Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N resonance assignments for a 14 kD protein, GABA$_A$ receptor associated protein (GABARAP)

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Biological context

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) acts as the molecular trigger for the ligand-gated heteropentameric membrane-spanning chloride ion channels known as GABA receptors (McDonald and Olsen, 1994; Rabow et al., 1995). This superfamily of receptors is the target for a variety of therapeutic or mood altering agents such as benzodiazapine tranquillizers, barbiturates, steroids, general anesthetics and convulsants. Type A GABA receptors contain two α subunits, two β subunits and a single γ subunit. A partial clone (residues 36–117) of a 117 amino acid residue protein known as GABA receptor-associated protein (GABARAP) was identified in a yeast two-hybrid screen as coding for a protein that interacts strongly with the intracellular M3-M4 loop of the γ2 subunit of GABA$_A$ receptors (Wang et al., 1999). Subsequent studies have yielded evidence that full length GABARAP binds GABA$_A$ receptors both *in vitro* and *in vivo*. Intact GABARAP also has the capacity to bind tubulin *in vitro*, and GABARAP can be found in microtubule-associated protein (MAP)-rich preparations of tubulin from cellular sources. Tubulin binding of GABARAP is dependent upon the N-terminal 35 residues of the protein, and is strongly attenuated by high salt concentrations (Wang and Olsen, 2000). A peptide corresponding to the strongly basic first 22 amino acid residues of GABARAP promotes tubulin assembly. GABARAP is also found to be associated
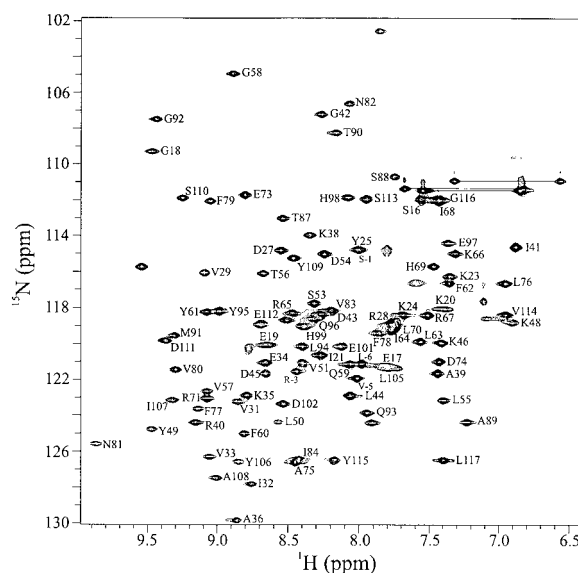

Figure 1. Assigned 2D ^{1}H-^{15}N HSQC spectrum of ^{15}N-labelled GABARAP recorded on a 500 MHz Varian UNITYplus spectrometer at 293 K.

with microtubules and microfilaments in intact cells. Tubulin and γ2 subunit of GABA$_A$ receptors can bind GABARAP simultaneously. These findings point to a role for GABARAP in the linkage of GABA$_A$ receptors with the cytoskeleton, possibly in a capacity to promote trafficking and high density clustering of receptors at the post-synaptic cell membrane.

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Methods and results

Sample preparation

Uniformly ^{15}N- and ^{13}C/^{15}N-labelled hexahistidine-tagged recombinant GABARAP was over-expressed in *E. coli* BL21 (DE3) grown on M9 minimal medium with 0.5 g/l (^{15}NH$_4$)$_2$SO$_4$ and 2 g/l ^{13}C$_6$-glucose, with subsequent purification by immobilized nickel ion affinity chromatography and size exclusion chromatography. For NMR studies, samples of 0.5–1.0 mM GABARAP in 20mM phosphate buffer (pH 7.0), 100 mM NaCl and H$_2$O/D$_2$O (9:1) were prepared.

NMR spectroscopy

NMR spectra were acquired at either 293 K or 303 K on Varian UNITYplus spectrometers (operating at nominal ^{1}H frequencies of 500 MHz and 600 MHz) equipped with a triple resonance (^{1}H, ^{13}C, ^{15}N) probe including Z-axis pulse field gradients. Sequence-specific resonance assignments were obtained by combining the data from the following 3D gradient sensitivity enhanced triple resonance experiments: HNCO, HNCA, HN(CO)CA, HNCACB (Yamazaki et al., 1994), and CBCA(CO)NH (Muhandiram and Kay, 1994). The backbone assignments were confirmed through the observation of sequential NH(i)-NH(i+1) and NH(i)-Hα(i+1) interproton NOEs identified in a ^{15}N-edited 3D NOESY-HSQC (100 ms mixing time) experiment. All spectra were processed using NMRpipe/NMRDraw (Delaglio et al., 1995) and analyzed using ANSIG v3.3 (Kraulis et al., 1994). Chemical shifts were indirectly referenced to DSS.

Extent of assignments and data deposition

Analysis of the triple resonance experiments allowed identification and sequential assignments for 91 out of the 110 GABARAP (117 less 7 prolines) backbone ^{15}N and amide proton resonances (i.e., not including the hexahistidine tag). Definitive assignments have not been obtained for residues Met1-His9, Phe11-Arg15, Arg22, Lys47, Glu100, Phe103, and Phe104. Conformational slow exchange is observed as NH cross peak doubling for residues Glu17, Glu19, Lys20 and Leu105. Nine backbone NH cross-peaks in the HSQC spectrum, including two that display evidence of slow conformational exchange, remain unassigned to specific residues. The absence of an unambiguously identifiable NH crosspeak for 10 residues is tentatively attributed to exchange broadening. Examination of the spectrum under a variety of sample conditions (temperature, pH, peptide ligands, co-solvents) has failed to reveal these 'missing peaks'. Figure 1 shows an assigned 2D ^{1}H-^{15}N HSQC spectrum of GABARAP, recorded at a ^{1}H frequency of 500 MHz.

From the assigned amide resonances, we were able to obtain 98% (86% of the total possible resonances), 96% (84%), 88% (78%) and 88% (78%) of the possible Cα, Cβ, CO, and Hα chemical shifts, respectively. GABARAP is highly homologous (57% identity) to a protein known as GATE-16 (16 kD Golgi-associated ATPase enhancer) that is implicated in intra-Golgi vesicle transport. The X-ray crystal structure of GATE-16 was recently reported at 1.8 Å resolution (Paz et al., 2000). Overall, the secondary structure elements revealed by the secondary chemical shifts and interproton NOEs of GABARAP are essentially identical to those observed for crystalline GATE-16, consistent with an ubiquitin-like fold with extra N-terminal helical segments.

The chemical shifts for backbone resonances of GABARAP have been deposited in the BioMagResBank (accession number 5064).

Acknowledgement

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