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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of human GABA receptor associated protein

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Biological context

Rapid signaling at the neuromuscular junction and synapses between neurons are mediated by small molecules, called neurotransmitters. Among those, the most prominent are acetylcholine and glutamate for excitatory synapses, and glycine and γ-aminobutyric acid (GABA) for inhibitory signaling, respectively. Receptors for these neurotransmitters are important targets for drugs used to treat mental disorders or to modulate sleep and mood. Consequently, drugs such as the benzodiazepines which directly bind to specific sites at the GABA type A ($GABA_A$) receptor, the principal GABA-gated ion channel, offer both medical and economic potential. Recently, a novel protein was identified as a binding partner for the γ2-subunit of $GABA_A$ receptor, termed GABARAP ($GABA_A$ receptor-associated protein; Wang et al., 1999). GABARAP is also reported to bind tubulin, gephyrin (Kneussel et al., 2000) and ULK1 (Okazaki et al., 2000). It is closely related to light chain-3 (LC-3) of microtubule-associated proteins 1A and 1B (MAP-1A and 1B) and to the 'late acting intra-Golgi transport factor', termed GATE-16, of which an X-ray structure was reported recently (Paz et al., 2000). In contrast to GABARAP, however, GATE-16 does not interact with gephyrin and $GABA_A$ receptor γ2-subunit (Kneussel et al., 2000). GABARAP is postulated to have an important role for early steps in movement and sorting of $GABA_A$ receptors (Kneussel & Betz, 2000) and for $GABA_A$ receptor clustering at the postsynaptic membrane. Binding affinity of GABA to $GABA_A$ receptors, as well as kinetics of inactivation and desensitization of the receptors are dependent on the clustering state of the GABA receptor, which appears to be strongly modulated by GABARAP (Chen, 2000). Modulation of GABARAP binding to its interaction partners provides a new avenue for pharmacological intervention of receptor activity and neurotransmitter action at the synapse. We therefore started a structural investigation of GABARAP and its complexes in solution by nuclear magnetic resonance (NMR) spectroscopy and report here the ^{1}H, ^{13}C and ^{15}N resonance assignment of GABARAP.

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Methods and experiments

A DNA fragment coding for GABARAP was amplified by PCR and cloned into the vector pGEX-4T-1 (Amersham) using *Bam*HI and *Not*I restriction sites introduced by PCR. Sequence analysis of the resulting expression plasmid pGEX-GABARAP confirmed 100% identity with human GABARAP (TrEMBL MM46, accession number O95166). This construct provides a Thrombin cleavage site to remove the N-terminal glutathione-S-transferase (GST) affinity tag from GABARAP.

The GST-GABARAP fusion protein was expressed in *E. coli* strain BL21 transformed with pGEX-GABARAP. Cells with an O.D. at 600 nm of about 0.6 were induced with 1 mM IPTG and harvested after 3 hours growth at 37 °C. For ^{15}N and ^{13}C labeling M9 minimal medium was used with $^{15}NH_4Cl$ and $^{13}C_6$-glucose as sole nitrogen and carbon sources, respectively. GST-GABARAP fusion protein was initially purified from the soluble extract by GST affinity

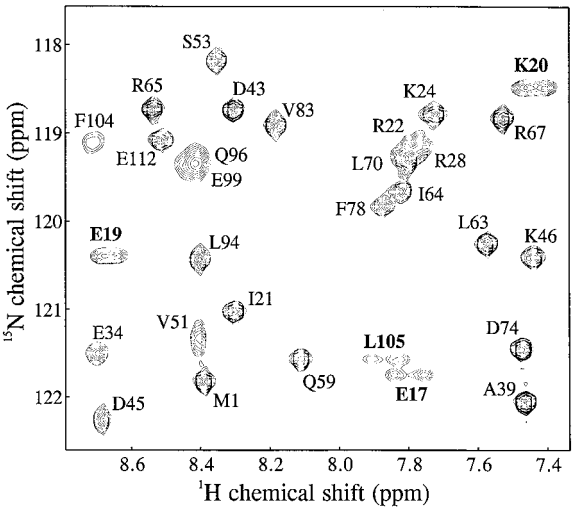

Figure 1. Selected region of the $^{1}H,^{15}N$-HSQC spectrum of GABARAP. Resonances for residues E17, E19, K20 and L105 appear at two different proton frequencies. Residues are numbered according to their position in GABARAP irrespectively of the glycine serine residues attached to the N-terminus.

chromatography (Glutathione Sepharose from Amersham). Thrombin (Merck) cleavage was performed in solution yielding full-length GABARAP with additional glycine and serine residues at the N-terminus. For final purification, the sample was applied to a Superdex 75 prepgrade gel filtration column equilibrated with 400 mM NaCl and 50 mM phosphate buffer, pH 7 and 5% (per volume) glycerol. The sample was concentrated by ultrafiltration (1 kDa cutoff, Pall-Gelman). During the concentration procedure, buffer conditions were changed to the desired NMR buffer of 25 mM sodium oxalate, 100 mM NaCl, 100 mM KCl, pH 6.9, in 95% H_2O/5% D_2O. This was achieved by multiple additions of the NMR buffer to the protein solution during filtration.

NMR samples contained 0.8 mM protein in the NMR buffer with the addition of 100 μM PMSF, 0.02% (by weight) sodium azide and 50 μM EDTA. All NMR spectra were recorded at 298 K on a Varian Unity INOVA 750 MHz spectrometer equipped with a triple-axis pulse-field-gradient (PFG) $^{1}H/^{15}N/^{13}C$ probe, except for the HC(CO)NH-TOCSY spectrum which was recorded at 298 K on a Bruker DMX 600 MHz spectrometer equipped with a triple-axis PFG $^{1}H/^{15}N/^{13}C$ probe. $^{1}H,^{15}N$-HSQC, HNCACB, CBCA(CO)NH, HNCO, HC(CO)NH-TOCSY, HCCH-TOCSY, HCCH-COSY, ^{15}N- and ^{13}C-edited NOESY spectra were recorded to obtain resonance assignments. The data were processed using NMRPipe (Delaglio et al., 1995) and assigned using NMRView (Johnson & Blevins, 1994). Backbone amide resonances for residues E17, E19, K20 and L105 appear at two different proton frequencies as shown in the $^{1}H,^{15}N$-HSQC spectrum (Figure 1). Some other resonances are broadened. This may indicate slow to intermediate conformational exchange in regions of the protein. Preliminary inspection of chemical shift data by TALOS (Cornilescu et al., 1999) yielded similar secondary structure elements as reported for the X-ray structure of GATE-16 (Paz et al., 2000).

Extent of assignments and data deposition

The backbone ^{1}H, ^{15}N, ^{13}C and ^{13}CO assignments are essentially complete for GABARAP. Backbone amide ^{1}H and ^{15}N resonances for 99 out of 110 (117 residues minus 7 prolines) possible amide resonances were assigned (90%). Furthermore, 114 out of 117 $^{13}C_{\alpha}$ resonances and their corresponding $^{1}H_{\alpha}$ resonances (97%) could be assigned. Residues H9, R14 and E100 could not be assigned, but their presence has been confirmed by DNA sequencing of the respective gene. Nearly all sidechain resonances are assigned. The ^{1}H, ^{15}N and ^{13}C chemical shifts of GABARAP have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5058.

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