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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments and ^{15}N-^{1}H residual dipolar couplings for the α-adaptin ear-domain

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Biological context

Endocytosis via clathrin-coated pits and vesicles constitutes the major mechanism of protein internalization into cells (Conner and Schmid, 2003). Central to this process is the clathrin adaptor protein-2 (AP-2), a heterotetramer composed of α-, β2-, μ2-, and σ2-adaptin subunits (Brodsky et al., 2001). Clathrin-mediated membrane budding also functions at the *trans*-Golgi network where it mediates the transport of cargo to the endosomal/lysosomal system (Brodsky et al., 2001). Here, a crucial role is played by the clathrin adaptor protein-1 (AP-1), which has a similar structure to AP-2, composed of γ-, β1-, μ1-, and σ1-adaptin subunits (Brodsky et al., 2001). At their C-termini, α-, β- and γ-adaptins contain a globular structure termed the appendage or 'ear' domain. The crystal structure of the ear domain of α-adaptin (α-ear) has been determined at 1.4 Å resolution and is composed of an N-terminal β-sandwich and a C-terminal mixed α-β platform subdomains (Owen et al., 1999; Traub et al., 1999). The α-ear binds to a number of components of the endocytic regulatory machinery (Conner and Schmid, 2003). These proteins contain short consensus peptide motifs, DPF/W and F×D×F that mediate α-ear binding, and cocrystallization studies have examined the interactions between these peptides and the α-ear (Brett et al., 2002). Interestingly, we have recently uncovered a new α-ear binding motif, WVQF in the NECAP proteins (adaptin-ear-binding coat-associated proteins), novel components of clathrin-coated vesicles (Ritter et al., 2003). Here, we report NMR resonance assignments for the 27 kDa α-ear, which allows for detailed solution studies of the interactions of these important endocytic structures with the various peptide motifs (Wasyak et al., 2003).

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Methods and experiments

The ear-domain of mouse α-adaptin C (residues 700–938) was amplified by PCR from cDNA gi:90292 (gift of Dr. Stephane Laporte), transferred into pGEX-2TK and expressed as a GST-fusion protein in *E. coli* BL21. Cultures were grown at 37 °C on minimal M9-media supplemented with ^{15}N ammonium chloride and ^{13}C-glucose (Cambridge Isotopes Laboratory, Andover, MA) to produce uniformly ^{15}N- or ^{15}N, ^{13}C-labeled proteins. Following a 6 h induction with 1 mM IPTG at 25 °C, soluble GST-α-ear protein was purified by affinity chromatography using glutathione-Sepharose 4B column and cleaved with thrombin for 2 h. The NMR samples contained 0.3–1.0 mM protein in 90% H_2O/10% D_2O, 25 mM sodium phosphate (pH 7.3), 75 mM NaCl, 0.5 mM EDTA and 5 mM DTT. NMR spectra were acquired at 30 °C on a Bruker DRX-600 spectrometer equipped with a triple resonance cryoprobe and pulsed field gradients.

The TROSY (Fernandez and Wider, 2003) and regular versions of 3D-experiments were used for backbone HN, N, C^{α}, CO and side chain C^{β} resonance assignments: HNCA, HN(CO)CA, HNCACB, CBCA(CO)HN, HNCO and ^{15}N-edited-NOESY (with mixing time of 90 ms). NMR spectra were processed using XWINNMR (Bruker) software and analysed with XEASY (Bartels et al., 1995). ^{15}N-^{1}H residual dipolar couplings were extracted from IPAP-HSQC

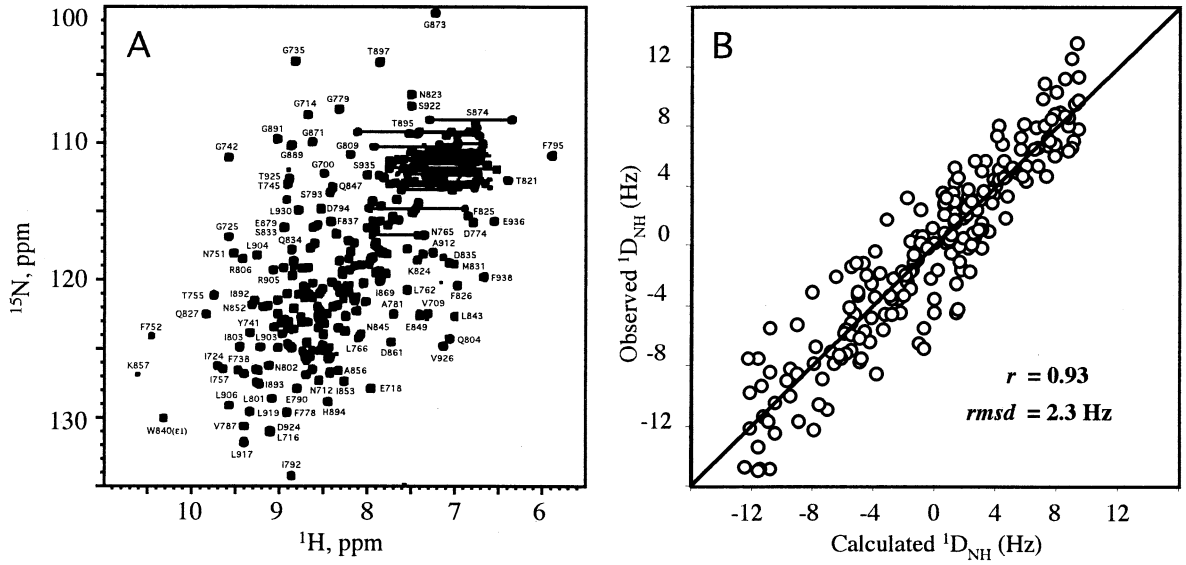

Figure 1. (A) 600 MHz ^{1}H-^{15}N HSQC spectrum of the α-adaptin ear-domain at 30 °C. Peaks arising from the two protons of amide groups in Asn and Gln side chains are connected. Amino acid labels were omitted from the middle of HSQC for clarity. (B) Observed versus calculated RDCs for the best fit of the crystal structure (PDB entry: 1B9K) to couplings measured in Pf1 phage. The axial A_a and rhombic A_r components of the alignment tensor are equal to 5.3×10^{-4} and 1.8×10^{-4}, respectively.

experiments (Ottiger et al., 1998) on an isotropic sample and a sample containing 2.5 mg/ml Pf1 phage.

Extent of assignments and data deposition

Nearly complete backbone ^{1}H and ^{15}N resonance assignments of the α-ear were obtained for all signals detected in ^{1}H-^{15}N HSQC spectra (Figure 1A). The C^{α}, C^{β} resonances were assigned for all residues except Ser-701, Pro-772, Gly-808, Pro-859, Glu-907 and Pro-908; CO resonances were not assigned for residues preceding proline residues. The secondary chemical shifts for C^{α} confirmed location of α-helices and β-sheet strands in the α-ear. ^{15}N-^{1}H residual dipolar couplings (RDC) for 205 residues were measured with a precision of ±1.5 Hz. Observed RDCs were compared with back-calculated values using MODULE software (Dosset et al., 2001). The result of the comparison (Figure 1B) demonstrated that the solution structure of α-ear is very close to the recently determined crystal structure, and that the β-sandwich subdomain is rigid with respect to the distal α-β platform. Independent fitting of the two α-ear subdomains gave the same alignment tensor as the global fit. The assignments and RDC values were deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6034.

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