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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone assignments for the C-terminal globular domain of agrin

Andrei T. Alexandrescu[a,*], Mark W. Maciejewski[b], Markus A. Rüegg[c], Jürgen Engel[d] & Richard A. Kammerer[e]

[a]*MCB, University of Connecticut, Storrs, CT 06269, U.S.A.;* [b]*Department of Biochemistry, University of Connecticut Health Center, Farmington, CT 06032, U.S.A.;* [c]*Department of Neurobiology/Pharmacology, and* [d]*Department of Biophysical Chemistry, Biozentrum, University of Basel, Basel, CH-4056, Switzerland;* [e]*Wellcome Trust Centre for Cell-Matrix Research, School of Biological Sciences, University of Manchester, Manchester M13 9PT, U.K.*

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Biological context

Agrin is a proteoglycan associated with the basal lamina of a wide range of tissues (Ruegg and Bixby, 1998; Sanes and Lichtman, 1999). Agrin, released from the motor nerve terminal has been shown to act as a signal that induces the postsynaptic organization of the neuromuscular junction, including the aggregation of acetylcholine receptors (ACHRs) during nerve-muscle synapse development. The C-terminal portion of agrin contains three laminin G-like domains (G1 to G3), separated by EGF repeats (Denzer et al., 1998). The G1-G2 domain tandem binds α-dystroglycan. Induction of AChR clustering at neuromuscular junctions requires only the last globular domain (agrin-G3). The three agrin G domains are predicted to belong to a superfamily of protein modules with a β-jellyroll fold, which includes laminin G, thrombospondin N, and the pentraxin family (Beckmann et al., 1998, Hohenester et al., 1999). Alternative mRNA splicing of the agrin gene results in several isoforms. The 'B' isoforms differ by up to 19 residue inserts in the sequence of the agrin-G3 domain (Sanes and Lichtman, 1999). Isoforms containing 8, 11, or 19 residue inserts (B8-B19) are specifically expressed in neurons and induce AChR clustering. The B0 isoform, that lacks the sequence inserts, is expressed in non-neuronal cells and shows no AChR clustering activity.

*To whom correspondence should be addressed. E-mail: andrei@uconnvm.uconn.edu

Methods and experiments

The agrin-G3 used for NMR comprises residues 1748-1940 of the chicken agrin protein (Swiss-Prot: P31696, B0 splice isoform). Recombinant agrin-G3 was expressed in *Escherichia coli* JM109 cells using the expression vector pGSTHis, a derivative of pGEX-1 that encodes GST, a 6×His tag, and a thrombin cleavage site preceding agrin-G3. For the preparation of $^{2}H/^{13}C/^{15}N$ and ^{15}N uniformly labeled samples, cells were grown in modified New Minimal Medium (Wiltscheck et al., 1997) containing 3 gm/l d_7-$^{13}C_6$-D-glucose, 99.9% D_2O and/or 1 g/l $^{15}NH_4Cl$. The fusion protein was purified by affinity chromatography on glutathione Sepharose. After thrombin cleavage, agrin-G3 was separated from the 6×His-tagged GST by affinity chromatography on Ni^{2+}-Sepharose.

NMR data were recorded on a 600 MHz spectrometer at 25 °C. 3D ^{15}N HSQC-NOESY-HSQC (200 ms mix time), and deuterium-decoupled TROSY versions of 3D HNCACB, HNCO, and HN(CA)CO data were collected on a 1.0 mM $^{2}H/^{13}C/^{15}N$-labeled agrin-G3 sample containing 4 mM $CaCl_2$, 10 mM imidazole-d_4 (pH 6.5). A 1.0 mM ^{15}N-labeled agrin-G3 sample containing 6 mM $CaCl_2$, 10 mM phosphate (pH 7.1) was used to collect 3D ^{15}N TOCSY-HSQC (39 ms mix time) and ^{15}N NOESY-HSQC (100 ms mix time) data. Internal DSS was used for chemical shift referencing. Aliphatic proton chemical shifts were obtained from the ^{15}N-labeled sample. All other chemical shifts were obtained from the $^{2}H/^{13}C/^{15}N$-labled sample, and the

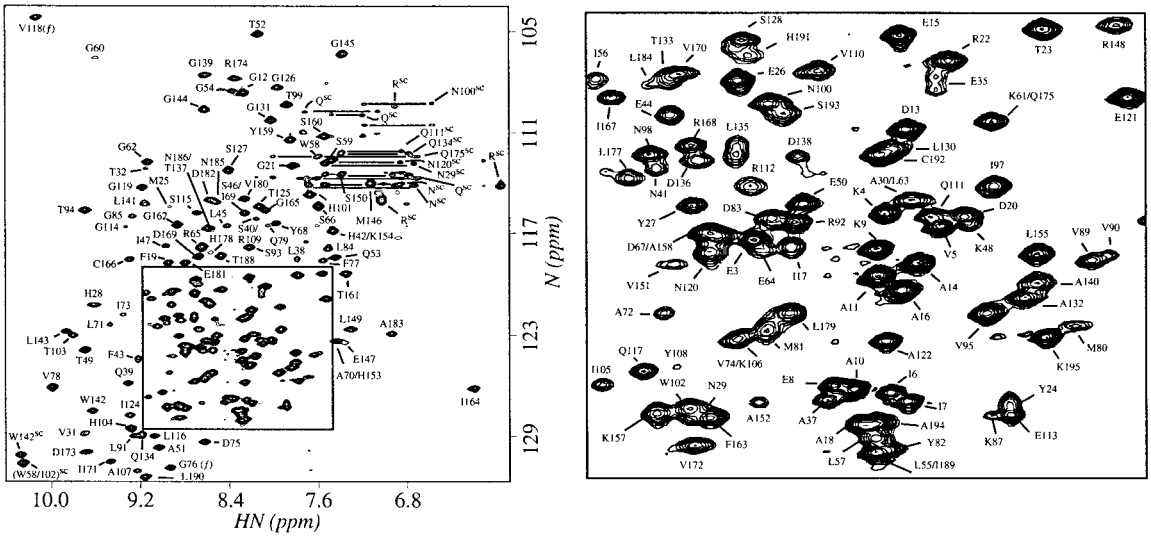

Figure 1. ^{1}H-^{15}N TROSY spectrum of the Ca^{2+}-bound B0 isoform of agrin-G3 (1 mM ^{2}H/^{13}C/^{15}N agrin-G3, 4 mM $CaCl_2$, 10 mM imidazole-d_4, pH 6.5, 25 °C). The panel on the right shows an expansion of the region indicated by the rectangle. Side chain correlations are marked 'sc' (some side-chain correlations could only be assigned to residue type). The arginine H^{ε}-N^{ε} correlations ('R^{sc}') and the backbone correlations for G75 and V118 (marked '*f*') are aliased in the ^{15}N dimension. Backbone correlations for Ser34 and Ser86 are only visible at lower contour levels, and those for Gly1, Ser2, Lys33, Lys36, and Glu176 are not assigned.

reported shift values are not corrected for ^{2}H isotope effects.

Extent of assignments and data deposition

The assignment coverage statistics are: $^{1}H^{N}$ (97%), ^{15}N (94%), $^{13}C\alpha$ (94%), $^{13}C\beta$ (97%), ^{13}CO (96%), $^{1}H\alpha$ (91%), when all 195 residues in the 21 kDa protein are considered. Chemical shift deviations indicate a predominantly β-sheet structure consistent with the prediction of a β-jellyroll fold. Initial NMR work was done on agrin-G3 without Ca^{2+}. About 15% of the fingerprint ^{1}H-^{15}N HSQC peaks expected from the protein sequence were broadened beyond detection under these conditions. Following the observation that many members of the pentraxin and laminin G families share a conserved Ca^{2+} binding site (Beckmann et al., 1998; Hohenester et al., 1999) an NMR titration was used to establish that the agrin-G3 domain is similarly a Ca^{2+}-binding protein. Addition of Ca^{2+} led to marked improvements in the quality of NMR spectra. Most NMR signals from regions near the putative Ca^{2+}-binding site (Glu64-Trp58) and the B-insert site (Leu38-Met25) are only detectable in the presence of Ca^{2+}. NMR signals from these regions remain weaker in the Ca^{2+}-bound form. Future studies will examine how Ca^{2+}-binding and B sequence inserts modulate the flexibility and function of the agrin-G3 domain. NMR assignments for the Ca^{2+}-bound B0 isoform of agrin-G3 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4978.

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