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Slowing of Contractile Kinetics by Myosin-Binding Protein C Can Be Explained by Its Cooperative Binding to the Thin Filament

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Abstract

Cardiac myosin binding protein C (cMyBP-C) is a thick filament-associated protein that participates in the regulation of muscle contraction. Simplified in vitro systems show that cMyBP-C binds not only to myosin, but also to the actin filament. The physiological significance of these separate binding interactions remains unclear, as does the question of whether either interaction is capable of explaining the behavior of intact muscle from which cMyBP-C has been removed. We have used a computational model to explore the characteristic effects of myosin-binding versus actin-binding by cMyBP-C. Simulations suggest that myosin-cMyBP-C interactions reduce peak force and Ca^{2+} sensitivity of the myofilaments, but have no appreciable effect on the rate of force redevelopment (k_{tr}). In contrast, cMyBP-C binding to actin increases myofilament Ca^{2+} sensitivity and slows k_{tr} at sub-maximal Ca^{2+} values. This slowing is due to cooperation between cMyBP-C 'crossbridges' and traditional myosin crossbridges as they bind to and activate the actin thin filament. We further observed that an overall recapitulation of skinned myocardial data from wild type and cMyBP-C knockout mice requires the interaction of cMyBP-C with both of its binding targets in our model. The assumption of significant interactions with both partners was also sufficient to explain published effects of cMyBP-C ablation on twitch kinetics. These modeling results strongly support the view that both binding interactions play critical roles in the physiology of intact muscle. Furthermore, they suggest that the widely observed phenomenon of slowed force development in the presence of cMyBP-C may actually be a manifestation of cooperative binding of this protein to the thin filament.

Introduction

Cardiac myosin binding protein C (cMyBP-C) is a 137 kDa thick filament protein which is located in the C-zone of the sarcomere, the central region of the A-band [1] (Fig. 1A & B). The study of cMyBP-C and its physiological role in muscle regulation has intensified with the realization that cMyBP-C mutations account for a large portion (34%) of mutations found in patients with familial hypertrophic cardiomyopathy [2,3]. This interest is driven by the expectation that greater understanding of normal cMyBP-C function will shed light on the precise mechanisms that cause cMyBP-C-associated cardiac hypertrophy [4].

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The cardiac isoform of cMyBP-C consists of eight immunoglobulin-like domains and three fibronectin type-3-like domains (numbered C0 to C10), with the C terminus being strongly bound to myosin and titin [1,5]. The N terminus is free to diffuse away from the thick filament backbone, and contains a well-established binding site to myosin S2 [6]. Some conceptual models of cMyBP-C function envision this ‘tethering’ of myosin heads as its chief regulatory mechanism [6]. Tethering appears to be abolished by phosphorylation of the protein’s M domain [7,8] thereby creating the potential for cMyBP-C to regulate cross bridge formation, maximal force development and Ca^{2+} sensitivity in response to β adrenergic stimulation [8-10].

Although cMyBP-C binding to actin was observed decades ago [11], this interaction has been studied in detail only recently. These studies provide broad support for specific binding of cMyBP-C’s N-terminal domain to actin filaments. Using a yeast two-hybrid system, Bhuiyan *et al.* provided evidence that the C1 and M domains of cMyBP-C can bind to actin [12]. Evidence supporting actin-cMyBP-C interactions has also been found using co-sedimentation binding assays [13], in vitro motility assays [14], and small-angle X-ray scattering [15]. Although the hypothesis that the thin filament can be activated through binding of cMyBP-C to actin has been controversial [6], further experimental evidence has emerged. Three-dimensional helical reconstruction of decorated actin filaments show cMyBP-C holding tropomyosin in its Ca^{2+} -activated position on actin even at low Ca^{2+} levels, suggesting that cMyBP-C has the ability to activate the thin filament upon binding [16]. Furthermore, using bifunctional rhodamine probes on TnC, Kampourakis *et al.* showed that the N terminal fragments of cMyBP-C (C1mC2) lead to a change in TnC structure that is similar to the Ca^{2+} -activated state [17]. This led them to conclude that MyBP-C can stabilize the active state of the thin filament in the absence of Ca^{2+} .

In spite of accumulating evidence that cMyBP-C interacts with both myosin and actin, it is unclear whether these interactions are sufficient (alone or in combination) to explain intact muscle observations [18,19]. Several groups have measured contractile function and kinetics of cardiac muscle preparations from mice in which cMyBP-C is genetically ablated. These studies universally report increased rates of force development upon removal of cMyBP-C [20-22], indicating that the presence of cMyBP-C slows force development, particularly at low levels of Ca^{2+} [20]. On the other hand, consensus has not been reached for effect of cMyBP-C on myofilament Ca^{2+} sensitivity [23]. Depending on the experimental procedure, the presence of cMyBP-C has been observed as causing no change [20], an increase [21,24] or even decrease in Ca^{2+} sensitivity [7,25]

To help clarify the relationship between cMyBP-C’s molecular binding activity and muscle-level observations, we added an explicit representation of this protein to a previous model of myofilament activation (Aboelkassem *et al.*, submitted). This allowed us to examine the unique effects of myosin-cMyBP-C and actin-cMyBP-C binding individually or in tandem on myofilament Ca^{2+} sensitivity and kinetics. We also used the model to search for binding properties capable of explaining the effects of cMyBP-C ablation on skinned myocardium.

Methods

We modeled the cardiac thin filament as a series of 26 interacting actin regulatory units (RUs), each of which obeys a six-state model [26] (Fig. 1, B & C). As established in early models of myofilament activation [27], we allow each RU to take on one of three conformations: a blocked (B) state in which tropomyosin (Tm) sterically blocks S1 binding sites on actin, a closed (C) state in which S1 can bind weakly to actin, and an open (M) state in which S1 binds strongly to actin, thereby generating force. This depiction of crossbridge cycling, effectively one crossbridge per RU, has been shown to be functionally equivalent to the more computationally expensive representation of three independently cycling crossbridges under each RU [27]. B, C, and M states have both Ca^{2+} -free and Ca^{2+} -bound states (denoted by subscripts 0 and 1, respectively), reflecting a 'loose coupling' regulatory scheme in which Tm can transition between states even in the absence of Ca^{2+} , albeit only rarely. Such Ca^{2+} -free transitions, representing the spontaneous dissociation of TnI, are dependent on the parameter λ [see Aboelkassem et al., submitted]. This parameter scales the Ca^{2+} -independent activation rate such that when $\lambda = 0$, Ca^{2+} -independent transitions are completely prohibited. If $\lambda > 0$, some Ca^{2+} transitions are allowed (Fig. 1C). RU transitions between B, C, and M states are dependent upon the current states of each RU's nearest neighbors as detailed in Campbell *et al.* [28]. These interactions represent Tm-Tm overlap between adjacent RUs, and they impart realistic cooperative behavior to the model. RUs outside of the C-zone behave according to the six-state model, subject to the status of nearest neighbors (Fig. 1C).

To approximate the region of our modeled filament that lies in the C-zone, we assign one cMyBP-C molecule to each of nine consecutive RUs at the distal end (Fig. 1B). This assignment is motivated by structural data showing that RUs occur every 38 nm [29], while cMyBP-C occurs every 42.9 nm across nine C-zone stripes [19]. This is an obvious simplification of the true stoichiometry, as it assumes a sarcomere length that gives full overlap of the C-zone with the thin filament and further that the three-dimensional configuration within the lattice reduces to an effective concentration of one cMyBP-C molecule per RU in the C-zone. On the other hand, this simplification seems reasonable for a model limited to the resolution of RUs and not individual actin monomers.

In the model we allow cMyBP-C to reside in one of three states: free, actin-bound and myosin-bound. We define the forward and reverse rates for actin binding as $k_{\text{cp.a}}^+$ and $k_{\text{cp.a}}^-$ respectively, and the forward and reverse rates for myosin binding as $k_{\text{cp.m}}^+$ and $k_{\text{cp.m}}^-$ respectively. We also define the equilibrium constants corresponding to these reactions: $K_{\text{cp.a}} = k_{\text{cp.a}}^+/k_{\text{cp.a}}^-$ and $K_{\text{cp.m}} = k_{\text{cp.m}}^+/k_{\text{cp.m}}^-$. We assume that the cMyBP-C associated with a given RU cannot interact with actin and myosin simultaneously.

A further assumption is that when cMyBP-C binds to either actin or myosin, it limits the states that may be occupied by that particular RU (Fig. 1D). These assumptions can be summarized with the following set of specific rules: First, when cMyBP-C is bound to myosin, the associated RU cannot enter the M_0 or M_1 states, as myosin is tethered and cannot reach actin for binding. The reasoning behind this assumption is based on the idea that was first presented by Starr and Offer [30]. It is now well established that the

unphosphorylated N terminal C1-m-C2 region of cMyBP-C can bind to the S2 segment of the myosin rod [1,31,32], which is commonly understood to prevent the pair of myosin heads from reaching the thin filament (Fig. 1A) [18]. We therefore assume that this tethering prevents transition of the RU into its open (M) state in an all-or-nothing manner. The second assumption is that cMyBP-C binding to actin is only possible when its associated RU is not in a blocked (B_0 or B_1) state, as we assume that Tm blocks cMyBP-C binding sites on actin when in the B_0 or B_1 states. Such an assumption seems reasonable given recent findings: Experiments suggest that N terminal fragments of cMyBP-C compete with Tm for the same region on actin [15,33,34]. Simulations with 3D reconstructions of thin filaments support the same hypothesis [16]. In the blocked state, Tm covers this putative cMyBP-C binding site on actin, suggesting that Tm must transition into the blocked or closed state prior to cMyBP-C binding. The third assumption is a logical extension of these same findings, namely that an RU cannot enter a blocked (B_0 or B_1) state when its associated cMyBP-C is bound to actin, because this attachment prevents Tm from shifting back to the blocked position. A similar assertion has been raised by others [35]. The fourth assumption is that if cMyBP-C binds to myosin while myosin is also bound to actin, this assembly immediately detaches from the thin filament (as indicated in Fig. 1C). This is a simplifying assumption, made in order to reduce the number of free parameters in the model.

This model as described can be implemented using a Monte Carlo scheme in which the state of each of the 26 RUs is explicitly tracked in time. At each time step, the Tm state and cMyBP-C binding status are updated according to numbers drawn randomly from a uniform distribution ($0 \rightarrow 1$). The interval of R is divided into subintervals: one for each permissible transition out of the given state, plus one interval corresponding to no state transition. For instance, the interval of R is divided into three subintervals for the state B_0 : one representing the transition to B_1 , one for the transition to C_0 , and one for remaining in B_0 . The size of each subinterval is proportional to the governing rate constant, and describes a transition probability $P_i = k_i \Delta t$, where k_i is the relevant rate constant and Δt is the simulation time step (as detailed in Aboelkassem et al., submitted). For each simulation, we initialized all 26 RUs to the B_0 state and all 9 cMyBP-C molecules to the free state. We then allowed force to develop to steady state by running the simulation for 9 seconds at a specified concentration of Ca^{2+} (Fig. 2). After 9 seconds, a slack-restretch (k_{tr}) maneuver was simulated by breaking all actin-myosin attachments and actin-MyBP-C attachments instantaneously. This involves placing any RUs residing in the M_0 or M_1 states back into C_0 or C_1 states, respectively. Similarly, any RUs in the C-zone which happen to have cMyBP-C attached to actin at the time of the k_{tr} maneuver are returned to the unbound, free state. We assume that the k_{tr} maneuver does not directly disrupt myosin-cMyBP-C interactions in any way, as cMyBP-C shares the thick filament with myosin (Fig. 1A). After the k_{tr} maneuver, the simulation was continued for 3 seconds to allow the time-course of force redevelopment to be observed. From each force-time record, we extracted two scalar quantities: the steady-state force (F_{ss}) and the time constant of force redevelopment (k_{tr}). F_{ss} was calculated by averaging force values over the interval between 8 and 9 seconds post-activation. k_{tr} was calculated from the time $t_{1/2}$ required to reach one half of F_{ss} after the k_{tr} maneuver according to the following equation [36]:

$$k_{tr} = \ln(2) / t_{1/2}.$$

Simulations were repeated at multiple pCa values in order to construct curves showing the Ca^{2+} dependence of F_{ss} and k_{tr} .

For some cases, we performed simulations designed to mimic skinned fiber experiments in the presence of N-ethylmaleimide-modified myosin S1 (NEM-S1), which are soluble, chemically modified myosin heads that bind with very high affinity to the thin filament without generating force [20]. We assumed that an RU containing NEM-S1 would forcibly occupy the C_0 state at the beginning of the simulation, and would be prohibited from transitioning to the B_0 or B_1 state at any point. For each repetition of each simulation, RUs containing NEM-S1 were randomly assigned at a probability of 3.5%, a density that yielded 13% maximal force at pCa 6.2. This level of force coincides with data from mouse myocardial preparations [20].

t for simulations was selected such that the cumulative probability for transition out of any given state never exceeded 70%. This gave time steps of $1.37 \mu\text{s}$ for the parameter sets used, requiring a total of 1,575,000 time steps in a typical simulation ($0.7 \mu\text{s}$ for twitches). The stochastic nature of the solution technique required numerous repetitions and time-averaging of the results in order to obtain sufficiently smooth force-time traces (Fig. 2). Each F_{ss} - and k_{tr} -pCa value we report was extracted from a force development record formed by averaging 19,200 individual repetitions. The time required to compute repeated simulations was greatly reduced by using GPU parallel processing. Due to the GPU architecture, the number of repetitions performed is constrained to be a multiple of 3840. Numerical convergence was guaranteed for all reported simulations after 5×3840 repetitions, or 19,200 total repetitions.

To establish baseline parameter sets, we optimized model parameters such that simulation output matched muscle properties reported in the literature. We chose data from studies on cMyBP-C knockout myocardium in order to establish contractile parameters in the absence of cMyBP-C activity [20]. Thereafter, introducing its binding interactions with myosin and actin could be interpreted straightforwardly as modifications to the baseline cMyBP-C null behavior. We used particle swarm optimization [37] with 10 particles, a velocity clamping factor of 2 and 100. Model parameters were constrained to ranges listed in Supplemental Table 1, with initial seed values taken from previously published models [28]. Baseline (cMyBP-C null) skinned fiber data were matched by minimizing the least-squares error between model output and experimentally reported values of F_{ss} -pCa and k_{tr} -pCa relationships for cMyBP-C knockout mice by using the following cost function: $c = \sum[(F_{ss.exp} - F_{ss.sim})^2 / F_{ss.exp}] + \sum[(k_{tr.exp} - k_{tr.sim})^2 / k_{tr.exp}]$. The resulting parameters are shown in Parameter Set 1, Table 1.

A second baseline parameter set was produced by fitting the model-predicted twitch force response to an average twitch measured in engineered mouse myocardium in which cMyBP-C had been genetically ablated [38] (Parameter Set 2, Table 1). Initial parameter values were selected from a recently determined set (Aboelkassem et al., submitted), which corresponds to data collected at room temperature in adult mouse papillary muscle. The twitch data used

here were obtained at a higher temperature (37°C) and originate from neonatal mouse myocytes grown in vitro, thereby necessitating some adjustment (seed values and ranges detailed in Supplemental Table. 1). The optimization was performed by minimizing the cost function $c = \sum [(F_{\text{exp}} - F_{\text{sim}})^2 / F_{\text{exp}}]$. Values for cMyBP-C binding parameters were hand-fitted to data using the results of sensitivity analyses, as detailed below.

Results

Having added a representation of cMyBP-C binding events to our model of myofilament activation, we examined the consequences of myosin-cMyBP-C and actin-cMyBP-C interactions separately at first. The effects of myosin-cMyBP-C interactions on the Ca^{2+} -dependence of force and k_{tr} were studied by repeated simulations at several values of the association constant $K_{\text{cp,m}}$ while setting the binding rate $k_{\text{cp,m}}^+$ constant to 0.001 ms^{-1} . This sweep of parameters produced a comprehensive range of responses (Fig. 3). Binding of cMyBP-C to actin was eliminated in these simulations in order to observe only those effects arising from its interactions with myosin. Raising $K_{\text{cp,m}}$ decreases Ca^{2+} sensitivity of the myofilaments as well as the maximum Ca^{2+} -activated force (Fig. 3B). This effect saturates at high myosin-cMyBP-C affinities because all myosins in the C-zone are effectively neutralized by tethering, and further affinity increases cannot have additional impact. As tethering reduces the numbers of attached myosin crossbridges, it prevents them from participating in cooperative activation of the thin filament. As a consequence, Ca^{2+} sensitivity also drops. Although the absolute binding rate selected for myosin-cMyBP-C should have no effect on steady-state force (Fig. 3B), we considered it likely that binding rate could have a substantial impact on a dynamic response such as k_{tr} . For this reason, we repeated simulations under fast and slow regimes (relative to the rate of crossbridge binding, f_{rel}) of the binding rate $k_{\text{cp,m}}^+$ (0.1 vs. 0.001 ms^{-1}), and examined the effects on k_{tr} (Fig. 3, C & D). Interestingly, neither of the parameter regimes slowed the rate of force redevelopment, and in some cases made k_{tr} more rapid. These results run counter to the intuitive expectation that myosin-cMyBP-C interactions should slow force development kinetics.

In like manner, we examined the effects of actin-cMyBP-C binding under different affinities and kinetic regimes and in the absence of any myosin-cMyBP-C interactions (Fig. 4). The effect of actin-cMyBP-C binding on the Ca sensitivity of force is opposite that of myosin-cMyBP-C binding in many respects. Actin-cMyBP-C binding acted to increase Ca^{2+} sensitivity by holding RUs in their active states even at low Ca^{2+} (Fig. 4B). Unlike myosin binding, actin-cMyBP-C binding had striking effects on k_{tr} (Fig. 4, C & D). Simulations showed that actin-cMyBP-C binding is capable of accelerating or slowing k_{tr} , depending on its assumed binding rate. Compared with k_{tr} values predicted in the absence of actin-cMyBP-C binding (black traces, Fig. 4), rapid actin-cMyBP-C binding accelerated force redevelopment, whereas relatively slow binding (on the order of crossbridge attachment) caused k_{tr} to be slower (Fig. 4, C & D; $k_{\text{cp,a}}^+$ values of 0.1 or 0.001 ms^{-1} , respectively).

After examining the separate effects of myosin and actin binding with cMyBP-C, a logical explanation of experimental results in cMyBP-C null myocardium emerged. Measurements of F_{ss} and k_{tr} were reported in wild type (WT) and cMyBP-C $-/-$ (KO) myocardium by

Stelzer *et al.* [20]. If WT fibers can be thought of as illustrating the effects of ‘adding’ cMyBP-C to the system, Stelzer *et al.* determined that cMyBP-C had no effect on Ca^{2+} sensitivity of F_{ss} , but that it reduced k_{tr} at sub-maximal concentrations of Ca^{2+} . In view of our initial results (Figs. 3 & 4), we hypothesized adding a combination of actin and myosin binding by cMyBP-C would result in no net change in Ca^{2+} sensitivity. We further speculated that an assumption of slow actin-cMyBP-C binding (as in Fig. 4D) would reproduce the slowing of k_{tr} relative to the KO condition observed in experiments. A faster actin-cMyBP-C rate was deemed unlikely as in isolation this regime was seen as having the opposite effect on k_{tr} .

We sought computational support for these hypotheses by running simulations in which cMyBP-C binding to both actin and myosin were permitted (Fig. 5). After adjusting baseline model parameters to match the F_{ss} and k_{tr} -pCa relations reported by Stelzer *et al.* [20] for KO fibers (blue traces and open circles, Fig. 5 A & C), we gradually increased the affinity of cMyBP-C for actin, using the assumption of slow actin binding. As suggested by the sensitivity analysis (Fig. 4D), an actin-cMyBP-C affinity recapitulating the observed slowing of k_{tr} at submaximal activation was ultimately reached at $K_{cp,a} = 2$ (red trace, Fig. 5C). To compensate for the increased Ca^{2+} sensitivity produced by this actin-cMyBP-C binding (e.g. Fig. 4B), we increased $K_{cp,m}$ until the result of no net Ca^{2+} sensitivity change in F_{ss} was also achieved ($K_{cp,m} = 4$, red trace in Fig. 5A). The non-zero equilibrium constants obtained for both $K_{cp,m}$ and $K_{cp,a}$ support substantial affinity for both targets in the intact sarcomere. The final rate constants to achieve the fits to WT data were $k_{cp,a}^+ = 0.001 \text{ ms}^{-1}$, $k_{cp,m}^+ = 0.2 \text{ ms}^{-1}$, $k_{cp,m}^- = 0.0005 \text{ ms}^{-1}$, and $k_{cp,m}^- = 0.05 \text{ ms}^{-1}$. In summary, the simulations suggest that in the intact sarcomere cMyBP-C exerts its effects through binding with roughly equal affinity to both actin and myosin targets, but with substantially different rates.

The action of chemically modified myosin S1 subfragments (NEM-S1) on WT and KO fibers was also reported by Stelzer *et al.* [20], and we used these results to seek an independent validation of the proposed mechanisms of cMyBP-C action. NEM-S1 was randomly incorporated into the simulated thin filament at an effective binding level of 3.5 %, and new response curves were generated (Fig. 5 B & D). The model predictions showed good qualitative agreement with reported behavior.

Seeing that a combination of both actin- and myosin-cMyBP-C binding interactions seemed to explain data from skinned myocardium, we sought evidence that a similar mechanism could explain observations of twitch dynamics in intact cells. Recently, de Lange and colleagues reported average twitch dynamics measured in engineered myocardial strips formed from neonatal cardiomyocytes from both WT and KO mice [38]. A representative intracellular Ca^{2+} transient was digitized from their data set, and used as an activating input into the model (Fig. 6A). Baseline model parameters were fitted such that they reproduced the average KO muscle twitch (blue trace and circles, Fig. 6B). This required, among other changes, increases in crossbridge cycling rates to reflect the higher temperature at which these intact preparations were characterized (Parameter Set 2, Table 1). This scaling factor was used to estimate initial cMyBP-C binding kinetic under these same conditions. Then, the cMyBP-C parameters were adjusted until error between simulation output and the reported WT twitch was minimized (Parameter Set 2, Table 1; red trace in Fig. 6B).

Although the absolute parameter values differ substantially between Sets 1 & 2, reflecting differences in temperature and experimental preparations (skinned vs. intact cells), the relative relationship between rates and affinities of the two binding interactions were similar. That is, in both parameter sets the affinities of cMyBP-C to myosin and actin are roughly equal, and the rate of cMyBP-C binding to myosin is faster than its binding to actin.

Discussion

In spite of abundant in vitro evidence of actin-cMyBP-C interactions [12,14,16,35,39,40], their relevance to intact muscle physiology is subject to active debate in the field [6,19,35]. Several authors are inclined toward an earlier conceptualization of the cMyBP-C regulatory mechanism, namely that it functions by tethering myosin heads and preventing their attachment to actin [7,25,41]. Going by intuition alone, this notion seems in line with the universal observation that normal cardiac muscle exhibits contractile kinetics that are slower than muscle that lacks cMyBP-C [20,21,42]. And yet, our results suggest that myosin tethering has little if any effect on the force redevelopment rate (Fig. 3, C & D). Strikingly, only the assumption of relatively slow actin-cMyBP-C binding was capable of producing the slower k_{tr} values indicated by experimental data (Figs. 4D & 5D). Our simulations therefore suggest that the apparent slowing of kinetics in the presence of cMyBP-C observed in many studies may actually be a manifestation of significant actin-cMyBP-C interactions within the intact sarcomere.

Because the model recapitulates experimental measurements, it suggests new hypotheses detailing how cMyBP-C exerts its functional effects. In particular, when actin-cMyBP-C interactions are represented in simulations, they end up slowing contractile kinetics through a process of cooperative recruitment. This subtle and somewhat counter-intuitive mechanism is analogous to the one elucidated by K.B. Campbell to explain the activation dependence of k_{tr} [43]. It can be described as follows: Force production at low levels of Ca^{2+} is augmented by the formation of actin-myosin crossbridges because they prevent Tm from returning to the blocked state, exposing additional sites where other crossbridges may bind; therefore, directly after a k_{tr} maneuver (in which crossbridges are broken) the thin filament drops to a level of activation that is lower than the steady-state (crossbridge-enhanced) value. Initially, crossbridges can re-bind only to this small number of active RUs and force remains well below the steady-state level. However, after these first crossbridges bind they promote activation of neighboring RUs and the binding of still more crossbridges. Each crossbridge that binds promotes more activation in a process that can be viewed as a form of positive feedback. This process continues until the ultimate crossbridge-enhanced level of activation is finally attained. Because re-recruitment of active RUs is required to reach the ultimate steady-state activation level, force develops at a rate that can be far slower than the actual binding kinetics of individual myosin heads.

Our modeling results suggest that cMyBP-C could reasonably function in a similar way to reduce the rate of force redevelopment, provided that its binding to actin is slow (Fig. 4D). In effect, we are proposing that cMyBP-C acts as a kind of ‘second crossbridge’, participating in the cooperative recruitment of other crossbridges and thereby slowing

contractile kinetics at low levels of Ca^{2+} . The simulations shown here provide evidence that such a mechanism is quantitatively feasible (Fig. 5D).

On the other hand, the model does not support the idea that myosin tethering by cMyBP-C is capable of slowing contractile kinetics [10]. Initially, we too anticipated that this binding interaction would in fact slow k_{tr} . We reasoned that myosins forcibly detached from actin by the k_{tr} maneuver would bind instead to cMyBP-C. These sequestered myosins would then dissociate from cMyBP-C and re-enter the pool of cycling crossbridges gradually, thereby slowing the rate of force redevelopment. The model reveals however, that this intuition may be false – none of the parameter combinations attempted could produce slowing of k_{tr} (Fig. 3, C & D). The simplest way of explaining these results is to recall that in the model myosin crossbridges actually slow k_{tr} due to cooperative recruitment. If some crossbridges are eliminated by tethering to cMyBP-C, they cannot participate in cooperative recruitment, and their ability to slow force redevelopment is eliminated. Hence, our model predicts that increases in myosin-cMyBP-C affinity either increase k_{tr} or leave it unchanged.

Although myosin-cMyBP-C interactions do not appear to explain slowed contractile kinetics, our simulations suggest that they are nonetheless critical to explaining experimental observations. Without them, the actin-cMyBP-C interactions alone would cause an unrealistic sensitization of the myofilaments to Ca^{2+} (Fig. 4B). Hence, these results suggest that both types of binding play critical roles for in the physiology of cMyBP-C. This ‘dual binding’ hypothesis may also provide an explanation for a lack of consensus with regards to the impact of cMyBP-C on myofilament Ca^{2+} sensitivity. In the model, myosin-cMyBP-C and actin-cMyBP-C interactions had opposing effects on Ca^{2+} sensitivity (Fig. 3B vs. Fig. 4B) such that the final sensitivity was a balance between the effects of the two binding interactions. This delicate balance could be altered by variations in experimental conditions and muscle preparations that affect one binding interaction differently than the other (e.g. through phosphorylation status or isoform expression), leading to a diversity of outcomes.

Additional insight into the possible physiological benefits of dual binding targets for cMyBP-C can be gleaned from the model’s reproduction of twitch dynamics (Fig. 6B). In the model, the elimination of cMyBP-C binding interactions causes a shortening of twitch duration with a small increase in peak twitch force (5%, see inset to Fig. 6B). Experimentally, engineered myocardial constructs have also shown an increase or preservation of peak force upon deletion of cMyBP-C [38,44,45]. Overall, the modeling results lead us to hypothesize that the dual binding nature of cMyBP-C allows tuning of cardiac relaxation (lusitropy) without lessening the magnitude of twitch force. This type of regulatory paradigm would complement high heart rates by increasing diastolic function while maintaining contractility.

While providing useful insights and new hypotheses, the model presented here includes several simplifying assumptions whose consequences should be carefully considered. Most critical is the simplified stoichiometry used when describing myosin-cMyBP-C interactions. Within the C-zone, we assume that a single cMyBP-C molecule regulates the binding of one myosin crossbridge, when in fact roughly three crossbridges are capable of binding to an actin regulatory unit. We demonstrated in previous work that explicitly representing three

myosins per regulatory unit had no unique impact on cooperativity and k_{tr} [28]. Hence, we do not believe that this assumption affects our main conclusions, which center around cMyBP-C mediated changes to steady-state force and k_{tr} . However, this simplified stoichiometry does mean that the effective myosin-cMyBP-C binding parameters reported here cannot be straightforwardly related to the absolute values that might be measured using single-molecule techniques. It also means that we cannot meaningfully compare actin-cMyBP-C with myosin-cMyBP-C affinities implied by the fitted model parameters, beyond saying that both types of binding are required in some degree. The prediction of absolute myosin-cMyBP-C binding rates and affinities will require more complex models and additional free parameters. On the other hand, the hypothesis that actin-cMyBP-C binding and unbinding are relatively slow is not limited by model assumptions, insofar as the actin to cMyBP-C stoichiometry in the model is represented realistically.

We intentionally represented actin and myosin binding to cMyBP-C as simplistically as possible in order to capture broad properties of the resulting model. Although this resulted in reasonable fits to experimental data, there are more subtle mechanisms, equally reasonable, that are not represented here. For instance, it is possible that cMyBP-C binding to myosin does not absolutely prevent crossbridge formation in an all-or-nothing fashion. Rather, cMyBP-C when attached to myosin could alter the actin-myosin attachment rate, the properties of myosin power stroke, the effective crossbridge stiffness, and/or myosin detachment rate. The present model does not rule out these types of mechanisms from contributing to the functional effects of cMyBP-C. Depicting these effects in a future model is possible, but difficult to implement at present without supporting experimental or structural data.

One further limitation to the work presented here is that we have only considered isometric phenomena, and none of the effects that cMyBP-C likely has on sliding filament velocity in vitro [39,46]. These effects have been treated in detail recently in modeling work by Wolcott *et al.* [47]. They too argue that experiments manipulating cMyBP-C can be explained by the inclusion of actin-cMyBP-C interactions. Other experimental results can and should be subjected to model analysis that are not within reach using the present model. For instance, the presence of cMyBP-C has been shown to alter length-dependent activation, but our assumption of a constant, non length-dependent binding rate of cMyBP-C to actin prohibits a systematic consideration of this and other pertinent questions [42,48,49]. Finally, the accuracy with which some results can be fit using the model is limited due to the resolution with which the actin thin filament is represented. This is manifest in the case of k_{tr} values obtained at low Ca^{2+} , which are not as slow as experiments suggest (Fig. 5, C & D). Future models could address some of these limitations, but such improvements would increase model complexity. Their value must be carefully weighed against the accompanying costs, given that important insights are already offered by this initial simplified model.

Conclusions

The model we have created is based on cMyBP-C's known in vitro interactions with actin and myosin, with explicitly assigned effects on thin filament regulatory states (Fig. 1). If these basic interactions can be accepted as reasonable, the model predicts several relevant

phenomena: (1) Data from intact sarcomeres can only be explained if binding occurs in roughly equal measure between cMyBP-C's binding partners; (2) Acceleration of force development observed by many in response to cMyBP-C ablation cannot be explained by its binding to myosin, but can be explained by relatively slow cooperative binding to actin. (3) Dual interaction of cMyBP-C with myosin and actin binding sites allows cardiac lusitropy to be regulated without loss of contractility by modifying a single protein target, namely cMyBP-C. These modeling results offer specific hypotheses and mechanistic descriptions that should assist ongoing experimental work toward unraveling cMyBP-C's contribution to inherited cardiomyopathy as well as the role of its phosphoregulation in normal cardiac physiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- cMyBP-C has been shown in vitro to bind both myosin and actin
- A computational model was used to explore the effects of these binding interactions
- In the model, myosin/cMyBP-C binding alone did not explain skinned fiber measurements
- Adding actin/MyBP-C binding slowed force development and improved predictions
- Both myosin and actin binding seem to be integral to cMyBP-C regulatory activity

Figure 1.

Schematic diagram of the myofilament model, which includes cMyBP-C binding activity. (A) By binding to the S2 segment of myosin, cMyBP-C tethers the two myosin heads towards the thick filament; by binding to actin, cMyBP-C is thought to displace tropomyosin and expose myosin binding sites. (B) The model tracks the activity of an actin thin filament comprised of 26 regulatory units (RUs). Each RU contains a Ca^{2+} binding site and is assumed to interact with a single myosin crossbridge (XB). Nine RUs on the right-hand end of the filament (in the C-zone) are assumed to interact with cMyBP-C as well. (C) RUs

outside the C-zone are governed by the six-state Markov network described in previous work (Aboelkassem et al, submitted). (D) RUs in the C-zone are subject to the same six-state model when cMyBP-C is bound to neither actin nor myosin, but upon binding to either target the states accessible by that RU become limited. An RU in any of the six states may experience a cMyBP-C-myosin binding event. If the RU happens to be in the M_0 or M_1 state, the XB is instantly released from actin. Myosin cannot attach to actin again until released by cMyBP-C (grayed out states, blue box). cMyBP-C can only bind to the actin filament when the RU is out of the blocked (B_0 or B_1) states (red X). Once cMyBP-C is attached to actin, the RU cannot return to B_0 or B_1 .

Figure 2.

Typical simulation protocol. Simulations of skinned fiber mechanics were performed assuming an initial condition in which all RUs were in the B_0 state, and all cMyBP-C molecules were bound to neither actin nor myosin. At time zero, Ca^{2+} concentration was set to some non-zero value (pCa 5.8 in the trace shown here), and force was allowed to develop until reaching a steady state value (F_{ss}). Then, a slack-restretch maneuver was simulated by instantaneously removing all myosins and cMyBP-C molecules bound to the thin filament and allowing the system to return to steady state. The rate of force redevelopment (k_{tr}) was computed as the time required to redevelop force to half of the steady-state value [36].

Figure 3.

Effects of cMyBP-C-myosin interactions on the steady-state force-pCa and k_{tr} -pCa relationships. cMyBP-C-actin binding has been suppressed in these simulations to isolate the characteristics of cMyBP-C binding with myosin. (A) Traces showing force redevelopment after simulated slack-restretch with no myosin binding (KO, black trace), fast myosin binding (light grey), and slow myosin binding (dark grey). The equilibrium constant for cMyBP-C-myosin binding is the same for fast and slow traces. (B) The effect of cMyBP-C-myosin affinity ($K_{cp,m}$) on the steady-state force-pCa relationship. The $K_{cp,m}$ value associated with each curve is indicated by different line styles, as indicated. (C) The effect of cMyBP-C-myosin affinity on the k_{tr} -pCa relationship, under the assumption of rapid binding

kinetics relative to crossbridge attachment (the binding rate $k_{cp.m+} = 0.1 \text{ ms}^{-1}$ for all curves). Same line styles as shown in Panel B. (D) Same as Panel C, but assuming relatively slow cMyBP-C-myosin binding kinetics (the binding rate $k_{cp.m+} = 0.001 \text{ ms}^{-1}$ for all curves).

Figure 4.

Effects of cMyBP-C-actin interactions on the steady-state force-pCa and k_{tr} -pCa relationships. cMyBP-C-myosin binding has been suppressed in these simulations to isolate the characteristics of cMyBP-C binding with actin. (A) Traces showing force redevelopment after simulated slack-restretch with no actin binding (KO, black trace), fast actin binding (light grey), and slow actin binding (dark grey). The equilibrium constant for MyBP-C-actin binding is the same for fast and slow traces. (B) The effect of MyBP-C-actin affinity ($K_{cp,a}$) on the steady-state force-pCa relationship. The $K_{cp,a}$ value associated with each curve is indicated by different line styles, as indicated. (C) The effect of MyBP-C-actin affinity on the k_{tr} -pCa relationship, under the assumption of rapid binding kinetics relative to the

crossbridge attachment rate (the binding rate $k_{cp,a+} = 0.1 \text{ ms}^{-1}$ for all curves). Same line styles as shown in Panel B. (D) Same as Panel C, but assuming relatively slow MyBP-C-actin binding kinetics (the binding rate $k_{cp,a+} = 0.001 \text{ ms}^{-1}$ for all curves).

Figure 5.

Comparison of simulations with data from cMyBP-C knock-out mice. Stelzer et al. [20] published steady-state force-pCa and k_{tr} -pCa relationships measured in skinned myocardial samples from wild type (WT) and cMyBP-C $-/-$ (KO) mice (red and blue circles in all panels, respectively). Model parameters were initially adjusted such that output (blue traces, panels A & C) matched data from KO fibers (blue circles). Then, cMyBP-C binding affinities to both actin and myosin were gradually increased while keeping all other model parameters constant. In order to achieve no net alteration in myofilament Ca^{2+} sensitivity

(Panel A), it was necessary to balance cMyBP-C affinity to both targets. We further observed that the assumption of slow binding to actin produced decreases in k_{tr} at submaximal levels of activation, in good agreement with data (Panel C). To test the predictive ability of the model, the effects of NEM-S1 on myofilament activation and kinetics were simulated and compared with reported values (Panels B & D).

Figure 6.

Comparison of simulated twitch force transients with data from engineered myocardium. (A) Experimentally reported Ca^{2+} transient record digitized from de Lange et al. [38], which was used as input to drive a twitch contraction in our model. (B) Digitized twitch records measured in engineered myocardial constructs containing neonatal cardiomyocytes isolated from either WT or cMyBP-C $-/-$ (KO) mice [38] (red and blue circles, respectively). Model parameters were adjusted to reproduce the KO twitch while holding cMyBP-C binding rates at zero to reflect gene knock-out (blue trace; Parameter Set 2, Table 1). Then, c MyBP-C kinetic rates were adjusted to fit the measured WT twitch (red trace). The inset shows simulated twitch traces without normalization.

Table 1

Model parameter values. Parameter Set 1 was fitted to data from skinned mouse myocardium at 22°C [20]. Parameter Set 2 reproduces twitch data from engineered mouse myocardium at 37°C [37]. Thin filament and crossbridge parameters were determined using constrained particle swarm optimization, whereas cMyBP-C parameters were determined through hand fitting as described in Results.

	Parameter Name	Parameter Set 1 (Skinned Myocardium)	Parameter Set 2 (engineered myocardial strip)
Thin Filament	λ	0.055	0.05
	$k_{Ca+} (\mu M^{-1} ms^{-1})$	.09	21.83
	$k_{Ca-} (ms^{-1})$	0.11	60.59
	$k_{B+} (ms^{-1})$	19.68	333.99
	$k_{B-} (ms^{-1})$	0.24	1.54
	r	1	1.00
	q	0.5	1.00
	γ_B	68.5	161.95
	$\gamma_M = \mu_B$	7	1.28
	μ_M	2	1.13
Crossbridge	$f^{ref} (ms^{-1})$	0.0167	4.93
	$g^{ref} (ms^{-1})$	0.0408	4.49
cMyBP-C	$k_{cp,m}^+ (ms^{-1})$	0.2	6.5
	$k_{cp,m}^- (ms^{-1})$	.05	3
	$k_{cp,a}^+ (ms^{-1})$	0.001	0.7
	$k_{cp,a}^- (ms^{-1})$	0.0005	0.14