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# Effect of Cardiac Myosin-Binding Protein C on Tropomyosin Regulation of Actin—Myosin Interaction Using *In Vitro* Motility Assay

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We studied the modulating role of cardiac myosin-binding protein C (cMyBP-C) in tropomyosin regulation of the actin—myosin interaction. The effect of cMyBP-C on the velocity of actin-tropomyosin filament sliding over cardiac and slow skeletal myosins was evaluated using *in vitro* motility assay. The effect of cMyBP-C on the actin-tropomyosin filaments sliding depended on the type of myosin. The regulatory effect of cMyBP-C differs for cardiac and slow skeletal myosin because of the presence of specific essential light chain (LC1sa) in slow skeletal myosin isoform.

**Key Words:** *myosin; tropomyosin; cardiac myosin-binding protein C*

Thick filament of striated muscles in vertebrates comprises except contractile (actin and myosin) and regulatory (troponin and tropomyosin) proteins also other proteins such as myosin-binding protein C (MyBP-C). Three isoforms of MyBP-C have been identified. Cardiac isoform of protein C (cMyBP-C) is now intensively studied, because some hereditary cardiomyopathies (familial hypertrophic cardiomyopathy) are associated with mutations in the gene encoding this protein [9].

cMyBP-C molecule consists of 11 immunoglobulin and fibronectin-like domains. N-terminal C0, C1, and C2 domains and the regulatory M-domain bind to actin and/or to myosin S2 fragment; while C-terminal domains C7-C10 bind to thick filament [2]. cMyBP-C participates in the regulation of heart muscle contraction [2,5,12]. Structural data obtained with small angle X-ray scattering [15] suggest that cMyBP-C and tropomyosin compete for binding with actin. It was found that the C0-domain of N-terminal fragment with MyBP-C activates thin filament via displacement of tropomyosin from its inhibitory position on actin to its "open" state [2,5].

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Here we studied the modulating role of cMyBP-C in tropomyosin regulation of the actin—myosin interaction. Using *in vitro* motility assay [1,6,10] we evaluated the influence of cMyBP-C on actin-tropomyosin filament sliding velocity over cardiac and skeletal slow myosin.

## MATERIALS AND METHODS

Actin was isolated from *m. psoas* of rabbit using standard technique [7]. Cardiac tropomyosin was isolated from the left ventricle of bovine heart [13]. Rabbit *m. semimembranous* containing up to 80-90% of slow isoform of skeletal myosin served as the source of slow skeletal myosin [11]. Skeletal and cardiac myosin from the left ventricle of rabbit heart was prepared by standard method [4] and stored in 50% glycerol at -20°C. cMyBP-C was isolated from chicken heart by the method [3] with modifications [12].

The actin-tropomyosin filament was reconstructed by mixing 400 nM rhodamine-phalloidin-labeled filamentary actin (F-actin) and 100 nM tropomyosin at 4°C in AB buffer. AB buffer contained (in mM): 25 KCl, 25 imidazole, 4 $MgCl_2$, 1 EGTA, 10 dithiothreitol (pH 7.5).

The *in vitro* motility assay was conducted as described previously [6] in a 50-µl flow chamber with nitrocellulose surface of a standard design. First, 50 µl myosin solution in AB buffer with high ionic strength (0.5 M KCl) at concentration of 200 µg/ml was incubated in a flow chamber for 2 min. Then, the chamber was washed successively with AB buffer with high ionic strength and AB buffer, and then, 50 µl BSA (0.5 mg/ml) was added for 1 min. Then, 500 µg/ml of unstained F-actin in AB buffer containing 2 mM ATP was added for 5 min to block nonfunctional myosin heads. The chamber was washed three times with AB buffer and then, 50 µl of 10 nM rhodamine-phalloidin-labeled F-actin or actin-tropomyosin filament was added for 5 min. Then the final portion of AB buffer containing BSA (0.5 mg/ml), dithiothreitol (20 mM), ATP (2 mM), tropomyosin (100 nM), and oxygen-utilizing system (3.5 mg/ml glucose, 0.02 mg/ml catalase, 0.15 mg/ml glucose oxidase) was added. In the experiment with actin-tropomyosin filaments, AB buffer contained 100 nM of tropomyosin to prevent dissociation of tropomyosin from actin. cMyBP-C was added to the flow chamber together with myosin. The concentration of cMyBP-C was expressed as molar ratio to myosin. All the experiments were performed at 28°C.

Fluorescent-labeled filament was visualized using an Axiovert 200 inverted epifluorescence microscope with a 100x/1.45 oil Alpha Plan-Fluar lens (Carl Zeiss) and an EMCCD iXon-897BV camera (Andor Technology). In each flow chamber, 10 fields were recorded for 30 sec each.

The experiments were repeated three times. All values are presented as mean±standard deviation. Significance of differences was assessed by using paired *t* test or nonparametric Mann–Whitney test at $p<0.05$.

## RESULTS

Cardiac tropomyosin slows the velocity of F-actin (from 0.50±0.10 to 0.37±0.14 µ/sec) (Fig. 1). Addition of cMyBP-C in a physiological concentrations (cMyBP-C/myosin 1:5) did not affect the sliding velocity of F-actin and increased the velocity of actin-tropomyosin filament to 0.52±0.10 µ/sec.

To elucidate the molecular mechanisms of the effect of cMyBP-C on tropomyosin regulation of actin—myosin interaction, we compared the effects of cMyBP-C on the velocity of actin-tropomyosin filament sliding over cardiac myosin and slow skeletal myosin. Slow skeletal myosin contains the same heavy myosin β-chain as V3 isoform of cardiac myosin, because this chain is encoded by the same gene [14], and differs from the cardiac myosin only by the composition of light chains. Tropomyosin did not affect the sliding velocity of F-actin over slow skeletal myosin. Addition of cMyBP-C in physiological concentrations also had no effect on the velocity of actin-tropomyosin filament (Fig. 2).

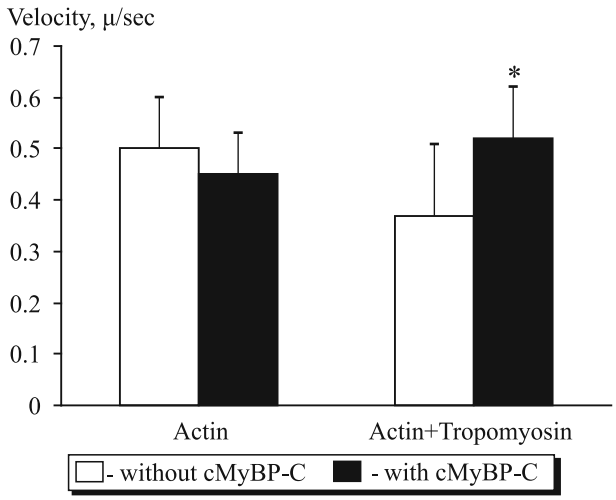


**Fig. 1.** Effect of cMyBP-C on the sliding of F-actin and actin-tropomyosin filament on cardiac myosin. Concentrations of myosin and cMyBP-C loaded into the flow chamber were 300 µg/ml (0.65 mM) and 20 µg/ml (0.13 mM), respectively, that corresponded to 1:5 cMyBP-C:myosin molar ratio. *$p<0.05$ in comparison with control (without cMyBP-C).

We showed that the addition of cMyBP-C in physiological concentration increased the sliding velocity of actin-tropomyosin filament over cardiac myosin, and did not affect its sliding over slow skeletal myosin. Thus, the effect of cMyBP-C on the movement of actin-tropomyosin filaments depends on the type of myosin.

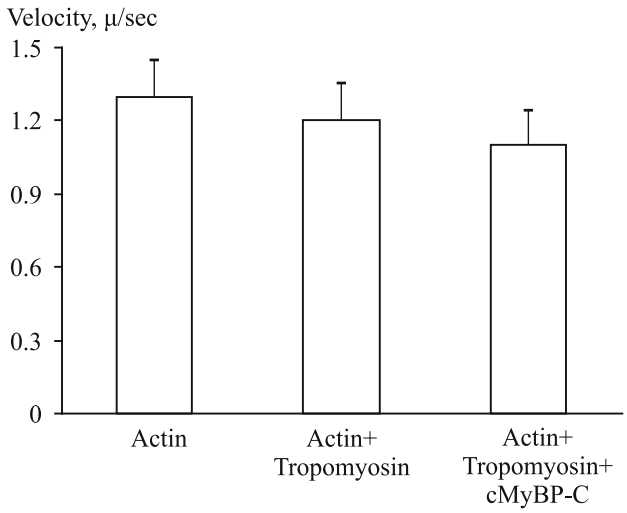


**Fig. 2.** Effect of tropomyosin on sliding of F-actin and effect of cMyBP-C on the velocity of actin-tropomyosin filament sliding over slow skeletal myosin. Concentrations of myosin and cMyBP-C loaded into the flow chamber were 300 µg/ml (0.65 mM) and 20 µg/ml (0.13 mM), respectively, that corresponded to 1:5 cMyBP-C: myosin molar ratio.

Slow skeletal myosin from rabbit muscles contains light chains LC1sa, LC1sb, and sLC2. Light chains LC1sb and sLC2 are also expressed in the myocardium. It was shown [8] that functional characteristics of slow filaments depend on the molar ratio of LC1sa to LC1sb. We found that the regulatory effect of cMyBP-C for cardiac and skeletal myosin isoforms differed due to the presence of specific essential light chain (LC1sa) in skeletal myosin isoform.

Thus, the effect of cMyBP-C on tropomyosin regulation is related to the type of myosin, namely amino acid sequence of myosin light chains. These data clarify some regulatory mechanisms of myocardial contractility and help to understand the molecular basis of myocardial function.

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