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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances of the C-terminal EF-hands of α-actinin in a 14 kDa complex with Z-repeat 7 of titin

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Biological context

Titin (also known as connectin) is an exceptionally large protein of M.W. ∼3 MDa, found in mammalian skeletal and cardiac muscle (Maruyama, 1997). A single molecule of titin spans half the sarcomere from the Z-disk to the M-line and binds to α-actinin (Ohtsuka et al., 1997; Sorimachi et al., 1997), a major component of the Z-disk, where actin filaments are anchored. The sequence of the C-terminal portion of α-actinin contains a calmodulin-like domain composed of four non-canonical EF-hand motifs, that have no ability to bind calcium. The C-terminal pair of EF-hands of α-actinin ('EF34') are necessary and sufficient for the binding to titin (Ohtsuka et al., 1997).

α-Actinin interacts with titin in the region localised in the Z-disk (Sorimachi et al., 1997; Young et al., 1998), in particular with the repeating 45–50 amino acid sequence motifs near the N-terminus of titin, termed 'Z-repeats' (Gautel et al., 1996). The number of Z-repeats in titin varies between species and between muscle types, the latter variation arising from alternative splicing and being correlated with the thickness of the Z-disk (Gautel et al., 1996). Only half of the last titin Z-repeat is required for binding to α-actinin (Ohtsuka et al., 1997) and this portion adopts an α-helical structure in the complex (Atkinson et al., 2000). Here, we report the assignment of the ^{1}H, ^{13}C and ^{15}N resonances of the 73 C-terminal amino acids of α-actinin ('EF34'), in a 14 kDa complex with the 51-amino acid Z-repeat 7 of titin ('ZR7'), and the deposition of the assignment of ^{1}H and ^{15}N resonances of ZR7 in the same complex.

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Methods and results

Sample preparation. Constructs were produced as fusion proteins with a His-tagged GST separated by a TEV protease cleavage site expressed in *E. coli*. The first residue of the natural sequences was replaced by Met and the dipeptide Gly-Ala was left on the N-terminal end of each protein after cleavage. ^{15}N- and ^{13}C/^{15}N-labelled samples were produced growing the bacteria in minimal medium using $^{13}C_6$-D-glucose and $(^{15}NH_4)_2SO_4$ as sole sources of carbon and nitrogen. The complex of EF34 and ZR7 was obtained by mixing the components following cell lysis and was purified by gel filtration. Samples were ∼0.7 mM in 20 mM phosphate buffer at pH 6.6 in Shigemi tubes.

NMR spectroscopy. ^{15}N-HSQC, ^{13}C-HSQC, HNHA, HNHB, HNCO, HNCA, HN(CO)CA, CBCA(CO)NH, CBCANH, HCCH-TOCSY and ^{15}N-edited NOESY spectra (Cavanagh et al. (1996) and references therein) were recorded at 300 K on either Varian Unity 600 or Varian Unityplus 500 NMR spectrometers equipped with triple-resonance gradient probes. ^{13}C-edited NOESY experiments were recorded at 300 K on a Bruker AVANCE DRX 800 spectrometer.

All spectra were processed using NMRPipe (Delaglio et al., 1995) and analysed using XEASY (Bartels et al., 1995). Typically, the acquisition dimension was multiplied by a Gaussian function, and other di-

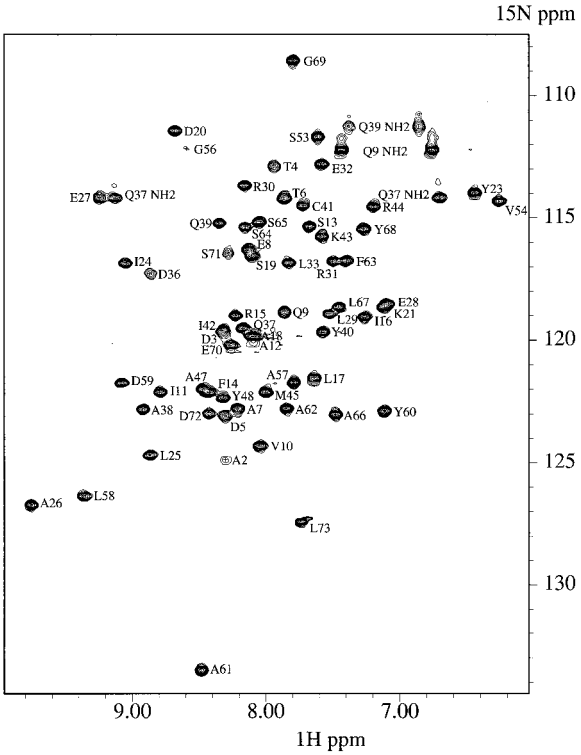

Figure 1. ^{15}N-^{1}H HSQC spectrum of EF34-ZR7 complex in which EF34 is ^{15}N-labelled. The cross peaks of assigned EF34 residues (except Gly50) are labelled, numbered as follows: 1-MADTD-TAEQV IASFRILASD KPYILAEELR RELPPDQAQY CIKRM-PAYSG PGSVPGALDY AAFSSALYGE SDL-73. The sequence of ZR7 is: 1-MGKVGVGKKA EAVATVVAAV DQARVREPRE PGLPEDSYAQ QTTLEYGYKE H-51.

mensions with a 90°-shifted sine-bell function. All dimensions were zero-filled at least to the next power of two, and linear prediction was used where required to extend the ^{13}C dimension.

A total of 71 cross peaks were observed in the ^{15}N-HSQC spectrum of the complex, of which six corresponded to side-chain amide protons of glutamine residues. A set of 68 cross peaks would be expected, of which 65 were observed. Exchange broadening and resonance overlap may account for not observing the full set of cross peaks.

Cross peaks in the HNCA, HN(CO)CA, CBCA (CO)NH and CBCANH spectra were used to identify sequential sets of resonances. Cross peaks in the HNHA and HNHB and HCCH-TOCSY spectra were exploited to extend side-chain assignments of both ^{1}H and ^{13}C resonances. Backbone carbonyl resonances were assigned from the HNCO spectrum.

Extent of assignments and data deposition

Resonances were assigned to all ^{1}H and proton-bearing ^{15}N and ^{13}C nuclei of all non-proline residues of EF34, with the exception of the N-terminal glycine residue, some of the exchangeable side-chain protons, the side-chain atoms of Lys21 beyond ^{13}Cβ and the backbone ^{1}HN and ^{15}N nuclei of Ala0, Ser49 and Gly52. Proline residues 22, 34, 35, 51 and 55 are only partially assigned. Backbone carbonyl resonances were assigned for all residues except those followed by proline or by one of the residues whose backbone ^{1}HN and ^{15}N nuclei could not be assigned. The ^{1}Hζ$_{}$O resonance of Tyr23 was observed and the ^{1}Hϵ_2N resonances of Gln37 were separated by 2.4 ppm.

The assignments have been deposited with the BioMagResBank under accession number 4453 (http://www.bmrb.wisc.edu)[1].

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[1]A study of the same complex in which ZR7 is ^{15}N-labelled while EF34 is unlabelled has been reported elsewhere (Atkinson et al., 2000). The assignment of ^{1}H and ^{15}N resonances and values of $^{3}J_{HNH\alpha}$ coupling constants have been deposited with the BioMagResBank under accession number 4454.