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Letter to the Editor: Sequence specific assignment of domain C1 of the N-terminal myosin-binding site of human cardiac Myosin Binding Protein C (MyBP-C)

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Biological context

The cardiac isoform of human myosin binding protein C (MyBP-C) is a member of large family of multidomain proteins fulfilling regulatory and scaffolding functions in muscle and other cytoskeletal structures (Winegrad, 2000). MyBP-C 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.

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 (Idowu et al., 2002, 2003) by structural and dynamics data. Domain cC1 of MyBP-C is a part of the cC1-mybpc-cC2 fragment that was shown to bind to the S2 subfragment of myosin (Gruen and Gautel, 1999). Domain cC1 was studied by NMR to enable interaction studies with myosin and because it contains several point mutations linked to hypertrophic cardiomyopathy, namely, D228N, Y237S, H257P and E258K (see *www.angis.org.au/Databases/Heart/* website for references). We assume that these mutations might affect the stability, the dynamics or the binding to myosin of this domain. Such changes may have dramatic consequences on the function of MyBP-C *in vivo*.

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Methods and results

The domain cC1 was cloned comprising residues 151-260 from the human cardiac MyBP-C cDNA (EMBL accession code Q14896 and Q16410) (Carrier et al., 1997). The fragment was cloned into a pet8a vector linking it N-terminally to a His tag (additional sequence: MHHHHHHSS). All protein expression was carried out in BL21* (Invitrogen). For the production of ^{15}N and $^{15}N/^{13}C$ labelled protein expression was carried out in minimal medium modified as described previously (Marley et al., 2001; Idowu et al., 2001). Cells were grown to an OD600 = 0.8 (in minimal media 1.0) and expression was induced with 50 μg/L IPTG. Expression was continued at 37 °C for 4 h before cells were harvested. Cells were resuspended in 40 mM Tris buffer, 70 mM NaCl, 2 mM β-mercaptoethanol and opened by three cycles of french press treatment. Cell debris was removed by centrifugation at 20,000 g for 60 min. The supernatant was diluted 1:2 and then applied to TALON resin (Clontech) for metal chelating affinity purification in gravity flow. Usually four columns of 4 ml resin were used in parallel per 1 L of cell culture. The resin was washed 6 times with 15 ml wash buffer (40 mM Tris buffer pH 7.5, 70 mM NaCl, 2 mM bimercaptoethanol) and bound protein was then eluted with 8 mL wash buffer containing 500 mM imidazole. Yields were usually around 10 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-16 column (Pharmacia) on a Pharmacia FPLC system. Protein was stored at 4 °C before use. Samples were concentrated and dialysed against NMR buffer in concentrator (Vivaspin) with 5 kD molecular weight cutoff.

NMR experiments were carried out on samples with protein concentrations ranging from 0.5 to

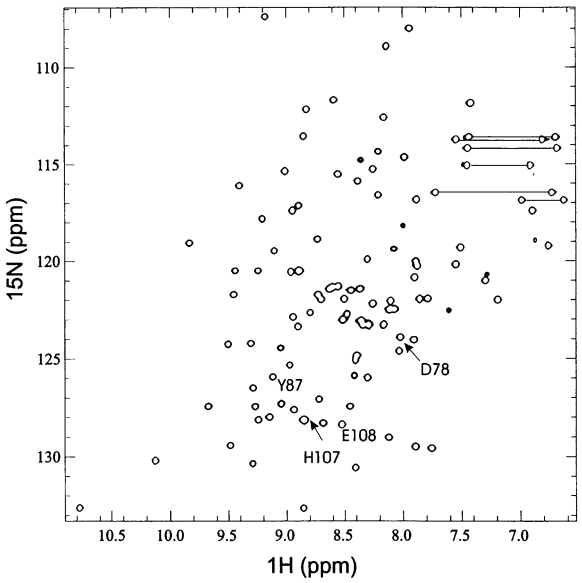

Figure 1. ^{15}N HSQC spectrum of cC1 at T = 298 K and 600 MHz. The residues shown with the spectrum are FHC mutations (D78N, Y87S, H107P and E108K; numbering for cC1 alone). The position of the residues in full sequence of human cardiac MyBP-C are D228, Y237, H257 and E258.

0.8 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 3D HSQC-NOESY and HSQC-Aromatics 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 = 298 K and at a proton frequency of 600 MHz on a Bruker 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 optimized using ^{15}N labeled protein. An optimized ^{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 labeled sample. These spectra were supplemented by ^{15}N resolved 3D NOESY-HSQC and TOCSY-HSQC spectra recorded on a purely ^{15}N labeled 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 (Vuister and Bax, 1992) and HCCH-TOCSY experiments and connected to the backbone resonances through NOEs.

Essentially complete assignments were obtained for ^{1}H, ^{15}N and ^{13}C resonances. It is worth mentioning that the NMR spectra were of such a quality that we were able to identify two sequencing errors in the database sequence of MyBP-C, S236G and E248D. 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/), under accession number 6015.

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References

Andersen, P.S., Havndrup, O., Bundgaard, H., Moolman-Smook, J.C., Larsen, L.A., 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, M., 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.

Idowu, S.M., Gautel, M., Perkins, S.J. and Pfuhl, M. (2003) *J. Mol. Biol.*, **329**, 745–761.

Iodwu, S.M., Gautel, M. and Pfuhl, M. (2002) *J. Biomol. NMR*, **22**, 199–200.

Marley, J., Lu, M. and Bracken, C. (2001) *J. Biomol NMR*, **20**, 71–75.

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.