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Letter to the Editor: Assignment of the ^{1}H, ^{13}C, and ^{15}N resonances of holo isoform 4 of *Lethocerus indicus* Troponin C

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Biological context

Muscle contraction is generally regulated by changes in the concentration of Ca^{2+} in the fibres. In many insect species, however, the wing-beat frequency is too high for individual contractions to be activated by Ca^{2+}. The muscle fibres are instead activated by periodic stretches synchronized with the wing beat (Pringle, 1978). The wings are moved by resonant changes in the shape of the thorax produced by indirect flight muscles (IFMs). At the molecular level, the action of stretch on the IFM has been shown to act through the tropomyosin–troponin regulatory system on thin filaments (Agianian et al., 2004).

The giant water bug *Lethocerus indicus*, is being used as a suitable model for understanding insect muscle contraction. *Lethocerus* troponin consists of a tropomyosin-binding subunit (TnT), the Ca^{2+} binding subunit (TnC) and TnH in which the inhibitory subunit (TnI) is fused to a proline and alanine rich extension. TnT has a negatively charged extension to the C-terminus not present in vertebrate TnTs, but found in other arthropod sequences. Three different TnC isoforms are present in adult *Lethocerus*, two of them, in different amounts, are in the IFMs while the third one is in the leg muscles. One of the two isoforms in the IFMs (isoform 4, or LiTnC4; hereafter referred as F1) is predicted to be composed of four EF hand motifs, as are all the TnCs but is unusual in having a single Ca^{2+}-binding site near the C-terminus (in the fourth EF hand) (Qiu et al., 2003). The site has high affinity for Ca^{2+} and binds Mg^{2+} with much lower affinity. F1 is necessary for stretch activation of IFM, while the second TnC isoform (F2) is necessary for activating the muscle at higher Ca^{2+} concentration without stretching (Agianian et al., 2004).

To provide new insights into the molecular determinants of the calcium TnC interaction and to find out how the F1 isoform responds to stretch in asynchronous muscles might respond to stretch, we have started a project which aims at the structure determination of the TnC subunit in solution. Here we report an essentially complete backbone and side-chain assignment of the NMR spectrum of the holo form of F1.

Methods and experiments

The cDNA containing the sequence of *Lethocerus indicus* F1 (accession number AJ512939; SwissProt nomenclature TPC4_LETIN) was inserted into the NwI/EcoRI or NcoI/Hind III sites of a modified pET24d(M11) expression vector (Novagen) containing an N-terminal hexahistidine (His6) tag followed by a tobacco-etch-virus (TEV)-protease cleavage site. The recombinant protein was grown in a modified *E. coli* strain BL21(DE3)pJY2 and the soluble protein was purified from the lysate of sonicated cells on a Ni^{2+}-nitriloacetate (Ni-NTA)-agarose column. The six-His-tag cleaved from purified protein with TEV protease and the protein was run through the column again to remove the tag.

Isotopically $^{13}C/^{15}N$ double and ^{15}N-labelled samples were over-expressed in *E. coli* host strain BL21 (DE3) grown on a minimal media containing 2 g l^{-1} ^{13}C-glucose and/or 1 g l^{-1} ^{15}N-ammonium

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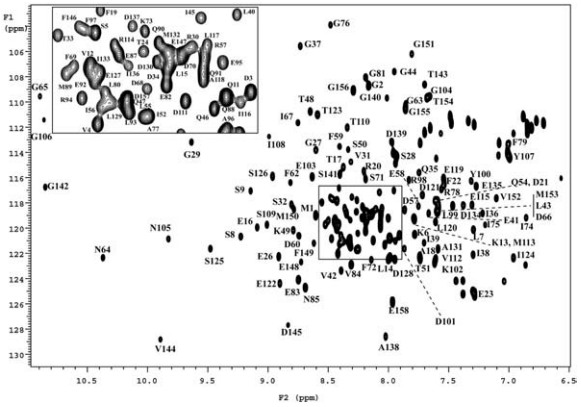

Figure 1. Two dimensional ^{1}H,^{15}N-HSQC spectrum of F1 in its Ca^{2+}-loaded form as recorded at 25 °C on a 600 MHz INOVA Varian instrument. A sample containing 0.9 mM F1 in 100 mM KCl solution at pH = 6.8 was used. The assignment of the backbone resonances is indicated.

sulphate purified as previously described (Qiu et al., 2003).

NMR samples of calcium loaded F1 contained: 0.9 mM protein in 100 mM KCl solution (pH = 6.8), 10 equivalent of calcium added as $CaCl_2$ in H_2O/D_2O (9:1 v/v). All NMR experiments were acquired at 25 °C on Varian Inova spectrometers operating at 600 MHz and 500 MHz.

^{15}N-HSQC, HNCA, HN(CO)CA, HNCO, HNCACB experiments were employed to obtain sequence specific ^{1}HN, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$ and $^{13}C'$ backbone assignments (Muhandiram and Kay, 1994). Side chain aliphatic proton and carbon assignments were achieved by a combination of 3D ^{15}N-edited TOCSY- and NOESY-HSQC (Fesik and Zuiderweg, 1988), H(CCO)NH and HCCH-TOCSY (Kay et al., 1993). For the assignment of the large number (12) of aromatic side chains present in F1, $(H_\beta)C_\beta(C_\gamma C_\delta)H_\delta$ and $(H_\beta)C_\beta$ $(C_\gamma C_\delta)H_\varepsilon$ experiments were used in combination with ^{13}C-HSQC, and HCCH-TOCSY (Kay et al., 1993) tuned for the aromatic resonances.

All spectra were processed using NMRPipe/NMR Draw (Delaglio et al., 1995) and analysed using XEASY (Bartels et al., 1995).

Extent of assignment and data deposition

The holo form of the F1 protein resulted in NMR spectra of excellent quality. In the ^{15}N-HSQC (Figure 1), backbone NH resonances were observed for 157 out of an expected 158 non-proline residues (the HN correlation of Asn10 was not identified).

The NMR assignment of backbone resonances is complete except for $^{13}C\alpha$ and the C' atoms of Ser9 and the C' atoms of the residues followed by prolines. The assignment of side chain aliphatic ^{1}H and ^{13}C resonances is approximately 90% complete, a minor number remaining unassigned owing to missing correlations and/or resonance overlap. Aromatic proton assignment is also almost complete (55 out of 58). The ^{15}N resonance of residue Val144 is extremely down field shifted. A similar shift has been observed for the corresponding position in calmodulin and was attributed to its carbonyl being directly coordinated to the calcium ion (Biekofsky et al., 1998). The resonances of Ser9 are very weak, possibly because of internal mobility of these residues on a slow or intermediate timescale.

Available ^{1}H,^{13}C and ^{15}N chemical shifts of F1 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 6081.

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