% or 100% D_2O.

All NMR experiments were performed at 25 °C on Bruker DMX-500 and DRX-800 spectrometers. The following spectra were used for the ^{1}H, ^{15}N, $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ resonance assignments: ^{1}H-^{15}N HSQC, HNCA,

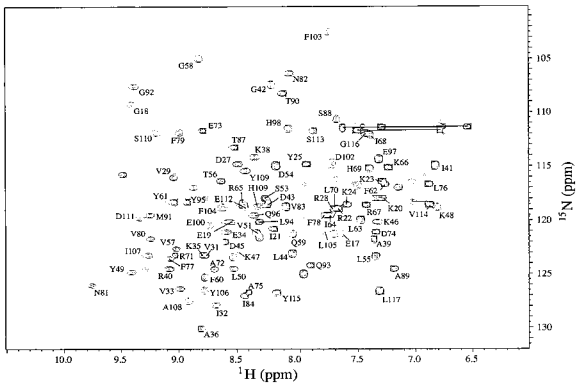

Figure 1. ^{1}H-^{15}N HSQC spectrum of GABARAP at 25 °C (pH 6.5). The assignments are presented alongside the corresponding signals. Several signals connected by horizontal lines correspond to the amide groups of the side chains of Asn and Gln, and remaining signals without residual number could not be assigned.

CBCANH, CBCA(CO)NH, H(CCO)NH, HCCH-TOCSY (43 ms mixing time), ^{15}N-edited TOCSY (43 ms), ^{15}N-edited NOESY (50 and 100 ms), and 2D NOESY (100 ms). An HNHA experiment was performed to obtain the $^{3}J_{\mathrm{HNH}\alpha}$ coupling constants.

^{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 with NMRPipe (Delaglio et al., 1995) and PIPP (Garrett et al., 1991) software on a Linux workstation.

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC spectrum of GABARAP gives well-separated signals (Figure 1), in which the assignments are indicated for individual cross peaks. In the case of the resonances overlapping on spectra obtained from ^{15}N-edited NMR experiments, the assignments were performed with a set of ^{13}C-edited NMR spectra. Consequently, the backbone resonance assignments were achieved for 101 of the 117 residues of GABARAP, while the resonances of 16 amino acids in the N-terminal region almost do not appear in the NMR spectra. The data of the ^{1}H, ^{13}C and ^{15}N chemical shift and the $^{3}J_{\mathrm{HNH}\alpha}$ coupling constants of the GABARAP have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5128.

As a result of the resonance assignments of GABARAP, we found that the ^{1}H$^{\alpha}$ resonances of Phe78, Ala108 and Tyr109 are abnormally shifted to downfield (5.91 ppm, 6.40 and 6.57, respectively), which may implicate unique features in the tertiary structure of GABARAP. In the case of Phe78, both of the neighboring residues are phenylalanines (Phe77 and Phe79). An analysis using the Chemical Shift Index (CSI) (Wishart and Sykes, 1994) shows that these residues form β-strand (data not shown), and the backbone amide H-D exchange experiment indicates that amide groups of Phe77 and Phe79 form hydrogen bonds. The side chain groups of residues constructing a β-sheet are generally perpendicular to the plane composed of backbone atoms. Under these conditions, the downfield-shift of the ^{1}H$^{\alpha}$ resonance of Phe78 might be due to the ring current effect of the aromatic rings of Phe77, Phe78 and Phe79.

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