

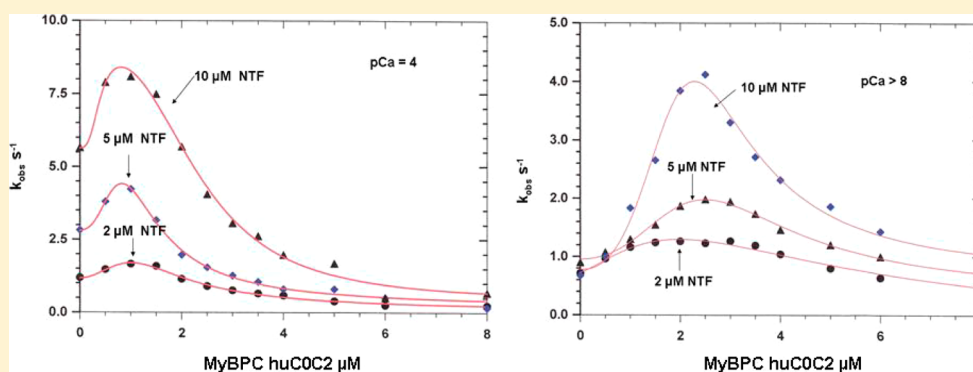
Modulation of Thin Filament Activation of Myosin ATP Hydrolysis by N-Terminal Domains of Cardiac Myosin Binding Protein-C

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S Supporting Information



ABSTRACT: We have used enzyme kinetics to investigate the molecular mechanism by which the N-terminal domains of human and mouse cardiac MyBP-C (C0C1, C1C2, and C0C2) affect the activation of myosin ATP hydrolysis by F-actin and by native porcine thin filaments. N-Terminal domains of cMyBP-C inhibit the activation of myosin-S1 ATPase by F-actin. However, mouse and human C1C2 and C0C2 produce biphasic activating and inhibitory effects on the activation of myosin ATP hydrolysis by native cardiac thin filaments. Low ratios of MyBP-C N-terminal domains to thin filaments activate myosin-S1 ATP hydrolysis, but higher ratios inhibit ATP hydrolysis, as is observed with F-actin alone. These data suggest that low concentrations of C1C2 and C0C2 activate thin filaments by a mechanism similar to that of rigor myosin-S1, whereas higher concentrations inhibit the ATPase rate by competing with myosin-S1-ADP-P_i for binding to actin and thin filaments. In contrast to C0C2 and C1C2, the activating effects of the C0C1 domain are species-dependent: human C0C1 activates actomyosin-S1 ATPase rates, but mouse C0C1 does not produce significant activation or inhibition. Phosphorylation of serine residues in the m-linker between the C1 and C2 domains by protein kinase-A decreases the activation of thin filaments by huC0C2 at pC_a > 8 but has little effect on the activation mechanism at pC_a = 4. In sarcomeres, the low ratio of cMyBP-C to actin is expected to favor the activating effects of cMyBP-C while minimizing inhibition produced by competition with myosin heads.

Myosin binding protein-C (MyBP-C) was first discovered by Offer,¹ who found that it was the next most common protein after myosin in crude preparations of skeletal myosin and that it could be separated from myosin by ion exchange chromatography. He and his colleagues also showed that antibodies to MyBP-C formed nine 43 nm striations on each side of the A bands of muscle fibers. A similar distribution of MyBP-C has also recently been demonstrated in cardiac muscle labeled with anti-cMyBP-C antibodies.² Although it was thought that MyBP-C was a component of the thick filament, possibly having a structural role as a trimeric collar to strengthen the thick filament, soon after its discovery, MyBP-C was also shown to bind to the I band in myofibrils and to bind to actin and inhibit the actin activation of myosin ATP hydrolysis *in vitro*.^{3,4} The ability to bind both actin and myosin suggested that MyBP-C might form cross-links between the thick and thin filaments of striated muscle fibers, but it was unclear what, if any, physiological significance these cross-links

might have. Since then, there has been increased interest in MyBP-C, especially over the past 18 years following the discovery that mutations in the gene encoding the cardiac isoform of MyBP-C (cMyBP-C) are a common cause of inherited cardiomyopathies.^{5,6} Subsequently, 187 mutations have been identified that result in cMyBP-C cardiomyopathies.⁷ This is second only to the number of mutations of the β -cardiac myosin heavy chain linked to cardiomyopathy.⁸

The primary sequence of mammalian cMyBP-C indicates that it has a structure composed of 8 IgG and 3 fibronectin-like domains, as shown in Figure 1. C-Terminal domains C7–C10 bind to 9 locations on each half of the myosin thick filament,^{1,2} and there is evidence that N-terminal domains of cMyBP-C bind to the S2 region of myosin.^{9,10} However, the same N-

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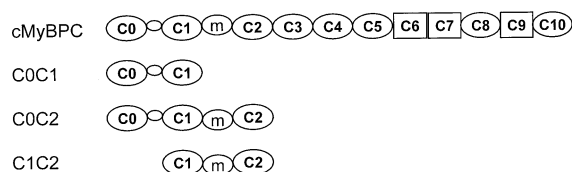


Figure 1. Domain structure of intact and internal domains of cMyBP-C. (Top) cMyBP-C is composed of repeating IgG-like domains (ovals) and fibronectin-like domains (squares), numbered C0–C10 beginning at the N-terminus of the protein. A proline–alanine-rich sequence (small oval) links the C0 and C1 domains. PKA phosphorylation sites occur in the m-linker domain. (Bottom) Schematics showing domain organization of the recombinant proteins used in this study: C0C1, C0C2, and C1C2.

terminal domains also bind to actin and the thin filament, as shown by solution binding studies, electron microscopy, and low-angle X-ray scattering.^{11–13} Multiple actin binding sites, present as individual subdomains (e.g., C0, C1, C2, and the m-domain), may bind stoichiometrically (one subdomain per actin subunit).^{13–15} Although there are some differences in the details of the binding geometries, all show binding modes that suggest overlap with the site of myosin binding to actin and are likely to interfere with the position of tropomyosin in the inhibited form of the thin filament. These data thus suggest an explanation for the apparently contradictory observations that cMyBP-C inhibits actomyosin activity by competing with myosin-ADP-P_i binding to actin⁴ and that it activates the thin filament by promoting the active conformation of tropomyosin.^{16,17}

The goal of the present study was to directly test the ability of the N-terminal domains of cMyBP-C to activate or inhibit steady-state myosin ATPase rates by either F-actin alone or by native thin filaments in the presence of high and low calcium. Both human and mouse recombinant cMyBP-C proteins were used because species-specific differences in the behaviors of mouse and human cMyBP-C have been reported.¹⁸ Effects of phosphorylation of the m-linker of human C0C2 were also determined. Results are consistent with a model in which the N-terminal domains of cMyBP-C bind specifically to actin and activate the thin filament in a manner analogous to the way in

which myosin S1 binds to the thin filament to activate contraction.

MATERIALS AND METHODS

Protein Preparation. Rabbit skeletal actin, native porcine cardiac thin filaments, N-terminal subdomains of cMyBP-C, and the A1 subfraction of skeletal myosin-S1 were prepared by previously published methods.^{17–20} SDS PAGE of these proteins are shown in Figures S2 and S3. In some experiments, the hexa-His tag encoded at the N-terminus of recombinant protein sequences (included to facilitate protein purification) was removed using the Tagzyme enzyme according to the manufacturer's instructions (Qiagen). Phosphorylation of recombinant cMyBP-C proteins was done using the catalytic subunit of bovine PKA according to published methods,¹¹ which has been shown to fully phosphorylate 4 sites on recombinant human and mouse C0C2.²¹

Steady-State ATPase Measurements. Steady-state ATP hydrolysis rates were measured by colorimetric measurement of phosphate production as described previously.^{22,23} Experimental conditions: ionic strength (50 mM), temperature (30 °C), and myosin-S1 (rabbit skeletal), actin, and thin filament concentrations were chosen to produce steady-state ATP hydrolysis rates that could be readily measured. Preliminary experiments determined that rates were proportional to the concentration of myosin-S1 (i.e., the activation was not dependent upon the ratio of S1 to actin or thin filament concentrations), and the amount of myosin-S1 used in individual experiments was adjusted so that between 0.25 and 0.5 of the ATP was hydrolyzed. Steady-state rate data (k_{obs}) for the inhibition of F-actin-activated ATP hydrolysis by N-terminal cMyBP-C domains were fit to eq 1 using simplex fitting routines in the Scientist graphics software package (Micromath Corp, St. Louis, MO), where V_0 is the rate in the absence of cMyBP-C, $[C]$ is the concentration of cMyBP-C recombinant protein, k_0 is the ATP hydrolysis rate at saturating cMyBP-C protein concentration, K_i is the concentration of cMyBP-C protein required for 50% inhibition, and n is the Hill coefficient.

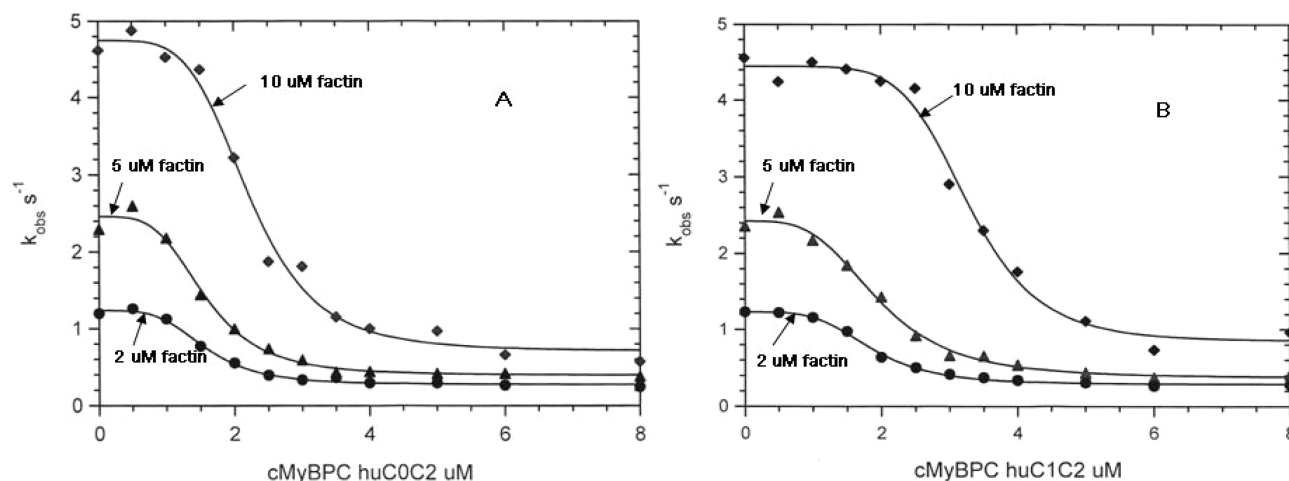


Figure 2. Effect of human C0C2 and C1C2 on the actin activation of ATP hydrolysis by myosin-S1. ATP hydrolysis was measured by colorimetric determination of phosphate as described in Materials and Methods. (A, B) Data were fit to eq 1 using the parameters listed in Table S5. Experimental conditions: 0.25–1.0 μM myosin-S1, F-actin and cMyBP-C at the indicated concentrations, 10 mM MOPS, 50 mM KAc, 3 mM MgCl_2 , pH 7.0.

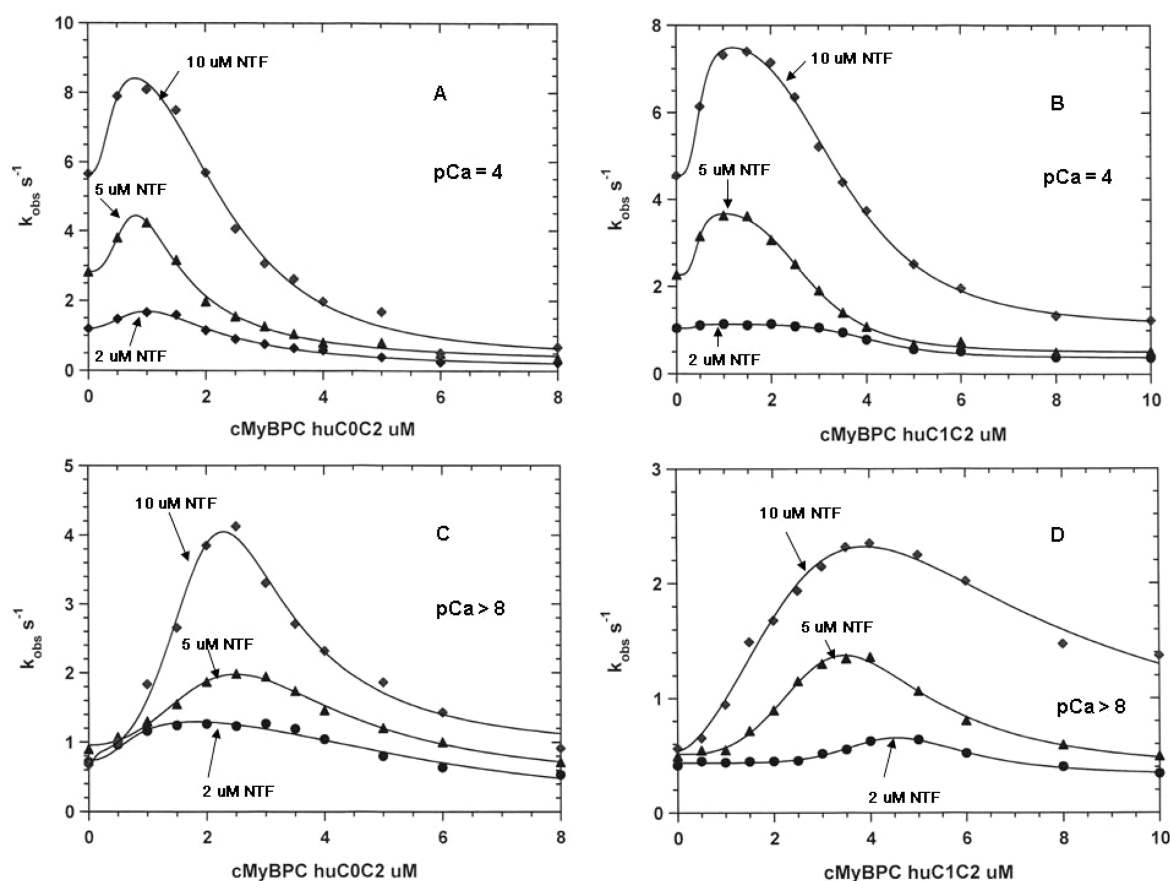


Figure 3. Effect of huC0C2 and huC1C2 on native thin filament activation of ATP hydrolysis by myosin-S1. ATP hydrolysis was measured by colorimetric determination of phosphate as described in Materials and Methods. Data were fit to eq 2 using the parameters listed in Table S5. Experimental conditions: 0.25–1.0 μ M myosin-S1, native cardiac thin filaments and MyBP-C at the indicated concentrations, 10 mM MOPS, 50 mM KAc, 3 mM MgCl_2 , pH 7.0, 30 $^\circ\text{C}$, and either 0.1 mM CaCl_2 (A, B) or 1 mM EGTA (C, D).

$$k_{\text{obs}} = V_0 / (1 + ([C]/K_i)^n) + k_o \quad (1)$$

A more complex steady-state relationship (eq 2) was required to account for the biphasic kinetics observed with thin filaments in which the hydrolysis rate is increased by low concentrations of cMyBP-C and inhibited at higher concentrations. Additional terms in eq 2 are V_a , the increase in ATPase rate by cMyBP-C; K_a is the apparent affinity of the activation by cMyBP-C, and n is the Hill coefficient.

$$k_{\text{obs}} = V_a / ((1 + (K_a/[C])^n) + (1 + ([C]/K_i)^n)) + V_0 / (1 + ([C]/K_i)^n) + k_o \quad (2)$$

RESULTS

Effects of cMyBP-C N-Terminal Domains C1C2 and C0C2 on the Steady-State Activation of Myosin ATP Hydrolysis by Actin and Native Cardiac Thin Filaments.

The dependence of the steady-state rate of myosin ATP hydrolysis on actin and the N-terminal domains of human cardiac MyBP-C are shown in Figure 2. Steady-state myosin-S1 ATPase rates (k_{obs}) increased with increasing concentrations of F-actin, as expected, from 2 to 10 μ M. However, ATP hydrolysis rates decreased with the addition of increasing concentrations of either N-terminal domain of human cMyBP-C (huC0C2 and huC1C2). These inhibitory effects are consistent with the inhibition of actomyosin ATP hydrolysis by native full-length skeletal MyBP-C previously reported by

Moos and colleagues,⁴ suggesting that binding of N-terminal domains to the actin filament is sufficiently strong to compete effectively with the binding of M-ADP-P_i. The data are poorly fit by a simple binding equation ($n = 1$ eq 1) but are fit by K_i of $\sim 2 \mu$ M and Hill coefficients of ~ 3 –4, as shown in Table S1. The Hill coefficient > 1 suggests cooperative binding of the N-terminal domains huC0C2 and huC1C2 of cMyBP-C to actin.

We next investigated the effects of cMyBP-C N-terminal domains on the steady-state rate of myosin ATP hydrolysis activated by native porcine cardiac thin filaments (NTF) at either high ($p\text{Ca} = 4$) or low ($p\text{Ca} > 8$) calcium. Figure 3 shows that at $p\text{Ca} = 4$ steady-state ATP hydrolysis rates were initially activated up to 50% by 1 molecule of huC0C2 per 5–10 actin subunits in the thin filament (panel A). However, with increasing concentrations of huC0C2, the ATPase was inhibited with an apparent K_i of approximately 2–4 μ M, similar to the apparent affinity measured for the inhibition of F-actin-activated ATP hydrolysis shown in Figure 2. Additional experiments using the huC1C2 domains of cMyBP-C (panel B) showed a similar concentration dependence of the inhibition by huC0C2 and huC1C2, indicating little, if any, additional contribution of the C0 and proline–alanine segments at $p\text{Ca} < 4$.

At low calcium ($p\text{Ca} > 8$) and in the absence of N-terminal domains of cMyBP-C, native cardiac thin filaments (2–10 μ M) only weakly ($\sim 20\%$) activated the steady-state rates of myosin S1 ATP hydrolysis. However, when N-terminal domains of cMyBP-C were also added (e.g., 2.5 μ M huC0C2), ATPase

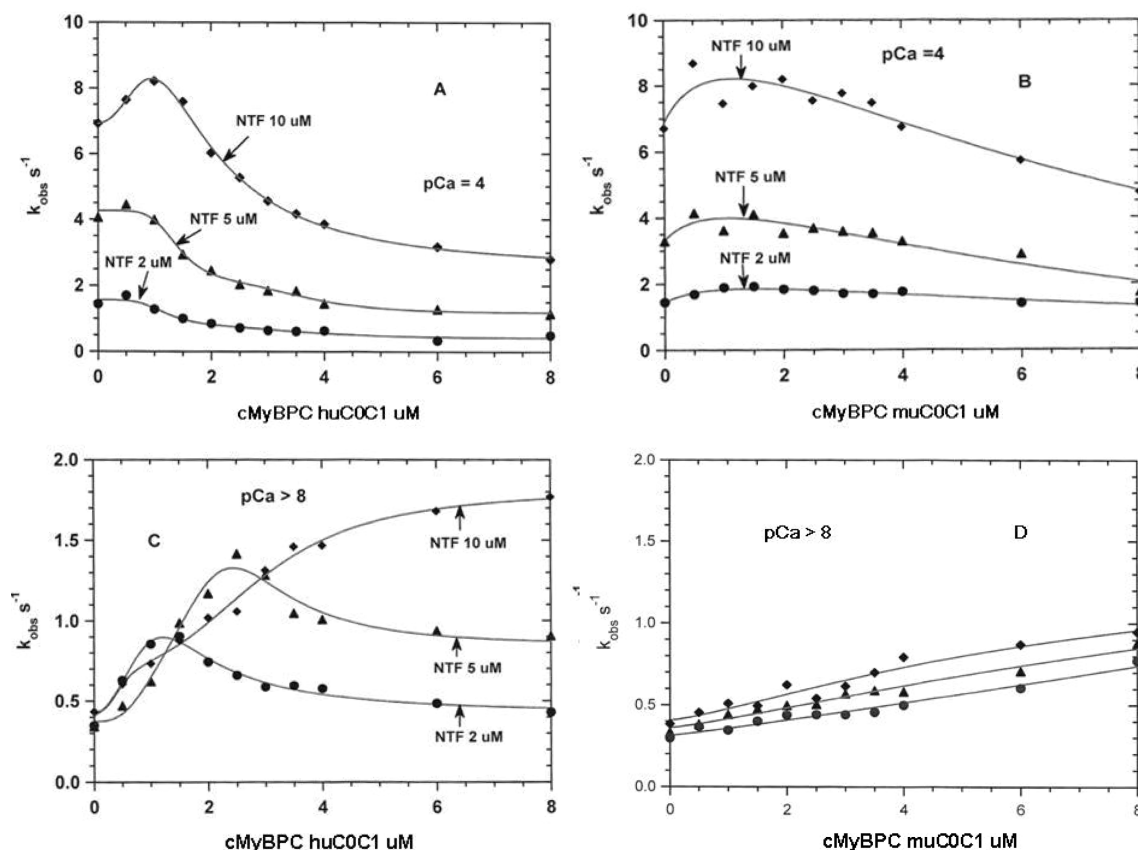


Figure 4. Comparison of the effects of muC0C1 and huC0C1 on native thin filament-activated ATP hydrolysis by myosin-S1. ATP hydrolysis was measured by colorimetric determination of phosphate as described in Materials and Methods. Data were fit to eq 2. Experimental conditions: 0.25 to 1.0 μM myosin S1, native cardiac thin filaments and muC0C1 or huC0C1 at the indicated concentrations, 10 mM MOPS, 50 mM KAc, 3 mM MgCl_2 , pH 7.0, 30 $^\circ\text{C}$, and either 0.1 mM CaCl_2 (A, B) or 1 mM EGTA (C, D).

rates were activated up to an additional 5-fold (Figure 3C,D). Effects were biphasic such that higher concentrations of huC0C2 and huC1C2 inhibited the observed rate of ATP hydrolysis with an apparent K_i of approximately 4 (huC0C2) and 8 μM (huC1C2). Similar biphasic patterns of activation and inhibition to those obtained for human C0C2 and C1C2 were also obtained with mouse C0C2 and C1C2 (Figure S1).

Species-Specific Effects of C0C1. We next compared effects of the C0C1 domains of mouse and human cMyBP-C on the activation of steady-state ATP hydrolysis by native cardiac thin filaments. Maximum activation of myosin-S1 ATP hydrolysis by huC0C1 at $p\text{Ca} = 4$ and $p\text{Ca} > 8$ (Figure 4A,C) were similar to those observed for human huC0C2 and huC1C2, although higher concentrations of huC0C1 were required for both activation and inhibition. Thus, the apparent binding affinities of the human N-terminal domains at $p\text{Ca} > 8$ are in the order $\text{huC0C2} \sim \text{huC1C2} > \text{huC0C1}$. These results are similar to the observed effectiveness of N-terminal domains cMyBP-C at increasing the calcium sensitivity of force production in skinned cardiac fibers from mice.¹⁷ By contrast, the effect of mouse C0C1 (muC0C1) on the steady-state ATPase rates was considerably diminished compared to that of the huC0C1 N-terminal domains at both low and high calcium (Figure 4B,D). We used centrifuge binding experiments to make a direct comparison of the binding affinity of muC0C1 and huC0C1 to that of native cardiac thin filaments at $p\text{Ca} = 4$ and $p\text{Ca} > 8$ (Figure S4). The affinities of mouse and human C0C1 were identical, $2.2 \pm 0.2 \mu\text{M}$ at $p\text{Ca} = 4$ and $p\text{Ca} > 8$,

within experimental error and did not correlate with the large differences in the apparent affinities measured by the activation of ATP hydrolysis measured in Figure 4. These results were surprising and suggest that the activation of the thin filament by C0C1 occurs subsequent to binding and results from a more specific interaction with C0C1 that leads to activation of the thin filament.

Because actin is a notoriously “sticky” protein with a negatively charged exterior that facilitates binding to positively charged proteins including myosin and the N-terminal domains of cMyBP-C, we wanted to investigate the influence of charge on the specificity of the interactions between the N-terminal domains of cMyBP-C and the thin filaments. In particular, we wanted to investigate whether the removal of positive charges added to the N-terminus of recombinant proteins via expression of a hexa-His tag affects the observed rates of thin filament activated myosin-S1 ATP hydrolysis. Comparison of the results in Figures 5A and 3A demonstrates that removal of the His tag sequence had only modest effects on rates of ATP hydrolysis at $p\text{Ca} = 4$ and that the overall pattern of activation and inhibition of cMyBP-C proteins on ATP hydrolysis by native cardiac thin filaments was similar before and after removal of the six His residues. A comparison of the results at $p\text{Ca} > 8$, Figures 5B and 3C, shows a less than 2-fold increase in apparent K_i after removal of the His tag. Thus, the observed binding is not an artifact produced by the His-tag, but the increase in positive charge does appear to contribute slightly to the binding affinity.

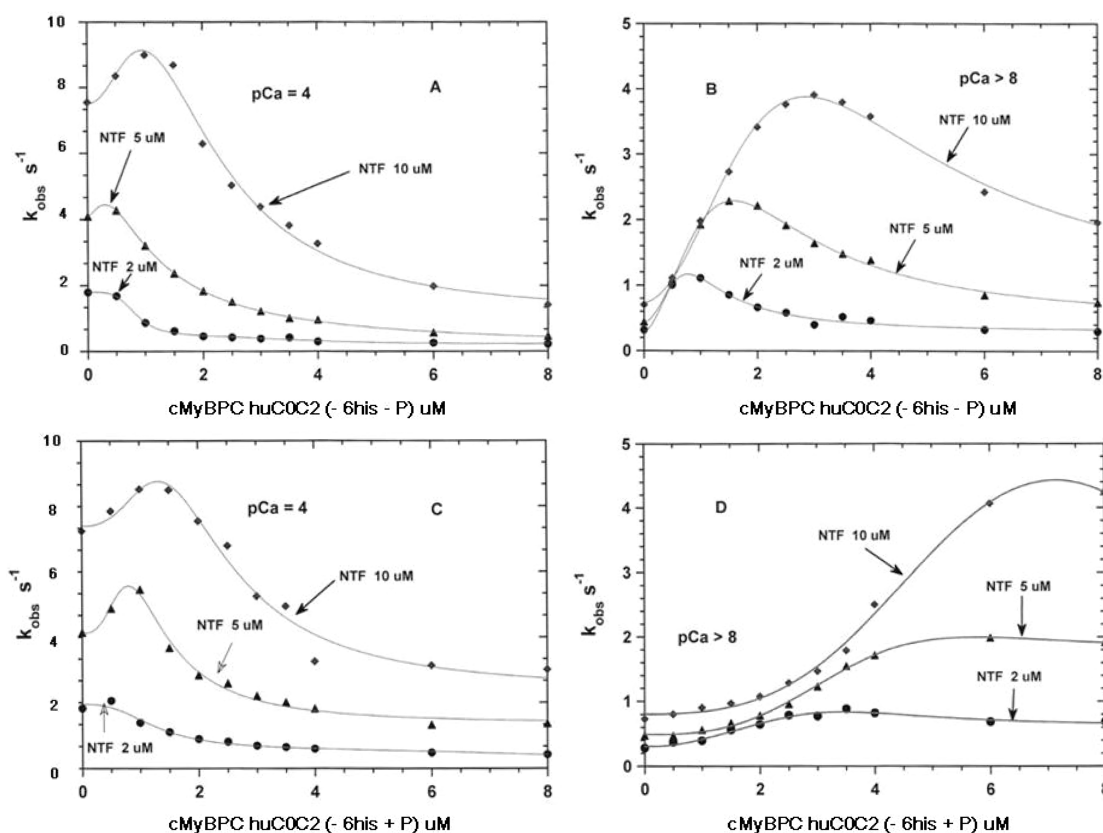


Figure 5. Effect of removal of the hexa-His N-terminal tag and phosphorylation of the m-linker of N-terminal C0C2 domains of human cMyBP-C on native thin filament-activated ATP hydrolysis by myosin-S1. ATP hydrolysis was measured by colorimetric determination of phosphate as described in Materials and Methods. Experimental conditions: 0.25 to 1.0 μ M myosin-S1, native cardiac thin filaments and cMyBP-C at the indicated concentrations, 10 mM MOPS, 50 mM KAc, 3 mM MgCl₂, pH 7.0, 30 °C, and either 0.1 mM CaCl₂ (A, C) or 1 mM EGTA (B, D).

Table 1. Charged Amino Acids in N-Terminal cMyBP-C Domains

	C0	PA	C1	m	C2	C0C2	C0C1	C1C2
mouse pK _i	9.26	3.76	7.99	9.10	6.77	6.21	5.59	8.54
glu + asp	14	10	11	18	12	65	35	41
lys + arg	17	1	12	20	12	62	30	44
net charge ^a	+3	−9	+1	+2	0	−3	−5	+3
human pK _i	9.30	3.77	8.75	8.26	6.64	7.63	6.91	8.48
glu + asp	13	6	10	19	12	60	29	41
lys + arg	16	1	12	20	12	61	29	44
net charge ^a	+3	−5	+2	+1	0	+1	0	+3

^aNet charge on the individual domains was calculated from (lys + arg) − (glu + asp).

Phosphorylation of the regulatory m-domain has also been suggested as a primary means of regulating cMyBP-C interactions with either myosin S2 or with actin via the introduction of negative charges that disrupt electrostatic binding interactions.^{11,24,25} Consistent with this idea, as shown in Figure 5B,D, higher concentrations of phosphorylated huC0C2 were required to obtain the same extent of activation of ATP hydrolysis (Figure 5D) as that required by the nonphosphorylated huC0C2 (Figure 5B) at low Ca²⁺ (pCa > 8). These results are in good agreement with conclusions that specific electrostatic charge interactions with the m-linker domain contribute to the binding and activating effects of the N-terminal domains of cMyBP-C with actin, as reported previously.^{11,26} However, as shown in Figure 5A,C, at pCa = 4 there are only modest effects of phosphorylation on thin filament activation by huC0C2.

DISCUSSION

We have shown that the N-terminal domains of cMyBP-C are sufficient to inhibit steady-state actin-activated ATP hydrolysis by a mechanism similar to that previously shown by Moos for intact skeletal MyBP-C.³ Furthermore, N-terminal domains of human cMyBP-C, C0C2, C1C2, and C0C1, all produce biphasic activating and inhibitory effects on thin filament-activated ATPase activity when native cardiac thin filaments (F-actin plus regulatory proteins, troponin, and tropomyosin) are used to activate myosin ATPase activity. That is, steady-state ATPase is activated by low concentrations of N-terminal cMyBP-C domains in the presence of thin filaments at low Ca²⁺, whereas higher concentrations of the N-terminal proteins inhibit ATPase activity at both low and high Ca²⁺ (pCa > 8 or = 4, respectively). The biphasic behavior strongly suggests a mechanism in which the low occupancy binding of N-terminal

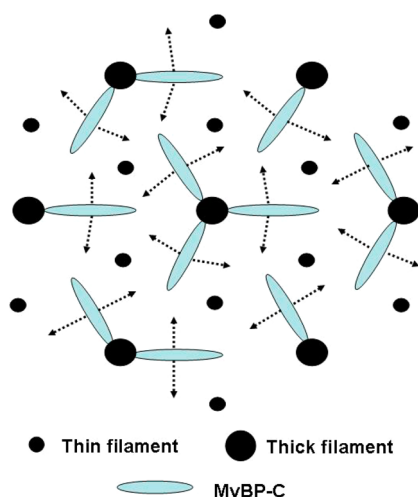


Figure 6. Cartoon of the cross-sectional geometry of a myofibrillar lattice through the 43 nm MyBP-C repeat showing the geometry of the actin and myosin filaments and MyBP-C cross-links. Arrows indicate variability in the position of the MyBP-C cross-links relative to the thin filaments.

domains of cMyBP-C activates the thin filaments by a mechanism similar to the activation of the thin filament by rigor S1 heads and NEM-S1, which shifts the position of tropomyosin from the inactive to active position at low ratios of S1 to actin.^{27,28} Higher concentrations of cMyBP-C binding compete with the less strongly binding M-ADP-P_i for actin sites in the thin filament. We similarly observe here that higher concentrations of N-terminal domains of cMyBP-C function as competitive inhibitors by blocking M-ADP-P_i binding to actin. Alternatively, N-terminal domains of cMyBP-C could potentially bind to and interfere with the inhibitory function of troponin. However, because activation of contraction was still achieved using N-terminal domains of cMyBP-C in permeabilized myocytes following extraction of TnC,¹⁶ a mechanism involving troponin is unlikely to account for all of the activating effects of cMyBP-C. Other interactions with troponin are also possible. For instance, it is an intriguing possibility that cMyBP-C interactions with the thin filament could mediate coordinated signaling between thin and thick filaments during activation and relaxation.^{29,30}

The idea that N-terminal domains can both activate and inhibit interactions of the thin filament with myosin is supported by electron microscopy of complexes of N-terminal domains of cMyBP-C and actin, which indicate that the binding site of the N-terminal domains of cMyBP-C overlaps with both the inhibitory position of tropomyosin on the thin filament and the binding site of myosin on actin.^{15,31} Strikingly, the extent of activation of myosin-S1 ATPase by N-terminal domains of cMyBP-C measured in this work is similar to that produced by rigor S1 activation of native cardiac thin filaments under similar conditions,³² further supporting the premise that the N-terminal domains of cMyBP-C interact in a highly specific manner with the thin filament.

Table 1 compares the charges on the constituent domains and linkers of the N-terminal subdomains of mouse and human cMyBP-C. The overall similarity observed for functional effects of mouse and human C1C2 is not surprising considering the 94% sequence similarity, with most of the changes being conservative. The net charge (+3) and the total number of positive (+44) and negatively (−41) charged amino acids are

the same in the human and mouse C1C2 sequences (Table 1). Results from this study (Figures 2–4 and S1) also showed that both mouse and human C0C2 or C1C2 sequences were equally effective at activation of native cardiac thin filaments but that muC0C1 is much less effective than huC0C1. These results thus agree with similar observations reported previously using motility assays¹⁸ and indicate that species-specific sequences contribute to functional differences of cMyBP-C. The findings reported here reconcile differences between different lab groups that have used either human or mouse N-terminal constructs of cMyBP-C.^{33,17}

Although the human and mouse C0 and C1 domains share ~80 and 90% sequence identity, respectively, the proline–alanine-rich region shares only ~40% identity across the two species.²⁹ Sequence differences in the PA region (possibly the 4 additional negative charges in the PA linker of muC0C1) are likely to be responsible for its reduced ability to activate the thin filament. Myosin-ADP-P_i binding to actin is enhanced by a favorable ionic interaction between negatively charged side chains on the actin and positively charged side chains on the myosin in the actomyosin binding site, even though the charge of both proteins is negative at physiological pH.^{34,35} It is therefore likely that similar local charged interactions are a component of N-terminal cMyBP-C binding to actin and that the net negative charge in the PA sequence is responsible for the reduced ability of muC0C1 relative to huC0C1 to activate thin filaments.

Crystal and NMR structures of cardiac C0, C1, and C2 domains show that the charges are fairly uniformly distributed on the surfaces of all 3 domains.^{36–38} These data suggest that all of the domains in human cardiac myosin binding protein C0C2 contribute to the activation of cardiac thin filaments and increase the binding affinity, whereas the increased negative charge in the PA region of the N-terminal domains of mouse myosin binding protein C reduces the ability of muC0C1 to activate the thin filament.

It is important to note that the activating and inhibitory effects of the N-terminal domains of cMyBP-C reported here cannot be attributed to interactions with the S2 segment of myosin. This is because the work reported here was all done using chymotryptic myosin-S1, which does not contain the S2 segment of myosin and thus avoids the possibility that interactions between MyBP-C and S2 are responsible for the increase in the actomyosin ATP hydrolysis rate observed in Figures 2–5. While our data does not provide evidence for or against an interaction between the S2 region of myosin and cMyBP-C, it does show that such interactions are not required for the activation of the thin filament by cMyBP-C N-terminal domains, as observed in this work.

Physiological Significance. The physiological significance of the activating and inhibitory effects of cMyBP-C are not completely understood, but a role for activating effects to prime the thin filament, to contribute to cooperative activation, and/or to counteract deactivation of the thin filament during muscle shortening are all possibilities. Conversely, inhibitory effects of cMyBP-C may contribute to cardiac contractile reserve where reversal of cMyBP-C inhibition by phosphorylation augments contractile activity in response to inotropic stimuli. However, the dual effects of cMyBP-C at different concentrations described here could provide an explanation for the puzzling occurrence of cMyBP-C in sarcomeres at limited stoichiometry relative to myosin because the cooperative activating effects of cMyBP-C would be expected to predominate while competi-

tion with myosin S1 heads should be minimized. This is because there is approximately one cMyBP-C/actin troponin/tropomyosin repeat in the C-zone, which comprises approximately 60% of the myosin filament-containing cross-bridges. A cartoon of the geometry of the actin and myosin filaments with the MyBP-C cross-links in striated muscle in Figure 6 illustrates that there is one MyBP-C per thin filament in the C-zone. The 43 nm distance between the MyBP-C repeats is just slightly longer than the 7 actin subunit repeat of the troponin tropomyosin. Thus, the stoichiometry of one cMyBP-C per 8 actin subunits in the C-zone is similar to that which we have observed here as being required to obtain maximal activation of myosin S1 hydrolysis by native cardiac thin filaments in solution, but it is not present at a sufficiently high concentration to compete with M-ADP-P_i for a significant fraction of the actin subunits.

■ ASSOCIATED CONTENT

● Supporting Information

Tabulated fitting parameters for kinetic data in Figures 2 and 3 (Table S5); additional kinetic data using mouse N-terminal domains (Figure S1); acrylamide gels of the proteins used in this work (Figures S2 and S3); measurement of the affinity of mouse and human cMyBP-C C0C1 to thin filaments by centrifuge binding experiments (Figure S4). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

MyBP-C, myosin binding protein C; cMyBP-C, cardiac myosin binding protein C; myosin-S1, myosin subfragment1; hu, human; mu, mouse; C0C1, N-terminal domains C0, the proline/alanine rich linker, and C1 of myosin binding protein C; C1C2, N-terminal domains C1, the M-domain linker, and C2 of myosin binding protein C; C0C2, N-terminal domains C0, the proline/alanine rich linker, C1, the M-domain linker, and C2 of myosin binding protein C; M-ADP-P_i, myosin ADP and P_i bound to active site; N-terminal, amino terminal

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