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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of full length human ADP Ribosylation Factor 1 (ARF1) using triple resonance connectivities and dipolar couplings

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Biological context

ADP Ribosylation factors (ARFs) comprise a family of 21kDa, Ras-related GTPases ubiquitously expressed in eukaryotic cells. The name ARF originates from the first identified activity, that of a cofactor in the cholera toxin-catalyzed ADP-ribosylation of the heterotrimeric G protein, Gs_{α} (Kahn and Gilman, 1986). Like other Ras-related, GTP binding proteins, ARFs also regulate a wide variety of intracellular signaling pathways; specifically those impacting vesicular traffic.

An essential aspect of the actions of ARF *in vivo* is its ability to reversibly bind to biological membranes or lipid vesicles. The co-translational N-myristoylation of ARFs provides a permanent hydrophobic addition to the amphipathic N-terminal helix whose conformation is highly sensitive to the binding of guanine nucleotides. In this way, the binding of GTP to ARF is tightly correlated with increased association of both the myristate and the N-terminal helix to the membrane lipids.

Crystallography has been used to produce structures for full length human ARF1 in its GDP bound non-myristoylated form (Amor et al., 1994), and a truncated form bound to GTP that also lacks the amino terminal helix (Goldberg, 1998). The membrane associated structure of the isolated N-terminal helix and its corresponding myristoyl chain have been obtained by nuclear magnetic resonance (Losonczi et al., 1998). These data have led to proposed models for structural rearrangements accompanying membrane binding and GDP-GTP exchange, but without information on the full length protein in association with lipids, these models remain speculative. The ^{1}H, ^{15}N and ^{13}C assignments for the non-myristoylated, but full length GDP form of ARF reported here represent a step toward acquiring these data.

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Methods and experiments

Full length human ARF1 was expressed in bacteria after transformation of the pOW12 plasmid into BL21 Star DE3 cells. Stable isotopes (^{13}C, ^{15}N) were incorporated using minimal media supplemented with 1 g l^{-1} $(^{15}NH_4)_2SO_4$ and 2 g l^{-1} ^{13}C-glucose. Protein expression and purification followed the methods described in Kahn et al. (1989). The sample used for triple resonance experiments was prepared as 0.5 mM ARF1 in 10 mM sodium phosphate (pH 6.5) and 100 mM NaCl, supplemented with 1 mM $MgCl_2$ and 1 mM GDP. 10% $^{2}H_2O$ was added to provide a lock signal. A sample, which used bicelles as an alignment medium was prepared to be 0.5 mM ARF1, labeled only with ^{15}N, in the same buffer. The 7% (w/v) bicelle solution contained DMPC/DHPC at a 2.8/1 molar ratio doped with DMPG (15:1 DMPC:DMPG).

Spectra for the heteronuclear 3D as well as coupled ^{1}H-^{15}N HSQC experiments were collected on Varian 600 MHz and 800 MHz spectrometers, respectively, using pulsed gradient triple resonance probes (Varian

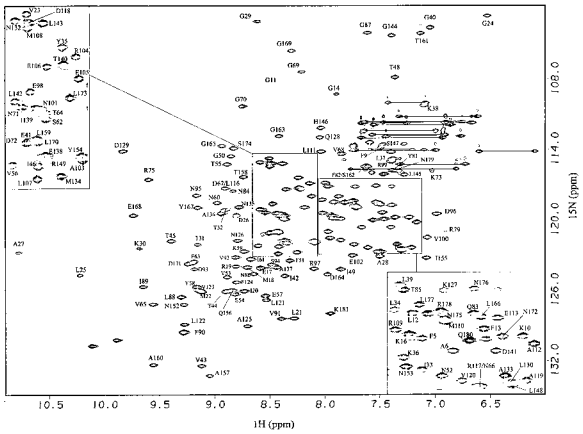

Figure 1. Annotated ^{1}H, ^{15}N-HSQC spectrum of 0.5 mM full length human ARF1 at a ^{1}H resonance frequency of 800 MHz.

Inc, Palo Alto, CA). Heteronuclear 3D experiments were run at 298 K using standard gradient assisted sequences as supplied as part of the Varian protein pack. The ^{1}H-^{15}N HSQC experiments were run in both isotropic (298 K) and partially aligned (308 K) conditions. For the measurement of residual dipolar couplings a version of the coupling enhanced HSQC experiment was used (Tolman and Prestegard, 1996).

Backbone sequential assignments were obtained for 90% of the residues using the following heteronuclear 3D spectra: HNCA, HNCACB, CBCACONH, HNCO, and HN(CO)CA.

The small number of remaining residues were assigned with the aid of residual dipolar couplings. Splittings were extracted for every set of peaks in the coupled ^{1}H-^{15}N HSQC experiments by visually determining peak centers using columns which run through peak maxima. Differences in the splittings from both aligned and isotropic conditions (308 K and 298 K) yielded residual dipolar couplings. Elements of the alignment tensor were found by analysis of all assigned peaks using the crystal structure (PDB ID code 1HUR, Amor et al., 1994) and a refined version of a previously published program (Losonczi et al., 1999). Residual dipolar couplings, ranging from -31 to 32 Hz, were then back-calculated for all N-H vectors using this alignment tensor.

Residual dipolar couplings were measured for 5 unassigned peaks. In 2 cases, unique matches to predicted couplings were found. In 3 additional cases, prediction of chemical shift allowed removal of ambiguities and assignment. For these cases backbone chemical shifts were calculated using the ShiftX program (Wishart and Case, 2001). We were unable to determine RDC values for the remaining unassigned peaks due to unresolved ambiguities and peak overlap.

Extent of assignments and data deposition

All of the ^{1}H,^{15}N, $^{13}C_{\alpha}$, $^{13}C_{\beta}$ and $^{13}C_{o}$ backbone resonances for peaks observed in the ^{1}H-^{15}N HSQC spectrum were assigned, except M1, G2, N3, N7, L8, K15, I74, L77, W78, H80, D114, E115, H150 as shown in Figure 1. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5368.

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