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Letter to the Editor: Sequence specific resonance assignment of the central domain of cardiac Myosin Binding Protein C (MyBP-C)

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Biological context

Myosin binding protein-C (MyBP-C) is a member of the family of large multidomain proteins fulfilling regulatory and scaffolding functions in muscle and other cytoskeletal structures (Winegrad, 2000; Bennett et al., 1999). MyBP-C is composed of 11 domains, six of these belong to the intracellular subset of the Immunoglobulin superfamily (IgI), five belong to the fibronectin type III family (fn3) (Gautel et al., 1995). We have initiated a comprehensive characterization of important domains from MyBP-C by NMR spectroscopy to enhance previous functional *in vitro* and *in vivo* studies (Gruen and Gautel, 1999; Kunst et al., 2000) by structural and dynamics data.

The cardiac isoform of MyBP-C is of particular interest for structural studies as it is linked by today about 30 point or truncation mutations to various instances of familial hypertrophic cardiomyopathy (FHC), also known as sudden cardiac arrest (Yu et al., 1998). As only little is known about the details of MyBP-Cs function, a link between clinical symptoms and combination of structural and functional studies is therefore promising to elucidate, domain by domain, the biological function of MyBP-C.

The domain C5 from the cardiac isoform was chosen to initiate the study as it shows as a single domain the greatest sequence divergence to the skeletal muscle isoforms. Furthermore, it contains two point mutations that are linked to FHC.

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Methods and results

The domain cC5 was cloned comprising residues 641-765 from the human cardiac MyBP-C cDNA (EMBL accession code Q14896 & Q16410) (Carrier et al., 1997). The fragment was cloned into a pet8a vector linking it N-terminally to a His tag (additonal sequence: mhhhhhhss). All protein expression was carried out in BL21(DE3) PLysS strain. For the production of ^{15}N and ^{15}N/^{13}C labelled protein expression was carried out in minimal medium modified as described previously (Chen et al., 1998). Cells were grown to an OD600 = 0.8 (in minimal media 1.0) and expression was induced with 50 μg l^{-1} IPTG. Expression was continued at 18 °C for 18 h before cells were harvested. Cells were resuspended in 40 mM Tris buffer, 70 mM NaCl, 2 mM β-mercaptoethanol containing 1 tablet of protease inhibitor mix (Boehringer) and opened by three cycles of french press treatment. Cell debris was removed by centrifugation at 20 000 *g* for 90 min. The supernatant was diluted 1:5 and then applied to TALON resin (Clontech) for metal chelating affinity purification in gravity flow. Usually bed volumes of 4 ml resin were used per 1 L of cell culture. The resin was washed 3 times with 15 ml wash buffer (40 mM Tris buffer pH 7.5, 70 mM NaCl, 2 mM β-mercaptoethanol, 0.02% NaN_3) and bound protein was then eluted with wash buffer containing 200 mM imidazole. Yields were usually around 15 mg from 1 L of rich medium. Protein purity was checked on tris/tricine gels and if necessary the protein was further purified by gel filtration using a Superose-12 column (Pharmacia) on a Pharmacia FPLC system. Protein was stored at 4 °C before use.

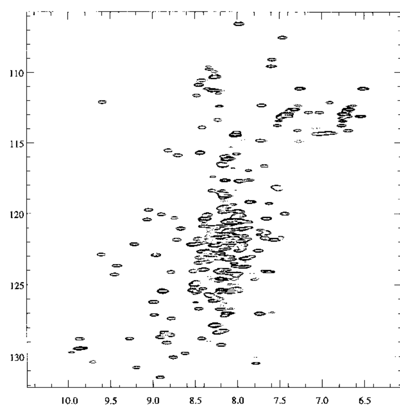

Figure 1. ^{15}N HSQC spectrum of cC5 at $T = 293$ K and 500 MHz.

Samples were concentrated and dialysed against NMR buffer in Schleicher & Schüll thimbles with 10 kD molecular weight cutoff.

NMR experiments were carried out on samples with protein concentrations ranging from 0.5 to 1.5 mM in 20 mM Tris/HCl or phosphate buffer pH 7.0, 50 mM NaCL, 2 mM DTT, 1 mM EDTA, 0.02% NaN_3, 90% H_2O/10%D_2O. The HCCH-TOCSY and ^{13}C resolved NOESY experiments were repeated in a buffer containing 99% D_2O as well. All double and triple resonance experiments used in the assignment were recorded at a temperature of T = 293 K and at a proton frequency of 600 MHz on a Varian Unity*Plus* spectrometer equipped with a 5 mm triple resonance/shielded Z-gradient probe and three RF channels.

Spectra were processed with nmrPipe (Delaglio et al., 1995) and analysed with Ansig (Kraulis, 1989). Chemical shifts for ^{1}H, ^{15}N and ^{13}C were referenced to TSP = 0 ppm, as proposed by (Wishart et al., 1995)

Extent of assignments and data deposition

Experimental conditions were initially optimised using ^{15}N labelled protein. An optimised ^{15}N HSQC spectrum is shown in Figure 1. The assignment was then performed based on a complete set of backbone triple resonance experiments comprising HNCA, HN(CO)CA, HNCO, HN(CA)CO, HNCACB and HN(CO)CACB (Kay et al., 1990; Grzesiek and Bax, 1992) recorded on a ^{15}N/^{13}C labelled sample. These spectra were supplemented by ^{15}N resolved 3D NOESY-HSQC and TOWNY-HSQC spectra recorded on a purely ^{15}N labelled sample. Assignments were extended into sidechains by means of 3D HCCH-TOCSY and ^{13}C resolved NOESY-HSQC spectra. Aromatic side chains were assigned from adapted ^{13}C CT-HSQC experiments (Vuister and Bax, 1992) and connected to the backbone resonances through NOEs.

Essentially complete assignments were obtained for ^{1}H, ^{15}N and ^{13}C resonances. Partially lacking are sidechain carbonyl resonances and sidechhain ^{15}N resonances in lysine and histidine residues. The assignment is deposited with the BioMagResBank (http://www.bmrb.wisc.edu/), accession number 5014.

The protein contains a large insertion of about 30 residues, very untypical for the general IgI fold. this inserion is rich in prolines some of which seem to undergo cis-trans isomerisation on a slow time scale. As a result, multiple conformers for some stretches could be assigned for parts of the insertion itself but also for loop regions adjacent to the insertion. These observations indicate the presence of a complex dynamic that will be investigated further using ^{15}N relaxation studies.

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References

Bennett, P.M., Fürst, D.O. and Gautel, M. (1999) *Rev. Physiol. Biochem. Pharmacol.*, **138**, 204–234.

Carrier, L., Bonne, G., Bahrend, E., Yu, B., Richard, P., Niel, F., Hainque, B., Gruand, C., Gary, F., Labeit, S., Bouhour, J.B., Dubourg, O., Desnos, M., Hagege, A.A., Trent, R.J., Komadja, M., Fiszman, M. and Schwartz, K. (1997) *Circ Res.*, **80**, 427–434.

Chen, H.A., Pfuhl, A., Davis, B. and Driscoll, P.C. (1998) *J. Biomol. NMR*, **12**, 457–458.

Delaglio, F., Gzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Gautel, M., Zuffardi, O., Freiburg, A. and Labeit, S. (1995) *EMBO J.*, **14**, 1952–1960.

Gruen, M. and Gautel, M. (1999) *J. Mol. Biol.*, **286**, 933–949.

Grzesiek, S. and Bax, A. (1992) *J. Magn. Reson.*, **96**, 432.

Kay, L. E., Ikura, M., Tschudin, R. and Bax, A. (1990) *J. Magn. Reson.*, **89**, 496.

Kraulis, P. J. (1989) *J. Magn. Reson.*, **24**, 627–633.

Kunst, G., Kress, K.R., Gruen, M., Uttenweiler, D., Gautel, M. and Fink, R.H.A. (2000) *Circ Res.*, **86**, 51–58.

Vuister, G.W. and Bax, A. (1992) *J. Magn. Reson.*, **98**, 428–435.

Winegrad, S. (2000) *Circ Res.*, **86**, 6–7.

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.

Yu, B., French, J.A., Carrier, L., Jeremy, R.W., McTaggart, D.R., Nicholson, M.R., Hambly, B., Semsarian, C., Richmond, D.R., Schwartz, K. and Trent, R.J. (1998) *J. Mol. Graph.*, **35**, 205–210.