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Letter to the Editor: ^{1}H, ^{13}C and ^{15} N resonance assignments of the third spectrin repeat of α-actinin-4

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Biological context

The actin cytoskeleton plays a fundamental role in cellular processes such as the maintenance of cell morphology, adhesion, division, phagocytosis, and as a key part of the contractile apparatus. α-actinins dynamically cross-link actin filaments to determine the mechanical properties of the actin filament network. Four α-actinin isoforms have been characterized in either muscle (α-actinins-2 and -3) or non-muscle cells (α-actinins-1 and -4). Non-muscle α-actinins localize to different sub-cellular compartments - α-actinin-1 localizes with stable structures such as focal adhesions, cell contacts and ends of stress fibres whereas α-actinin-4 localizes to actively moving structures (Araki et al., 2000) and is upregulated upon enhanced cell movement (Honda et al., 1998). α-actinin-4 is particularly significant as it is suggested to be linked with the metastatic potential and invasiveness of human cancers (Honda et al., 1998) and has been observed to suppress tumourgenicity of human neuroblastoma cells (Nikolopoulos et al., 2000). It is likely that α-actinin-4 may act as an adaptor between cell-signaling proteins and the cytoskeleton and may provide a mechanism of regulation of cytoskeletal remodeling.

The α-actinins belong to the spectrin superfamily and contain four spectrin repeats (each composed of a three-helix bundle). Spectrin repeats function as a structural motif for the spatial coordination of cytoskeletal interactions or as a protein-recognition motif for a number of regulatory proteins. This latter role is particularly prominent among the spectrin repeats of the α-actinins (reviewed in Djinovic-Karugo et al., 2002). We recently identified an interaction between the third spectrin repeat of α-actinin-4 and the PH domain/Btk motif of the tyrosine kinase Tec (in preparation). Tec-family proteins transmit intracellular stimulatory signals that can lead to changes in gene expression or cytoskeletal remodeling. These signals can arise from various activated cell-surface receptors i.e., B- and T-cell, cytokine, G-protein-coupled, integrin and adhesion receptors (reviewed in Qiu and Kung, 2000). The precise function of Tec itself, however, remains unclear. As a prelude to the structural characterization of the interaction between Tec and α-actinin-4, we report here the ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the third spectrin repeat of α-actinin-4.

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Methods and experiments

The gene encoding residues T519-Q645 of human α-actinin-4, corresponding to the third spectrin repeat, was subcloned into the pGEX-4T2 vector and expressed in *E. coli* BL21 (DE3). Overexpression was performed in LB or MinA minimal media (supplemented with either $^{15}NH_4Cl$, or ^{13}C-glucose and $^{15}NH_4Cl$) at 37 °C. The culture was induced at $OD_{600nm} \sim 0.8$ with 0.2 mM IPTG and cells harvested after 3 h by centrifugation. Cell pellets were resuspended in lysis buffer (50 mM Tris pH8, 150 mM NaCl, 0.1% Triton-X100) and lysed by sonication. Cell debris was removed by centrifugation and the supernatant was passed through a glutathione-agarose column. Following washing with cleavage buffer (50 mM Tris pH8, 150 mM NaCl, 2.5 mM $CaCl_2$), the GST fusion protein was digested with thrombin for 2 h at 37 °C. The protein was eluted (in 50 mM Tris pH8, 150 mM NaCl) and exchanged into H_2O using a PD10 desalting column, prior to quantitation and concentration. The final cleaved and purified protein has additional residues (Gly1, Ser2) at its N-terminus derived from the vector cloning site, and a molecular weight of 14.8 kDa.

Samples for NMR spectroscopy were prepared at concentrations of 0.6–0.8 mM, in a buffer containing 25 mM NaH_2PO_4 pH 6.75, 100 mM NaCl, 0.01% NaN_3, and either 100% D_2O or 90% H_2O/10% D_2O. NMR experiments were performed using a Varian In-

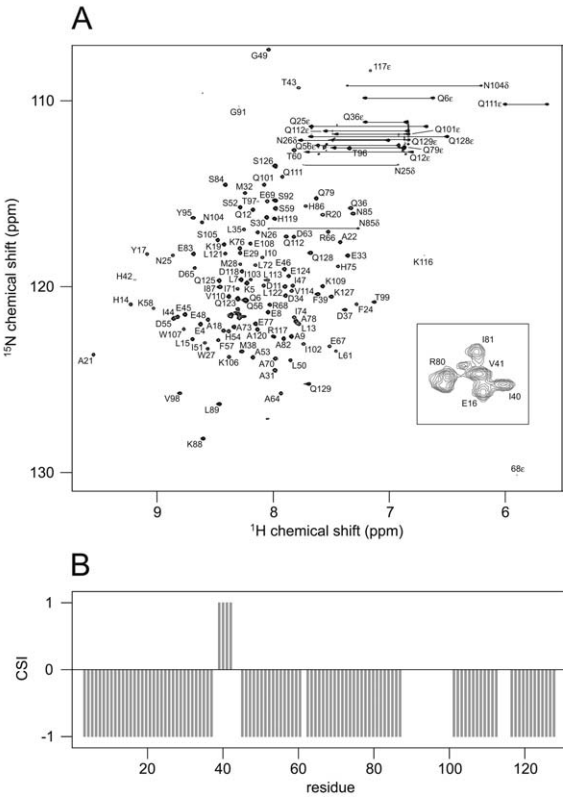

Figure 1. (A) ^{15}N/^{1}H-HSQC (600 MHz) spectrum recorded on recombinant human α-actinin-4 spectrin repeat 3. The sidechain NH_2 resonances of Asn and Gln residues are connected by lines. The H^{ε} resonances of Arg68 and Arg117 are folded in the ^{15}N dimension. (B) Chemical Shift Index (CSI) consensus plot for α-actinin-4 spectrin repeat 3, generated using $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO chemical shifts.

ova 600 MHz spectrometer at 25 °C. Backbone and sidechain assignments were made using the following experiments: HNCACB, CBCA(CO)NH, HNCO, C(CO)NH-TOCSY, H(CCO)NH-TOCSY, HNHA, HNHB, and ^{15}N NOESY-HSQC (150ms mixing time). Aromatic resonances were assigned using 2D ^{1}H TOCSY and NOESY experiments performed on unlabelled samples in D_2O, and 2D ^{13}C/^{1}H HSQC. Data were processed with NMRPipe (Delaglio et al., 1995) and analyzed using Sparky (Goddard and Kneller). ^{1}H, ^{13}C and ^{15}N chemical shifts were referenced to TSP at 0 ppm (Wishart et al., 1995).

Extent of assignments and data deposition

Figure 1A shows the 2D ^{1}H/^{15}N-HSQC spectrum of ^{15}N-labelled α-actinin-4 spectrin repeat-3. All non-proline ^{15}NH correlations have been assigned, except Ser90 and Asn93 which were absent in the spectra recorded. Most sequence-specific assignments were made using the combination of HNCACB and CBCA(CO)NH experiments. Assignments were confirmed and ambiguities resolved using NOE data derived from the ^{15}N-NOESY-HSQC. The ^{1}H and ^{13}C resonance assignments of sidechains were made using data from the H(CCO)NH- and C(CO)NH-TOCSY experiments. Aromatic ^{1}H and ^{13}C assignments were made using a combination of D_2O homonuclear TOCSY and NOESY experiments, and 2D ^{1}H/^{13}C-HSQC. In total, 94% of backbone, 96% of sidechain hydrogen, and 71% of sidechain carbon resonance assignments have been made.

The predicted fold consists of three α-helices and NOE data from the ^{15}N-NOESY-HSQC are consistent with this. The consensus chemical shift indices (Figure 1B) of all assigned residues were determined using the program CSI (Wishart et al., 1997) and indicate a break in the third α-helix. This is perhaps induced by the presence of a Pro residue (Pro115), with the weak intensity of the ^{15}NH correlation of Lys116 suggestive of enhanced amide exchange at this position. However, NOE data suggest this region is in fact α-helical. The ^{1}H, ^{13}C and ^{15}N resonance assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6013.

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