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Letter to the Editor: Semi-automated backbone resonance assignments of the extracellular ligand-binding domain of an ionotropic glutamate receptor

Robert L. McFeeters[a], G.V.T. Swapna[b], Gaetano T. Montelione[b] & Robert E. Oswald[a,*]
[a]*Department of Molecular Medicine, Cornell University, Ithaca, NY 14853, U.S.A.;* [b]*Center for Advanced Biotechnology and Medicine and Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ 08854, U.S.A.*

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Biological context

The AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) subclass of ionotropic glutamate receptors (GluR1 through GluR4) mediates a major portion of fast synaptic transmission in the central nervous system of higher vertebrates (Dingledine et al., 1999). AMPA receptors are multimeric, membrane-bound receptors with intrinsic ion channels composed of either four or five subunits of approximately 900 residues. Each subunit has 4 distinct modular domains (Wo and Oswald, 1995): An N-terminal domain, a ligand-binding domain, a pore forming region, and a C-terminal regulatory domain. The ligand-binding domain can be expressed in isolation and structural studies of this pharmacologically important domain are feasible (Arvola and Keinanen, 1996). The crystal structure of the ligand-binding domain of GluR2 has previously been reported and shown to consist of two lobes, which close upon agonist binding (Armstrong et al., 1998). Here we report the first backbone resonance assignment of an ionotropic glutamate receptor ligand-binding domain. We also demonstrate the utility of an automatic sequence assignment algorithm, AutoAssign (Moseley et al., 2001) in the assignment of a large (> 25 kDa) protein. The backbone assignments provide an essential first step in the analysis of the dynamics of the protein, high throughput screening of pharmacological agents directed toward glutamate receptors, and studies of lobe closure using residual dipolar coupling.

*To whom correspondence should be addressed. E-mail: reo1@cornell.edu

Materials and methods

A 263-residue version of the ligand-binding domain of GluR2 was obtained from Dr Eric Gouaux. All samples were purified as described earlier (Armstrong et al., 1998). ^{15}N backbone specific labeling of lysine, leucine, valine, phenylalanine and was performed (Shortle, 1994; Venters et al., 1996). Based on microdrop solubility, NMR samples were prepared in an acetate buffer at pH = 5.0. Protein function was confirmed in this buffer by competition binding assays. The K_D for kainate was within a factor of 2.5 of that previously reported for the GluR2 construct (Chen and Gouaux, 1997). Protein concentrations for NMR samples varied from 150 to 450 μM for specifically labeled samples and from 450 to 650 μM for uniformly labeled samples. All data were collected at 298K on a Varian Inova 600 with a triple resonance, z-gradient probe. Data were processed with nmrDraw/nmrPipe (Delaglio et al., 1995) and visualized using Sparky (T.D. Goddard and D.G. Kneller, UCSF).

TROSY-based ProteinPack (Varian Inc., Palo Alto, CA) sequences were used with the exception of a modified TROSY-based HN(CO)CACB experiment (Salzmann et al., 1998). Typically, 45 increments in the carbon dimension and 50 increments in the nitrogen dimension were collected, resulting in approximately 90 h per experiment. Linear prediction to twice the data size and zero filling to a final matrix size of 1024 by 256 by 128 points for the ^{1}H, ^{13}C, and ^{15}N dimensions, respectively, were performed

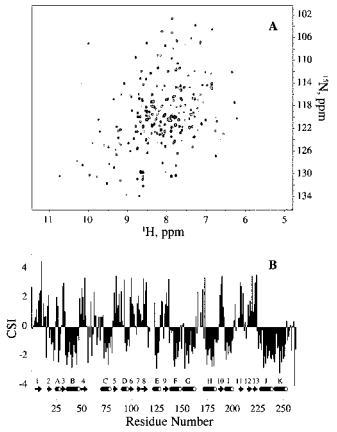

Figure 1. (A) ^{1}H,^{15}N-TROSY spectrum of the 263-residue extracellular ligand-binding domain of GluR2. Data acquired from the fully deuterated, ^{13}C,^{15}N-labeled sample. (B) Plot of the chemical shift difference from random coil values (CSI). A consensus value calculated from (($\delta_{C\beta}$-$\delta_{C\alpha}$-δ_{CO})/number assigned) was used. A positive value of the CSI generally indicates a β-strand and a negative value indicates a helix. Also shown are secondary structural elements derived from the crystal structure and the corresponding labels given by Armstrong et al. (1998). Arrows indicate β-sheets and cylinders represent α-helices.

for all triple resonance data. The ^{1}H dimension was referenced to an external DSS sample and referencing for the other dimensions was calculated based on the carrier offset and spectrometer frequency. Deuterium based chemical shift effects on ^{13}C resonances were corrected by applying a global ^{13}C shift to align the fully deuterated spectra with the partially deuterated spectra. A ^{1}H,^{15}N-TROSY spectrum of the fully deuterated ^{13}C,^{15}N-labeled sample is shown in Figure 1A. Resonance identification from this sample was nearly complete for all inter- and intra-residue C^α, C^β and inter-residue CO resonances.

With three potential connectivity pathways, AutoAssign was effectively used to make sequential assignments. After several iterations of adding weaker resonances, resolving overlap, adding manual assignments, and including selective labeling data, the final AutoAssign run resulted in the assignment of backbone ^{15}N, ^{13}CO and/or $^{13}C^\alpha$ resonances for 232 residues. Of these, the assignments for 12 residues had to be corrected by interactive manual analysis. The total amount of time required to complete processing, manual peak picking, peak editing, and to assign more than 95% of the backbone of this 29 kDa protein was approximately five weeks, about twice the amount of time required to acquire all the data from the perdeuterated sample. Current efforts are in progress to automate the processing, peak picking and peak editing to reduce the time required for data analysis.

Extent of data deposition

Backbone assignments of $^1H^N$, ^{15}N, $^{13}C^\alpha$, $^{13}C^\beta$, and ^{13}CO resonances for 246 of the total 263 residues (7 of which are proline) were completed. $^{13}C^\alpha$, $^{13}C^\beta$, and ^{13}CO resonances for an additional 10 residues (5 of which are proline) were reported from inter residue data. Only residues T119-E122, P167, S168 and P205 remain completely unassigned. Approximately two-thirds of all expected H^α resonances were also obtained from an ^{15}N-TOCSYHSQC experiment, though no side-chain data were included in the current BRMB deposition. Rigorous side-chain assignments are currently in progress. All α-helices and β-sheets predicted from chemical shift indexing agree with observed secondary structural elements reported in the crystal structure (Figure 1B). The sequence specific resonance assignments have been deposited in the BMRB under accession number BMRB-5182.

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