Isoform-specific Interaction of the Myosin-binding Proteins (MyBPs) with Skeletal and Cardiac Myosin Is a Property of the C-terminal Immunoglobulin Domain*

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Full-length cDNAs encoding chicken and human skeletal MyBP-H and MyBP-C have been isolated and sequenced (1–5). All are members of a protein family with repetitive immunoglobulin C2 and fibronectin type III motifs. The myosin binding domain was mapped to a single immunoglobulin motif in cardiac MyBP-C and skeletal MyBP-H. Limited α-chymotryptic digestion of cardiac MyBP-C generated three peptides, similar in relative mobility to those of skeletal MyBP-C: 100, 40, and 15 kDa. Trypsin digestion of MyBP-H yielded two peptides: 50 and 14 kDa. Partial amino acid sequences proved that the 15- and 14-kDa fragments are located at the C termini of cardiac MyBP-C and skeletal MyBP-H, respectively. Only the 14- and 15-kDa peptides bound to myosin. Thus, the myosin binding site in all three proteins resides within an homologous, C-terminal immunoglobulin domain. Binding reactions (2) between the skeletal and cardiac MyBPs and corresponding myosin isoforms demonstrated saturable binding of the MyBP proteins and their C-terminal peptides to myosin, but there are higher limiting stoichiometries with the homologous isoform partners. Evidence is presented indicating that MyBP-H and -C compete for binding to a discrete number of sites in myosin filaments.

The thick myofilaments of vertebrate muscle in addition to myosin contain a number of other proteins. These include titin or connectin (6, 7), MyBP-C (C-protein) (8), MyBP-H (H-protein) (9, 10), myomesin (11), M protein (12, 13), skelemin (14), MM-creatine kinase (15, 16), AMP-deaminase (17), and X-protein (the slow-type isoform of MyBP-C) (18–20). The MyBPs, first isolated in crude myosin preparations by Offer and colleagues (21) in the early 1970s are a group of proteins distributed in the central two-thirds of the cross-bridge bearing region (C-zone) of the A-band (22). This zone contains a set of 11 transverse repeats of 43 nm axial spacing, distributed along the constant diameter region of the thick filaments. Seven to nine of these repeats are decorated with antibodies specific for one or more isoforms of MyBP-C (18, 20, 21). MyBP-C is found in a 1:8 molar ratio to myosin and binds to the rod portion of the latter molecule in both its light meromyosin and subfragment-2 domains (23). MyBP-C also binds to F-actin (24) and to regulated actin filaments in a Ca2+-dependent manner (25, 26).

Fast, slow, and cardiac isoforms of MyBP-C have been identified in adult muscles (8, 19, 27–29), and additional isoforms have been identified in embryonic tissues (30, 31). Some muscles, e.g. the posterior latissimus dorsi of chickens, contain at least two isoforms, fast and slow, in every sarcomere (29).

MyBP-H is a lower molecular weight protein, localized in the P- and C-zones of the A-band. It has been identified in rabbit (9), human (4), and chicken muscles (3, 10, 32), but its distribution in the A-band differs slightly in mammalian and avian species (18, 32). In fact, all of the MyBP isoforms exhibit subtle variations in A-band distribution that remain unexplained (18, 20, 21, 32). The molecular mechanisms and physiological implications for sorting of the MyBP isoforms remain obscure. Mammalian and avian MyBP-H exhibit anomalous electrophoretic mobilities in reducing SDS-PAGE; the molecular weight of MyBP-H has been estimated to be 86 kDa by SDS-PAGE† and ultracentrifugation (9), but the true masses of the chicken and human proteins, based on cDNA sequences, are 58,487 and 51,986 kDa, respectively (3, 4). This anomalous retardation in SDS gels is a property of the N-terminal 24 kDa of the protein, which contains two extended motifs of alternating alanine and proline residues, resembling the N terminus of skeletal muscle myosin light chain 1 (33). The C-terminal 40-kDa section of MyBP-H is comparable in structure to the C terminus of MyBP-C; the two proteins share 49.6% sequence identity and 17% conservative amino acids in this region (1–3).

The full-length cDNAs encoding chicken and human skeletal MyBP-H and MyBP-C have been cloned and sequenced (1, 3–5). The amino acid sequences reveal seven immunoglobulin (Ig) C2 sets and three fibronectin (Fn) type III motifs in MyBP-C and four IgC2 and two Fn type III repeats in MyBP-H. Thus, MyBP-C and MyBP-H are members of the Ig superfamily (34, 35). The IgC2 and Fn (III) repeats are conserved in human MyBP-C (36) and seem to be a common feature of other myosin-associated proteins (37–42). The myosin binding domain of chicken MyBP-C has been mapped to a 14-kDa C-terminal, α-chymotryptic peptide, containing one IgC2 repeat (2). In this report we demonstrate that a comparable myosin binding domain resides at the C terminus of cardiac MyBP-C and skeletal actin filaments in a Ca2+-dependent manner (25, 26).

† The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; Fn, fibronectin; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; FPLC, fast protein liquid chromatography.

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MyBP-H and also show that this domain plays a central role in the binding specificities between the different myosin isoforms and variant forms of the MyBPs expressed in cardiac and skeletal muscles. Evidence is presented for competitive binding of MyBP-C and MyBP-H to myosin.

EXPERIMENTAL PROCEDURES

Protein Preparations—Crude myosin was prepared from fresh chicken heart and skeletal muscle as described (19). Only ventricles were used to isolate proteins from the heart. The MyBPs were purified as described (2).

Generation and Purification of MyBPs' Fragments—α-Chymotryptic cleavage of skeletal and cardiac MyBP-C was performed with proteins predialyzed in 0.1 M KCl, 20 mM imidazole-HCl (pH 7.0), and 1 mM DTT. Cardiac or skeletal MyBP-C (0.5 mg/ml) was mixed with 1:250 w/w of α-chymotrypsin in 0.001 M HCl and then incubated at 23 °C for 30 min. The cleavage reaction was terminated by the addition of 1 mM PMSF. Tryptic cleavage of MyBP-H was performed after dialysis of MyBP-H against 0.1 M KCl, 20 mM imidazole-HCl (pH 7.0), 1 mM DTT. MyBP-H (0.2 mg/ml) was mixed with 1:500 w/w of trypsin in 0.001 N HCl and incubated at 23 °C for 30 min. The reaction was terminated by the addition of 1.3:1 w/v of soybean trypsin inhibitor (Worthington). To purify the proteolytic fragments under non-denaturing conditions, products of the cleavage reaction were fractionated on a Superose 12 FPLC column (Pharmacia Biotech Inc.; 10 × 300 mm; run at 0.5 ml/min) equilibrated with 0.1 M KCl, 20 mM imidazole-HCl (pH 7.0), 1 mM DTT, 1 mM EDTA, and 1 mM PMSF.

Sequence Analyses of the Proteolytic Fragments of MyBPs—After proteolytic cleavage, peptides in the reaction mixtures were separated electrophoretically by 15% SDS-PAGE. Electrophoresed peptides were transferred to polyvinylidene difluoride membranes as described (43). After electrophoresis each gel was soaked for 5 min in transfer buffer. Transfer of peptides was performed at 75 mA (constant current) at 4 °C for 8 h. The membranes were then stained with 0.1% Coomassie Blue R-250 in 40% methanol in 1% acetic acid for 10 min and destained with 90% methanol and then washed in water. Stained bands were excised and sent for immediate sequencing or stored at −20 °C in sealed Eppendorf tubes.

Binding of MyBPs and Their Fragments to Myosin—Myosin (cardiac or skeletal) stored in glycerol was dialyzed against 0.1 M KCl, 20 mM imidazole-HCl (pH 7.0), and 1 mM DTT to obtain synthetic (reconstituted) filaments. The filaments were then pelleted by centrifugation (15,000 × g for 1 h) and solubilized in 0.6 M KCl, 20 mM imidazole-HCl (pH 7.0), 1 mM DTT. Myosin concentrations were determined spectrophotometrically using an extinction coefficient at 280 nm of 0.58 mg/ml cm (44). In all cases the binding reactions were performed between the MyBPs and myosin in filament form. The final concentration of myosin in all experiments was 1.5 μM. Thus, myosin was aliquoted into an Eppendorf tube, the solution was diluted 6-fold with water (reducing the KCl concentration from 0.6 to 0.1 M), and the MyBPs or their proteolytic peptides added at varying concentrations (0–4 μM). The solution was incubated for 30 min on ice in a final volume of 100 μl. Myosin filaments with associated binding proteins were then sedimented in an Airfuge (Beckman Instruments, Palo Alto, CA) at 22 p.s.i. for 20 min at 4 °C, and the pellets were resuspended in 0.1 M KCl, 20 mM imidazole-HCl, 0.5 mM PMSF. Supernatants and pellets were displayed by SDS-PAGE, and the gels were stained with Coomassie Blue and scanned with a GC300 Hoffer (San Francisco) densitometer linked to a GS350 data processing system. The amount of bound proteins or their fragments was normalized to the myosin light chain concentrations. In all experiments the binding stoichiometry is described as mol MyBP or MyBP peptide bound per mol of myosin molecule. Curve fitting for all binding plots was performed with the Slide Write Plus version 4 program.

RESULTS

Comparative Binding of Cardiac and Skeletal MyBP-C and MyBP-H to Reconstituted Myosin Filaments Prepared from Skeletal and Cardiac Myosin—A solution phase binding assay (2) was used to examine the interactions between fast-type

FIG. 1. Binding of full-length myosin-binding proteins to reconstituted myosin filaments. A, binding assays were performed at equimolar concentrations (1.5 μM) of the MyBPs and myosin and at 0.1 M KCl. The figure illustrates SDS-PAGE gels of the pellet fractions. Lanes 1 and 5, skeletal and cardiac myosin, respectively; lanes 2, 3, and 4, binding of skeletal myosin to skeletal MyBP-H, skeletal MyBP-C, or cardiac MyBP-C, respectively; lanes 6, 7, and 8, binding of cardiac myosin to skeletal MyBP-H, skeletal MyBP-C, or cardiac MyBP-C, respectively. Asterisks indicate positions of the MyBPs. B, equilibrium binding reactions between MyBP-H, skeletal MyBP-C, and cardiac MyBP-C and reconstituted skeletal myosin filaments. Increasing concentrations of each MyBP (0–4 μM) were incubated on ice with a fixed concentration (1.5 μM) of reconstituted myosin filaments. Samples were sedimented in an Air-fuge, and the supernatant and pellet fractions were displayed by 15% SDS-PAGE. Coomassie blue-stained gels were densitometrically scanned, and the concentrations of bound protein were normalized to the myosin light chain concentrations. •, binding of skeletal MyBP-H to myosin filaments; ○, binding of skeletal MyBP-C to myosin filaments; ●, binding of cardiac MyBP-C to myosin filaments.
Myosin-binding Proteins

MyBP-C and reconstituted filaments prepared from fast-type skeletal myosin purified from the pectoralis major muscle of adult chickens. The same assay was also used to analyze the binding of cardiac MyBP-C and MyBP-H to different myosin isoforms. Briefly, binding of the MyBPs to myosin filaments was examined at constant myosin concentrations (1.5 μM) while varying the concentrations of the MyBPs (0–4 μM). The binding reaction was performed for 30 min, twice the time needed to reach reaction equilibrium (data not shown), and the filaments with associated proteins were then separated from the supernatant by centrifugation. Several experiments are illustrated in Fig. 1A in which the binding assays were performed at equimolar ratios of the MyBPs and myosin (1.5 μM:1.5 μM), and the pellet fractions were displayed by SDS-PAGE. Densitometric analyses of the pellets revealed that all of the MyBPs exhibited saturable binding to reconstituted filaments prepared skeletal or cardiac myosins, but there were differences in the amounts bound at saturation (the limiting stoichiometries), which was characteristic of the respective isoforms. Based on half-maximal binding values, only minor differences in relative affinities were apparent.

At saturation MyBP-H bound to skeletal myosin filaments with a molar ratio of 1:1 (Fig. 1B). Different results were obtained with the MyBP-C isoforms. Skeletal muscle MyBP-C bound to skeletal myosin filaments with a limiting stoichiometry of about 0.61, but with cardiac myosin it saturated at a molar ratio of 0.25:1. MyBP-H exhibited similar myosin isoform-specific binding values. At saturation 0.35 mol of MyBP-H bound/mol of cardiac myosin, as compared with the 1:1 binding ratios with skeletal myosin. Thus, both of the skeletal muscle MyBPs had significantly higher limiting stoichiometries with skeletal than cardiac myosin filaments.

The reverse was true for cardiac MyBP-C; almost twice as much cardiac MyBP-C bound to cardiac as to skeletal myosin filaments (Fig. 1B). At saturation, ~0.6 mol of cardiac MyBP-C bound to 1 mol of cardiac myosin, whereas ~0.3 mol of MyBP-C bound to 1 mol of fast skeletal myosin filaments.

Competitive Binding of the MyBPs to Myosin Filaments—To test if MyBP-C and MyBP-H bound to a common site on myosin, a series of competition binding assays were performed. Myosin, skeletal or cardiac (1.5 μM), was incubated with 1.5 μM of skeletal or cardiac MyBP-C for 30 min; this was twice the time required for the binding complex of myosin-MyBP to form (data not shown). Varying concentrations of skeletal MyBP-H were then added to this mixture (Fig. 2, A and C), and the reaction mixtures were incubated for another 30 min on ice. The samples were centrifuged as above, the supernatants and pellets were displayed by SDS-PAGE, and the Coomassie Blue-stained gels were analyzed by scanning densitometry. The results indicated that addition of MyBP-H reduced the binding of MyBP-C to myosin filaments (Fig. 2, A and C). Identical results were obtained when skeletal or cardiac myosin filaments were first incubated with saturating levels (3, 4, or 5 μM) of skeletal or cardiac MyBP-C for 30 min, and then 1.5 μM of skeletal MyBP-H was added to the mixture (data not shown). This reduction was most pronounced with the cardiac proteins, i.e. MyBP-H could displace greater amounts of cardiac MyBP-C from cardiac myosin filaments than skeletal MyBP-C bound to skeletal myosin filaments (Fig. 2). Competitive binding of the two proteins was independent of the order in which the two myosin-binding proteins were mixed. The presence of MyBP-C did not alter the amount of MyBP-H bound to the myosin filaments at saturation. Similar saturating amounts of MyBP-H bound to myosin filaments in both the competition experiments and in the direct binding assays performed in the absence of MyBP-C (~1 mol MyBP-H/mol of skeletal myosin and 0.55 mol MyBP-H/mol of cardiac myosin) (Figs. 1 and 2). Competitive binding of the two proteins was independent of the order in which the two myosin-binding proteins were mixed. Although MyBP-H was capable of displacing MyBP-C from myosin filaments, the reverse was not true (Fig. 2, B and D). In these experiments skeletal or cardiac myosin filaments were incubated with 1.5 μM of skeletal MyBP-H for 30 min, and then varying concentrations of MyBP-C were added to the mixture and incubated for another 30 min. Increasing amounts of MyBP-C, whether skeletal or cardiac, did not affect the...
binding of MyBP-H to either skeletal or myosin filaments. These results suggest that MyBP-H has a greater avidity for the binding sites on myosin filaments than does MyBP-C, although the half-maximal binding values appear rather similar.

Proteolytic Fragments of Cardiac MyBP-C and Skeletal MyBP-H—Digestion of skeletal MyBP-C with \( \alpha \)-chymotrypsin gives rise to three peptides with relative mobilities (\( M_r \) of 100,000, 28,000, and 14,000; the C-terminal \( M_r \) 14,000 fragment contains the major myosin binding domain (2). Because MyBP-C and MyBP-H share 50% sequence identity in the C-terminal third of each molecule (2, 3), we reasoned that selective proteolysis of MyBP-H and the other MyBP-C isoforms might release comparable C-terminal cleavage fragments, which could then be tested for binding to myosin filaments.

MyBP-C from chicken cardiac muscle was digested with \( \alpha \)-chymotrypsin, generating three proteolytic fragments, \( M_r \); 100,000, 40,000, and 15,000 (Fig. 3, A and B). These fragments were separated by FPLC chromatography and purity assessed by SDS-PAGE (Fig. 4 A). The purified fragments of \( M_r \) 40,000 and 15,000 were partially sequenced (Fig. 5, A and B), and the fragments positioned within the primary structure were established by cDNA sequencing (45). The \( M_r \) 40,000 fragment could be assigned to the N terminus of the molecule, the \( M_r \) 15,000 peptide to the C terminus, and the 100-kDa fragment placed in between (Fig. 6). The summed relative mobilities of the three peptides came to 155,000 matching the \( M_r \) of cardiac MyBP-C (46).

We were unsuccessful in obtaining chymotryptic peptides from MyBP-H isolated from chicken pectoralis muscle. However, tryptic digestion of skeletal MyBP-H, under identical conditions, generated two major proteolytic fragments with \( M_r \) of 50,000 and 14,000, respectively (Fig. 3B). These fragments were separated by FPLC gel filtration, and purity was assessed by SDS-PAGE (Fig. 4B). Partial amino sequencing was used to place the \( M_r \) 14,000 fragments along the derived amino sequences obtained from cDNA analyses. The \( M_r \) 14,000 peptide contained the C-terminal sequence of skeletal MyBP-H beginning at residue Val-429 (3) (Fig. 5). We were unsuccessful in obtaining sequence data from the \( M_r \) 50,000 fragment, suggesting a capped N terminus. As discussed by Vaughan et al. (3),

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**Fig. 4.** Superose 12 chromatography of cardiac MyBP-C \( \alpha \)-chymotryptic fragments (A) and skeletal MyBP-H tryptic fragments (B). Proteolytic fragments were fractionated under nondenaturing conditions. –1 mg of total protein was applied to the Superose 12 column and eluted by 0.1 M KCl, 20 mM imidazole-HCl (pH 7.0), 1 mM DTT, 1 mM EDTA, and 1 mM PMSF. Insets show SDS-PAGE gels of the fractions eluted from the columns.

**Fig. 5.** Amino acid sequence alignments of cardiac MyBP-C and skeletal MyBP-H derived from cDNA base sequences (3, 45) with proteolytic peptide sequences (see “Experimental Procedures”). A and B, proteolytic peptides derived from \( \alpha \)-chymotryptic digestion of cardiac MyBP-C. C, proteolytic peptide derived from tryptic digestion of skeletal MyBP-H.

**Fig. 6.** Schematic illustration of MyBP-H and MyBP-C illustrating the distribution of proteolytic fragments within the primary structure of each molecule and their relation to the internal IgC2 (●) and Fn type III (□) motifs.
the true mass of the $M_r 50,000$ fragment may be less than $M_r 50,000$. The deduced order of these peptides in the MyBP-H molecule is indicated in Fig. 6.

**Binding of Proteolytic Fragments of the MyBPs to Myosin Filaments**—When the total $a$-chymotryptic digest of cardiac MyBP-C was incubated with reconstituted filaments prepared from cardiac myosin, only the 15-kDa C-terminal fragment exhibited significant cosedimentation with the myosin filaments. The N-terminal 40-kDa peptide and the large internal 100-kDa fragment associated weakly if at all to the myosin filaments (Fig. 7A). An equivalent result was obtained when the trypic hydrolysate of skeletal MyBP-H was incubated with reconstituted skeletal myosin filaments; only the C-terminal 14-kDa fragment cosedimented with the myosin filaments (Fig. 7A). No detectable binding to myosin filaments was observed with the N-terminal 50-kDa fragment.

We then purified the C-terminal peptides under nondenaturing conditions and used them for solution-phase binding assays with reconstituted skeletal and cardiac myosin filaments (Fig. 3B). In all cases, saturable binding of the 14- and 15-kDa fragments with myosin was observed (Fig. 7B). At saturation $\sim 1.5$ mol of the C-terminal fragments of skeletal MyBP-C and skeletal MyBP-H bound/mol of skeletal myosin, whereas less than 1 mol ($\sim 0.6$ mol) of the 15-kDa fragment of cardiac MyBP-C bound to this same concentration of either skeletal or cardiac myosin. Of particular note was the observation that significantly less of the 14-kDa fragment of skeletal MyBP-C bound to cardiac than to skeletal myosin filaments ($0.35$ versus $1.5$ mol/mol). As noted above with the full-length MyBPs, significantly more MyBP-C peptide bound to skeletal than to cardiac myosin filaments. Thus, the 14-kDa proteolytic fragment of MyBP-C and the full-length molecule exhibit qualitatively similar binding reactions with these two myosin isoforms.

We did not obtain sufficient quantities of the 14-kDa fragment from skeletal MyBP-H to perform binding reactions with cardiac myosin. However, clear differences were observed between the C-terminal peptides of skeletal MyBP-C and cardiac MyBP-H when binding was tested with cardiac myosin. At saturation more cardiac MyBP-C peptide bound to cardiac myosin than did the skeletal MyBP-C peptide. Based on its half-maximal binding values, the cardiac C-terminal peptide had an $\sim 2$-fold greater affinity for cardiac myosin than did the skeletal muscle C-terminal peptide for this same myosin isoform. Thus, isoform recognition of the MyBPs for myosin appears to reside in the C-terminal Ig motif of both MyBP-C and MyBP-H.

**DISCUSSION**

We have established that only the 14-kDa fragment of skeletal MyBP-H and the 15-kDa fragment of cardiac MyBP-C, each located at the C termini of the respective molecules, account for the myosin binding properties of these molecules. These results confirm and extend the data of Okagaki et al. (2), which demonstrated that the myosin binding domain of skeletal MyBP-C resides in a C-terminal 14-kDa peptide. Because the proteolytic digestions and myosin binding assays were per-
formed under nondenaturing conditions, we assume that the released fragments retain tertiary structures present within the full-length, native molecules. That being the case, it is likely that each of these MyBPs has a related three-dimensional structure at its C terminus, the myosin binding domain of each molecule. Our observations have significance in clarifying the pathogenesis of those cases of familial hypertrophic cardiomyopathy with mutations of the cardiac MyBP-C gene on chromosome 17. Both published reports (47, 48) indicate that the mutations cluster in the C terminus of MyBP-C and would be expected to alter expression of a region of this molecule that is now demonstrated to bind myosin.

We have demonstrated that when varying combinations of skeletal or cardiac myosin are mixed with each MyBP or its C-terminal fragment, there is saturable binding between these reactants. It appears that all of the in vitro binding properties of MyBP-C and MyBP-H to myosin filaments can be accounted for by a single Ig motif at the C terminus of each protein. Cross-binding studies between isoforms from skeletal and cardiac muscles demonstrate that at saturation there is a higher limiting stoichiometry between homologous partners than between heterologous pairs. More skeletal MyBP-C or its C-terminal peptide bound to skeletal myosin at saturation than to cardiac myosin. The reverse was true for cardiac MyBP-C. In short, there is a degree of isoform specificity or molecular recognition in these binding reactions. Interestingly, more C-terminal peptide bound per mole of myosin at saturation than did the full-length protein. Twice as much of the C-terminal peptide of MyBP-C bound per mole of myosin than did the full-length MyBP-C. This same phenomenon was true for each of the MyBPs. At this time it is not possible to fully interpret these differences with certainty since they involve the interactions of a small protein molecule or peptide with a large polymer of myosin. Both differential affinities, stearic hindrances and changes in polymer structure during the binding reaction might interplay to affect the results observed. Because the 14-kDa C-terminal peptide of MyBP-C is approximately one-tenth the size of the full-length molecule, it might have greater accessibility to myosin filament cores than does full-length MyBP-C. Alternatively, upstream sites in MyBP-C might inhibit the binding of the C-terminal domain to the myosin rod. Available data cannot distinguish between these possibilities. Evidence exists that titin binds to MyBP-C and that this binding may occur within the last four domains of the MyBP (49). It is likely that titin forms a trimeric complex with myosin and MyBP-C, thereby facilitating and organizing the binding of MyBP-C to native myosin filaments. Because all of our binding studies were performed in the absence of titin, future analyses are warranted with native thick filaments stripped of MyBP-C or with reconstituted myosin filaments in the presence of added titin. These considerations are important for in vivo considerations of C-protein targeting (discussed below).

There has been one prior study on the binding of MyBP-H to skeletal myosin (50), using rabbit muscle proteins; it was found that 1.5 mol of MyBP-H bound per mol of myosin at saturation. That might be compared with the value of 1 mol/mol found in the present report. Considering the differences in species and binding assays, the results are reasonably consistent.

Competitive binding experiments indicate that chicken MyBP-H can inhibit the binding of both skeletal and cardiac MyBP-C to reconstituted myosin filaments. A different result was obtained in the prior study with rabbit muscle proteins (50). In the rabbit study binding of MyBP-C to myosin filaments was only partially inhibited by the presence of saturating levels of MyBP-H. These species-specific differences are informative. Immunoelectronmicroscopic studies by Bennett et al. (18) have shown that rabbit MyBP-H is confined to the P-zone of the A-band and does not overlap the distribution of MyBP-C in the C-zone. In contrast, Bahler et al. (32) have shown that chicken MyBP-H is distributed along the 43 nm stripes of both the P- and C-zones of the A-band. Thus, chicken MyBP-H must co-distribute with MyBP-C along the thick filament, whereas rabbit MyBP-H binds at different sites than does MyBP-C.

Several possibilities might be considered to account for these binding data: (a) myosin filaments contain only one docking site for the MyBPs that is capable of binding either MyBP-C or MyBP-H but with differing affinities; (b) myosin filaments contain two (or more) sets of related docking sites for the MyBPs, but these differ in relative affinity for MyBP-H and MyBP-C; (c) myosin filaments contain independent binding sites for these two proteins, but occupancy of one site affects the binding properties of the other, i.e. the two sites interact allosterically; (d) association of a MyBP with a myosin filament could alter the higher order structure of the filament, thereby changing its interactions with other proteins, including the MyBPs. Present data cannot discriminate between these possibilities, but the two-site model seems to account for most of our observations and those of Yamamoto (50).

We hypothesize that the existence in the chicken of two binding sites/mol of myosin in the thick filament that are capable of binding a MyBP molecule. MyBP-H binds to both of these sites with comparable affinity, but MyBP-C binds to only one of these sites and does so with a lower avidity than that of MyBP-H. In rabbit muscle we suggest that MyBP-C and -H bind to separate sites, and this might account for their in vivo sorting to different positions in the A-band. On the other hand, if the two MyBPs share common binding sites with equivalent avidities, additional protein interactions must define their final distributions in the cell. In this case, A-band distributions of the two proteins could be specified by additional interactions between myosin and other A-band components, e.g. titin. Recent studies from our laboratory demonstrate that the myosin binding domain (motif 10) of MyBP-C is required for targeting of this protein to the A-band, but this motif is not sufficient to do so alone (51). The three adjacent motifs (motifs 7, 8, and 9) are also required for proper targeting of MyBP-C to the C-zone of the sarcomere. Because these latter modules appear to bind titin (36, 49), it seems likely that insertion of MyBP-C into its native sites in the A-band requires the cooperative interaction of myosin, titin, and the MyBP. Thus, MyBP-H and MyBP-C could compete for binding to myosin, but their distinctive interactions with titin would modify their insertion in the sarcomere. Although MyBP-H and MyBP-C share considerable sequence homology over their C-terminal 400 amino acids and contain alternating Fn III and IgC2 motifs in this section of both molecules, the proteins differ substantially at their N termini. Conceivably, N-terminal regions of each molecule might define additional protein-protein interactions required for the final placement of each molecule in the sarcomere and function in their regulatory properties.

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