Hypertrophic cardiomyopathy causing mutations in myosin binding protein-C alter PKA phosphorylation

C.E. Oakley,[1,2] J. Hwang,[1] L.J. Brown,[3] M. Kekic,[1] P.G. Fajer[2] and B.D. Hambly,[1] [1]Discipline of Pathology, University of Sydney, NSW 2006, Australia, [2]Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306, USA and [3]Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW 2109, Australia.

Cardiac myosin binding protein C (cMyBPC) is a large regulatory protein within the sarcomere. cMyBPC phosphorylation increases systolic tension, and dissociates the N-terminal region of cMyBPC from the S2 neck region of myosin. cMyBPC mutations are associated with familial hypertrophic cardiomyopathy (FHC). We have cloned, expressed and purified the N-terminal region (immunoglobulin motifs C1 to C2) that encompasses the tri-phosphorylation sites (defined as sites A to C). Using *in vitro* mutagenesis we have also generated four FHC mutant forms of C1-C2. Four FHC-causing mutations are located in the phosphorylatable linker between Ig motifs C1 and C2; G278E, G279A, R326Q and L352P. The effect of these mutations on phosphorylation with PKA at 30oC was investigated. The G279A and R326Q mutations yielded the same rate of phosphorylation as the WT C1-C2; complete di-phosphorylation in less than 5 minutes and complete tri-phosphorylation by 2.5 hours. The G278E mutant was phosphorylated more slowly; complete di-phosphorylation by 30 minutes and complete tri-phosphorylation in > 4 hours. Surprisingly, the L352P construct was phosphorylated more rapidly than WT; complete tri-phosphorylation in 2 hours. In all cases the phosphorylation order was the same as WT (first site B, then A and finally C). Structure prediction provides insights into the mechanisms underlying the changes in phosphorylation rate. Together these data suggest that an alteration in cMyBPC phosphorylation rate may underlie the pathogenesis of FHC caused by some mutations, although paradoxically the rate can be increased or decreased.