Journal of Biomolecular NMR **27:** 283–284, 2003.

Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N backbone assignment of the first two Ig domains Z1Z2 of the giant muscle protein Titin

Christian Edlich[a] & Claudia Muhle-Goll[a,b,*]

[a]*European Molecular Biology Laboratory, Meyerhofstr.1, D-69117 Heidelberg, Germany;* [b]*Institut für Anästhesiologie & Operative Intensivmedizin, Universitätsklinikum Mannheim, Theodor-Kutzer-Ufer, D-68135 Mannheim, Germany*

Received 17 April 2003; Accepted 2 June 2003

Key words: heteronuclear NMR, Ig domain, resonance assignment, titin, t-cap

Biological context

Titin is the largest protein known to date with a molecular mass of more than 3.6 MDa. Its gene encodes a single polypeptide that spans the entire half of a sarcomere with its N-terminus attached to the Z-disc and its C-terminus lying in the M-line (for review see Trinick, 1994; Gregorio et al., 1999). The immediate N-terminal two domains of titin, named Z1 and Z2, belong to the Immunoglobulin I (Ig) family. They are located at the periphery of the Z-disk, a zone of the sarkomere where actin, nebulin and titin filaments from adjacent sarkomeres are crosslinked via a complex network of proteins. It was shown that the two domains Z1Z2 interact with the 19 kDa protein T-cap (Telethonin) (Gregorio et al., 1998). The latter bears a specific phosphorylation site near its C-terminus, but its physiological role just emerges. A null mutation that creates a shortened T-cap protein was found in a Brazilian family and linked to limb girdle muscular dystrophy (Moreira et al., 2000). Recently, T-cap was reported to bind to MLP, a muscle LIM protein. The interaction between MLP and T-cap turned out to be essential for maintaining correct Z-disk architecture (Knöll et al., 2002). It was proposed that the Z-disc MLP/T-cap complex is a key component of the *in vivo* cardiomyocyte stretch sensor machinery and that it stabilizes the correct anchoring of Titin at the Z-disc. Titin acts as a molecular spring element that generates passive tension (Labeit and Kolmerer, 1996). It is still unknown how the mechanical stress sensed by titin is translated via the T-cap/MLP complex into signalling pathways.

In order to identify the residues that constitute titin's binding site to T-cap we carried out the sequential backbone assignment of the first 195 residues of Titin that constitute the two Ig domains Z1Z2. However, binding to T-cap does not yield a 1:1 complex but higher order assemblies (Zhou et al., 2003) that rendered the identification of the binding site by NMR virtually impossible.

Methods and experiments

Z1Z2 was cloned into a modified pET 9d vector (Novagen) with an N-terminal tag containing a $(His)_6$-sequence. Proteins were expressed in the *Escherichia coli* strain BL21 (DE3). Uniform labeling with ^{13}C and ^{15}N and partial labeling with ^{2}D was achieved by growing the bacteria in minimal medium containing 0.5 g/l $^{15}NH_4Cl$ and 2 g/l [$^{13}C_6$]-glucose and 70% D_2O. The protein was purified from the supernatant using an affinity purification step on a Ni-NTA column with subsequent gelfiltration.

NMR samples were concentrated to 1 mM in 20 mM Na-Acetate buffer (pH 5.6), 50 mM Na_2SO_4 and 10% D_2O. All NMR experiments were performed on a Bruker DRX-600 spectrometer. Sample temperature was 37 °C. Quadrature detection in the indirect dimension of the multidimensional experiments was achieved by the echo/antiecho detection scheme for ^{15}N, and by the States-TPPI method for ^{1}H and ^{13}C. Sequential backbone assignment was achieved by recording the following set of experiments: 3D HNCA, 3D HNCOCA, 3D HNCACB

*To whom correspondence should be addressed. E-mails: edlich@embl.de; muhle@embl.de

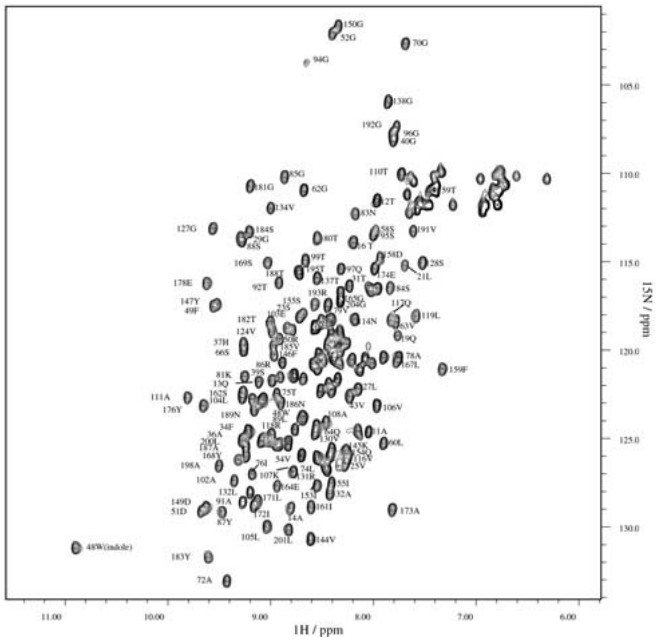

Figure 1. ^{1}H-^{15}N-HSQC-Spectrum of the first two Ig domains Z1Z2 of the muscle protein titin aquired on a Bruker DRX-600 spectrometer at 37 °C in 20 mM Na-Acetate buffer (pH 5.6), 50 mM Na_2SO_4. Resonances were labeled with the corresponding sequence positions. Amino acid labels were omitted from the middle of the HSQC for clarity.

and HNCOCACB. Proton chemical shifts were referenced with respect to DSS or calculated assuming $\gamma^{15}N/\gamma^{1}H = 0.1011329118$ and $\gamma^{13}C/\gamma^{1}H = 0.251449530$ (Wishart et al., 1995).

Extent of assignments and data deposition

Most of the main chain resonances have been assigned with the exception of the prolines. The first 8 N-terminal residues that belong to the $(His)_6$-tag are too weak to appear in the spectra probably due to their intrinsic flexibility. A couple of residues could only be partially assigned: the H^N and N resonances of Thr57, Phe67, Asp69, Ser190 and Val202 were missing from the spectra. Figure 1 shows the ^{1}H-^{15}N-HSQC-Spectrum of a uniformly ^{15}N labeled sample of Z1Z2. An analysis using the Chemical Shift Index (CSI) (Wishart and Sykes, 1994) shows that the two Ig domains are predominantly in β-sheet conformation (data not shown). Interestingly, the main chain resonances of two sequence stretches (Asn83 to Ala98 and Asp179 to Ala194) were difficult to distinguish from each other. Since the two stretches are located at homologous positions within the first and second Ig domain, this feature reflects the internal structural homology between the two domains. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5760.

Acknowledgements

We would like to thank Dr Michael Sattler for his technical support. We gratefully acknowledge the support by the Deutsche Forschungsgemeinschaft (MU 1606/1-1).

References

Gregorio, C.G., Granzier, H., Sorimachi, H. and Labeit, S. (1999) *Curr. Opin. Cell Biol.* **11**, 18–25.

Gregorio, G.C., Trombitas, K., Centner, T., Kolmerer, B., Stier, G., Kunke, K., Suzuki, K., Obermayr, F., Herrmann, B., Granzier, H., Sorimachi, H. and Labeit, S. (1998) *J. Cell. Biol.*, **143**, 1013–1027.

Knöll, R., Hoshijima, M., Hoffman, H.M., Person, V., Lorenzen-Schmidt, I. et al. (2002) *Cell*, **111**, 943–955.

Labeit, S. and Kolmerer, B. (1995) *J. Mol. Biol.*, **248**, 308–315.

Moreira, E.S., Wiltshire, T.J., Faulkner, G., Nilforoushan, A., Vainzogf, M., Suzuki, O.T., Valle., G., Reeves, R., Zatz., M., Passos-Bueno, M.R. and Jenne, D.E. (2000) *Nat. Genet.*, **24**, 163–166.

Trinick, J. (1994) *Trends Biochem. Sci*. **19**, 405–409.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.

Zhou, P., Gautel, M., Geerlof, A., Wilmanns, M., Koch, M.J. and Svergun, D.I. (2003) *J. Biol. Chem.*, **278**, 2636–2644.