N-TERMINAL FRAGMENTS OF CARDIAC MYOSIN BINDING PROTEIN-C INHIBIT ACTOMYOSIN MOTILITY BY TETHERING ACTIN

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Cardiac myosin binding protein-C (cMyBP-C) mutations are a leading cause of hypertrophic cardiomyopathy. cMyBP-C has 11 domains, C0 through C10, that bind sarcomeric proteins, including myosin and actin. A 29 kD N-terminal fragment (C0C1f) of cMyBP-C containing the first two domains C0 and C1 and the first 15 residues of the conserved MyBP-C motif is cleaved from cMyBP-C following ischemic-reperfusion injury (Sadayappan et al., JMCC 44:S44, 2008). Expressed C0C1f fragments inhibit actin velocities in the motility assay at a 2:1 molar ratio to myosin, similar to other N-terminal fragments: C0C3, C0C2, and C1C2. Interestingly, fragments containing only the C0C1 domains do not alter velocity, suggesting the additional 15 residues in C0C1f are necessary for inhibition. Adding C0C1 to the motility assay can partially reverse the C0C3-mediated inhibition of velocity, suggesting C0C1 may compete with C0C3 for actin binding. cMyBP-C fragments may affect motility by creating a tether between actin and the flowcell surface. To test this, motility experiments were performed under high ionic strength, saturating MgATP, and in the absence of methylcellulose, conditions in which most actin filaments diffuse away from the surface due to weak interactions with myosin. In the presence of C0C2, many actin filaments bound and translocated on the surface, confirming this fragment's tethering capacity. Additionally, in the laser trap we adhered C0C3 fragments to a bead in the absence of myosin and observed C0C3 transiently binding to a single actin filament with an ~100 ms attached lifetime. We also saw evidence that C0C3 may partially unfold under load. These experiments strongly suggest that N-terminal domains of cMyBP-C containing the MyBP-C motif tether actin filaments and provide one mechanism for modulating actomyosin motion generation, i.e. by imposing an effective viscous load within the sarcomere.