Myosin Binding Protein C, a Potential Regulator of Cardiac Contractility

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The role of myosin binding protein C (MyBP-C) in the formation and function of striated muscle is unclear, even though the demonstration of its presence in the thick filaments of striated muscle was made by Offer et al more than 25 years ago.2 Because of the inability of myosin to form normal thick filaments in the absence of MyBP-C and the temporal correlation between the appearance of MyBP-C, thick filaments, and sarcomeres in developing striated muscle,3–5 MyBP-C is assumed to be essential for the formation and normal structure of thick filaments. The cardiac isoform of MyBP-C has an additional feature that is absent from the skeletal isoforms. There are 3 phosphorylation sites located in the cardiac motif portion of the molecule in the N-terminal region.6 In addition to these phosphorylatable sites, a Ca2+/calmodulin-regulated kinase specific for cardiac MyBP-C is bound to the thick filament.7,8 The combination of these features suggests that the function of MyBP-C in cardiac muscle can be modified posttranslationally by phosphorylation. Such a phosphorylation could be an important regulator of cardiac contractility. At least 5 transmitter-based mechanisms for the regulation of cardiac contractility are associated with changes in the degree of phosphorylation of MyBP-C: increased or decreased phosphorylation with increased or decreased contractility.

MyBP-C is located in a series of 7 to 9 sites, each separated by 43 nm in the C zone of the sarcomere and most likely oriented perpendicular to the axis of the filament.9 Within the C zone, there are approximately 9 crossbridges per 3 MyBP-C molecules,9 but in the 50% of the thick filament outside the C zone there is no MyBP-C. The C-terminal region of MyBP-C contains a binding site for the rod (LMM) portion of myosin.10,11 Although the binding site itself is located in the C-terminal module (C10, cardiac MyBP-C contains 11 modules, C0 to C10, N- to C-terminus), the affinity of the binding is increased by the presence of C8 and C9.11 More recently, Gruen and Gautel12 have confirmed that a second binding site for myosin exists in the N-terminal region of MyBP-C. This site binds to subfragment 2 of myosin (S2), near the hinge of the crossbridge, the same region in which certain mutations of myosin cause familial hypertrophic cardiomyopathy.12,13 The binding occurs even with a fragment of MyBP-C containing only the C1 and C2 modules. Of particular importance is their demonstration that phosphorylation of the 3 sites in the MyBP-C motif within the C1C2 fragment inhibits the binding of the fragment to the S2 region of myosin in either skeletal myofibrils or cardiac myocytes.14 In isolated cardiac thick filaments containing the whole MyBP-C molecule, there is a change in crossbridge structure from phosphorylation of MyBP-C.15,16 The change in structure is of a type that could alter crossbridge cycling.

In the most recent work from Gautel’s group,14 published in this issue of Circulation Research, the effects of the binding of the C1C2 fragment to myosin on the mechanical properties of skinned skeletal muscles fiber have been studied. The fragment was allowed to diffuse into the skinned fibers in sufficient concentration to produce full or near full saturation of S2 both in and out of the C zone.

Kunst et al14 measured 6 parameters of contractility and found that 5 were changed by the unphosphorylated C1C2 fragment. None of these changes in mechanical performance occurred when the C1C2 fragment was fully phosphorylated before it diffused into the fiber.

Maximum Ca2+-activated force was decreased, but the stiffness during maximum Ca2+ activation was unchanged. Changes in stiffness are generally accepted to indicate an alteration in the number of crossbridges strongly attached to the thin filament. These results indicate that the number of crossbridges attached is unchanged by the addition of unphosphorylated C1C2, but the amount of force is decreased. Either each crossbridge develops less force or the duty cycle (fraction of crossbridge cycle during which force is generated) has been reduced by the binding of the C1C2 fragment.

The addition of C1C2 fragments to myofibrils increased rigor force and to a smaller extent rigor stiffness, indicating an increase in the number of rigor links. Because all crossbridges are believed to be attached to thin filaments during rigor,17 the experiments of Kunst et al14 show that the properties of the crossbridge in rigor are modified by C1C2 binding. The changes in Ca2+-activated force and rigor force could be explained by a decrease in the number and an increase in the stiffness of attached crossbridges from binding of C1C2, a not unreasonable effect from a protein fragment binding to the hinge region of the crossbridge.

The increased rate of formation of rigor links produced by C1C2 when ATP and creatine phosphate were withdrawn was attributed to a change in the kinetics of formation of rigor links.14 Even in the presence of C1C2 bound to S2, however, the rigor links formed over many seconds in the ATP-free
All of these effects produced by the unphosphorylated C1C2 fragment of cardiac MyBP-C and inhibited by the phosphorylation of the MyBP-C motif within the fragment are provocative and intriguing, because they raise the possibility of regulation of contractility of cardiac muscle by a variable interaction of the N-terminus of MyBP-C with the hinge region of the crossbridge. In interpreting these results, it is important to consider the stoichiometry of MyBP-C to myosin. Because there are about 3 myosin molecules per molecule of MyBP-C within the C zone only one third of myosin molecules there can interact with the N-terminus of MyBP-C. Given that almost half of the crossbridges lie outside the C zone, the vast majority of crossbridges cannot be regulated by direct interaction with MyBP-C. However, as a result of mechanical coupling of crossbridges within a thick filament, a change in the kinetics of cycling of as few as 5% to 10% of crossbridges can affect the cycling of all the crossbridges in the filament and lead to a change in the kinetics of the contraction of the fiber. Another possibility to be considered is that the high concentration of cardiac C1C2 could contribute to modification of crossbridge function by displacing some or even all of the native MyBP-C. This interpretation would explain the similarity of effects from the addition of unphosphorylated C1C2 and extraction of MyBP-C. It is possible that the results of Kunst et al. may be particularly relevant to familial hypertrophic cardiomyopathy caused by truncation of MyBP-C, in which a high concentration of the N-terminal fragment may be present. A high concentration of C1C2 could produce a decrease in the generation of force. The mechanical properties of the transgenic hearts of Yang et al., in which there is still normal as well as truncated MyBP-C, resemble those of the skinned fibers exposed to a high concentration of C1C2. This similarity raises the intriguing possibility that phosphorylation of the N-terminus of MyBP-C in myopathic hearts with a high concentration of N-terminal fragment might improve cardiac performance.

The results of Kunst et al. have added important new information and opened new ways of looking at the function of MyBP-C in cardiac muscle. Novel roles for this cryptic, but no doubt important, myofibrillar protein must be considered.

References


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