Dynamic Interaction Between Cardiac Myosin Isoforms Modifies Velocity of Actomyosin Sliding In Vitro

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To study the functional significance of cardiac isomyosin heterogeneity, active sliding of actin-myosin was studied using two different types of in vitro motility assay systems: (1) a sliding actin filament assay, in which fluorescently labeled actin filaments were made to slide on a myosin layer attached to a glass coverslip, and (2) a myosin-coated bead assay, in which myosin-coated latex beads were made to slide on actin cables of an alga. Two different isomyosins were obtained from 3-week-old (V₁) and hypothyroid (V₃) rat hearts and were mixed to form solutions with various mixing ratios (V₁/(V₁+V₃)). For these myosin mixtures, both ATPase activity and sliding velocity of actin-myosin were determined. As the relative content of V₁ increased, both ATPase activity and velocity increased. However, in contrast to the linear relation between the mixing ratio and ATPase activity, the relation between the mixing ratio and sliding velocity was sigmoid, suggesting the existence of mechanical interaction between different isomyosins. To clarify the nature of this interaction, sliding velocity was measured for mixtures of V₁ and p-NN'-phenylene-dimaleimide-treated V₁ myosin (pPDM-M). A convex relation was observed between the relative content of pPDM-M and velocity. Because pPDM-M is known to form a noncycling and weakly bound crossbridge with actin, it is expected to exert a constant internal load on V₁, in contrast to the actively cycling V₃. In conclusion, in actomyosin sliding, different isomyosins mechanically interact when they coexist. The interaction may be a dynamic one that cannot be explained by a simple load effect. (Circ Res. 1993;73:696-704.)

Key Words • myosin • isoform • actin • heterogeneity • in vitro motility assay

Three different isoforms (V₁, V₂, and V₃) have been identified in mammalian cardiac myosin based on their electrophoretic mobility on pyrophosphate polyacrylamide gels.¹ Biochemically, V₁ is characterized as having the highest ATPase activity, and V₃, the lowest. Later studies²,³ revealed that these isoforms consist of only two heavy chains named α and β; i.e., V₁ and V₂ are the homodimers of α and β, respectively, and V₃ is the heterodimer of α and β. Because the relative content of these isoforms changes (myosin heterogeneity) at different stages in development,⁴,⁵ in response to the thyroid state,⁶,⁷,¹¹,¹² and under the influence of the hemodynamic load,⁷,⁹,¹¹,¹² its functional meaning has been discussed repeatedly.⁷,⁹-¹¹ Although these studies unanimously demonstrated a positive correlation between ATPase activity of myosin and the maximum shortening velocity of the muscle or cell, the precise role of myosin heterogeneity remains to be fully understood because of the technical problems inherent in the preparations used in these studies. First, the parts of the tissue with which ATPase assay and mechanical assay were performed were not always identical. Second, because isoform distribution was difficult to control in vivo, only rough relations between isoform distribution and shortening velocity have been evaluated.⁵,¹³

An alternative method for overcoming these problems may be the in vitro motility assay technique, which has been developed recently.¹⁴,¹⁵ Because an isolated and purified myosin preparation is used to observe the actin-myosin sliding in these systems, an identical specimen can be used for both biochemical and physiological studies. Furthermore, by mixing different isomyosin preparations, we can obtain a myosin solution with the desired isoform composition for assays. Taking advantage of these assets, Sellers et al¹⁴ studied the in vitro actin-myosin sliding for mixtures of unphosphorylated and phosphorylated smooth muscle myosin, as well as for mixtures of skeletal muscle myosin and phosphorylated smooth muscle myosin. They found a nonlinear relation between the sliding velocity and the mixing ratio of different myosins. Warshaw et al¹⁵ also found a nonlinear relation between the velocity and the mixing ratio of different types of myosins, in contrast to the relatively linear relation between the ATPase activity of the solution and the mixing ratio. They discussed the existence of mechanical interaction between myosins of different properties as the mechanism of this nonlinearity. If this kind of interaction does indeed exist between cardiac myosin isoforms, reconsideration of cardiac mechanics and energetics would be required.

Accordingly, the purpose of this study was to examine the mechanical interaction between cardiac myosin isoforms using in vitro motility assays. We used two
different types of motility assay systems to measure the sliding velocity of cardiac actin-myosin for mixtures of V1 and V2 isoforms with various mixing ratios. The relation we obtained between the sliding velocity and the mixing ratio indicated the existence of mechanical interaction between cardiac myosin isoforms. Furthermore, to elucidate the nature of this interaction, similar measurement was performed on the mixtures of native and chemically modified cardiac myosin. The result suggested that the interaction between cardiac myosins may be a dynamic one that cannot be explained by a simple load effect.

Materials and Methods

Animal Preparation

To obtain V1 isomyosin, hypothyroidism was induced in 12-week-old male Wistar rats by adding 0.7 mg/mL of methimazole (Chugai Pharmaceutical Co, Japan) to drinking water over a period of 12 weeks.6,10 V1 cardiac isoamyosin was obtained from 3-week-old male Wistar rats, whose ventricular myocytes are known to contain only the V1 type of myosin.1,4,16,17

Myosin Preparation

Myosin preparation was performed in an identical manner for both V1 and V2 isoforms. Animals were anesthetized with an intravenous injection of sodium pentobarbital (40 to 50 mg/kg body wt), and the hearts were excised rapidly. All procedures described below were performed at 4°C. The hearts were washed in buffered saline (10 mmol/L sodium phosphate buffer and 0.9% NaCl, pH 7.2). Then, the atria and right ventricle were trimmed. Only the left ventricles were used for the extraction of myosin. Because the rat hearts were small, they were pooled for myosin extraction (10 hearts for 3-week-old rats and 5 hearts for hypothyroid rats). The heart muscle was homogenized in Tris–maleic acid buffer (20 mmol/L Tris [hydroxymethyl] aminomethane–maleic acid and 1 mmol/L EDTA, pH 7.0) and was centrifuged (SCR 20B centrifuge, Hitachi, Japan) at 1000g for 15 minutes. After the supernatant was discarded, the pellet was used for myosin extraction in two different ways. (1) For the in vitro motility assay and the determination of ATPase activity, the pellet was extracted with 3 vol of Guba-Straub solution (0.3 mol/L KCl, 100 mmol/L KH2PO4, 50 mmol/L K2HPO4, 1 mmol/L ATP, 5 μg/mL leupeptin, 5 mmol/L diithiothreitol [DTT], and 1 mmol/L EDTA, pH 6.5) for 10 minutes. After the extract was centrifuged at 11 000g for 15 minutes, the supernatant was collected, and 14 vol of ice-cold distilled water was added to precipitate the myosin. After 2 hours had passed, the myosin was collected by centrifugation at 11 000g for 15 minutes. The myosin was again dissolved in a high-ionic-strength solution (0.6 mol/L KCl, 10 mmol/L Tris-HCl, and 5 mmol/L DTT, pH 7.5), and the trace amount of actin was removed by centrifugation (L8M centrifuge, Beckman Instruments, Inc, Fullerton, Calif) at 12 000g for 2½ hours. The purity of the final myosin solution was confirmed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. Protein concentration was measured according to Lowry et al.18 Very fresh myosin, purified less than 48 hours beforehand, was used for the experiments. (2) For the isoform analysis, myosin was extracted by the method of Martin et al.6

Analysis of Myosin Isoform

We confirmed the homogeneity of each isomyosin solution obtained from 3-week-old (V1) and hypothyroid (V2) rat hearts by pyrophosphate gel electrophoresis, as described by Martin et al.5 Electrophoresis was carried out on 4% polyacrylamide gels at 3°C using an electrophoresis chamber (model GE4, Pharmacia, Uppsala, Sweden). The running buffer contained 20 mmol/L Na2HPO4, 10% (vol/vol) glycerol, 1 mmol/L EDTA, and 0.01% 2-mercaptoethanol, pH 8.8. Approximately 2 to 5 μg of myosin extracts was layered on each gel and run at a constant voltage gradient of 14 V/cm for 21 hours. The gels were fixed and stained with Coomassie brilliant blue R-250 and scanned by a laser densitometer (model LKB 2202, LKB Produkter AB, Sweden) at 600 nm.

Chemically Modified Myosin

Chemical modification of myosin with p-N,N′-phenylendimaleimide (pPDM) was performed as reported by Reisler et al.19 with some modifications. pPDM and ADP were purchased from Sigma Chemical Co, St Louis, Mo. V1 isomyosin in 0.6 mol/L KCl, 2 mmol/L Tris-HCl (pH 8.0), and 1 mmol/L Mg-ADP reacted with an eightfold molar excess of pPDM for 30 minutes at 4°C. The reaction was terminated by the addition of 200-fold molar excess of 2-mercaptoethanol.

Preparation of Myosin Mixture

Two homogeneous isomyosin solutions (V1, 2.5 mg/mL; V2, 2.7 mg/mL) were diluted with high-ionic-strength buffer to 2.0 mg/mL. Myosin preparations (concentration, 2.0 mg/mL) with variable isoform content [mixing ratio, V1/(V1 + V2)] were obtained by mixing these two solutions. V1- and pPDM-treated myosin was also mixed in a similar manner. The protein concentration of these mixtures was 1.5 mg/mL. These myosin preparations were used for the in vitro motility assays and for the determination of ATPase activity.

Determination of ATPase Activity

ATPase activity was determined using the method of Yazaki and Raben20 with some modifications. The reaction was run for 5 minutes at 25°C. The reaction solution contained 0.1 mg/mL myosin, 50 mmol/L Tris, 10 mmol/L CaCl2, and 1.5 mmol/L ATP, pH 7.5. Inorganic phosphate liberated in ATP hydrolysis was determined colorimetrically by the method of Youngburg and Youngburg.21

In Vitro Motility Assay

We used two different types of in vitro motility assay systems: (1) the sliding actin filament assay and (2) the myosin-coated bead assay.

Sliding actin filament assay. We used the method described by Kron and Spudich.22 Briefly, filamentous actin was obtained from an acetone powder of rat cardiac muscle by the method of Spudich and Watt23 and was incubated at 4°C overnight with a molar excess of rhodamine-phalloidin (Molecular Probes, Inc, Eugene, Ore) in a solution containing (mmol/L) KCl, 25; MgCl2, 6; HEPES, 25; and EGTA, 1.
Each myosin sample was diluted with a high-ionic-strength buffer (0.6 mol/L KCl and 50 mmol/L Tris-HCl, pH 7.5) to 0.8 mg/mL, 60 μL of which was applied on a nitrocellulose-coated coverslip (30 mm×30 mm, Matsunami Co., Japan) by a micropipette and covered by another smaller coverslip (18 mm×18 mm). On each edge of the smaller coverslip, 0.1 g of silicon grease (Dow Corning) was applied to create a fluid-filled flow cell (Fig 1). After a 15-minute incubation on ice, 180 μL of bovine serum albumin solution (0.5 mg/mL bovine serum albumin, 30 mmol/L KCl, and 20 mmol/L HEPES, pH 7.5) was applied to the flow cell to wash out unbound myosin and to coat the exposed nitrocellulose. According to Harada et al.24 when the concentration of the applied myosin solution is more than 0.5 mg/mL, the amount of myosin molecule bound to the coverslip is saturated at a density of 3.5 ng/mm², and the surface is expected to be almost maximally packed with a single layer of myosin heads. Next, actin filaments suspended in Mg-ATP solution (25 mmol/L KCl, 6 mmol/L MgCl₂, 25 mmol/L HEPES, 1 mmol/L EGTA, 1% of 2-mercaptoethanol, 4.5 mg/mL glucose, 216 μg/mL glucose oxidase, 36 μg/mL catalase, and 2 mmol/L ATP, pH 7.8) were introduced onto the myosin-coated coverslip. Then, 120 μL of Mg-ATP solution was perfused to wash out unbound actin filaments. Active slidings of fluorescently labeled actin filaments at 30°C were observed using an inverted fluorescence microscope (model TMD-EF2, Nikon, Japan) equipped with a ×100 oil immersion objective lens (numerical aperture, 1.3; Zeiss Neofluor, Germany), a 100-W super-high-pressure mercury lamp, and a rhodamine filter set. The fluorescent image of the filament was displayed on a TV monitor (model C1846-03, Hamamatsu-Photonics, Japan) via a high-sensitive silicon intensifier target camera (model C2400, Hamamatsu-Photonics) and was recorded with a video recorder (model BR-S601M, JVC, Japan) on videotape (model D44A, Sony, Japan). Velocity measurements were performed during a replay of the videotape recording. Each video frame was digitized at a rate of 3 frames per second into a 480×360 pixel array by a video grabber card (Personal Vision, Orange Micro Inc) equipped in a personal computer (Macintosh II fx, Apple). The investigator, using a mouse, located the leading edge of an actin filament in successive snapshots, allowing the computer to calculate the mean velocity of the filament from the movement distance and the elapsed time.

**Myosin-coated bead assay.** We used the method developed by Shimmen and Yano25 in which myosin-coated latex beads were made to slide on actin bundles of internodal cells of an alga, *Nitellopsis obtusa*. Briefly, latex beads (uniform latex particles, 2 μm in diameter, Dow Chemical Co) were suspended in a poly-t-lysine solution (5 mol/L poly-t-lysine [molecular weight, 70 000 to 150 000; Sigma] and 400 mmol/mL KOH) at 4°C for 3 hours and were washed four times by centrifugation in a buffer containing 400 mmol/L KCl and 5 mmol/L HEPES-KOH (pH 7.5). These poly-t-lysine-coated beads were mixed with myosin sample (2.0 mg/mL) in a high-ionic-strength buffer (400 mmol/L KCl and 10 mmol/L imidazole-HCl, pH 7.0). By reducing the ionic strength of the buffer solution, the myosin formed filaments and attached to the bead surface. Unbound myosin was removed by centrifugation, and the myosin-coated beads were suspended in Mg-ATP solution containing (mmol/L) ATP, 1; EGTA, 5; MgCl₂, 6; KOH, 70; PIPES, 30; and sorbitol, 200; pH 7.0.

The internodal cell of a green alga, *Nitellopsis obtusa* was used as an actin donor. This plant consists of long internodal cells connected in series, and each cell has well-organized rows of chloroplasts inside the plasmalemma. Because long straight actin cables are anchored on the inner surface of chloroplast rows, the analysis of the movement is greatly facilitated. In addition to the cell wall and plasmalemma that form the outer shell, there is an inner membrane system called the tonoplast. The tonoplast forms a vacuole in the core of the cell, and cytoplasmic streaming occurs in the space between the tonoplast and the actin cables.

Before each experiment, an internodal cell was isolated, trimmed free of branches, and stored in the artificial pond water (0.1 mmol/L each of KCl, NaCl, and CaCl₂; pH, approximately 5.6). To expose the actin cables, disintegration of the tonoplast was necessary. Both ends of an internodal cell were cut open, and a vacuole was perfused with the Mg-ATP solution. Because this solution contained 5 mmol/L EGTA, a drastic decrease in the calcium concentration in the vacuole resulted in a disintegration of the tonoplast. Although most of the endoplasm dispersed after this procedure, some remained attached to the actin cables and continued to stream. Then the cell was left on a perfusion bench, which was placed in a moist chamber to keep from drying. After 20 minutes, the cell was perfused again with an EDTA solution containing (mmol/L) EDTA, 5; ATP, 1; KOH, 71; PIPES, 30; and sorbitol, 200; pH 7.0. When the magnesium was depleted with the EDTA treatment, the remaining endoplasm containing the alga myosin was inactivated. Finally, the cell was perfused once again with the Mg-ATP solution to displace the inactivated endoplasm. The tonoplast-free cell thus prepared was used for the motility assay.

Myosin-coated beads suspended in the Mg-ATP solution were introduced into the tonoplast-free cell via intracellular perfusion. Both ends of the cell were ligated with polyester threads to avoid the passive movement of intracellular fluid, and the cell was placed on a slide glass in a solution containing (mmol/L) K₂SO₄, 0.1; NaCl, 1; CaCl₂, 0.1; and sorbitol, 150. The
movement of the beads was observed under a photomicroscope (Labophoto-2, Nikon, Japan) and was recorded on videotape (D44A, Sony) with a video camera (KV-24, Hitachi Denshi, Japan) and a videotape recorder (HR D-23, JVC). All experiments were carried out at 21°C to 23°C. The measurement of the velocity was performed during a replay of the videotapes using the same image-processing system previously described. The velocity measurement was performed on a continuum of smooth movement of the bead at least 5 μm long.

Statistical Analysis

In the sliding actin filament assay, the mean velocity for each myosin mixture was calculated from the velocities of 40 to 50 different actin filaments; in the myosin-coated bead assay, the mean velocity was obtained from the velocities of 10 to 20 different beads or bead aggregates. For each myosin solution, ATPase activity measurement was repeated four times. Data were indicated as mean±SD. The correlation between the ATPase activity and the relative content of unmodified V, isomyosin was studied by linear regression analysis. A value of P<.01 was considered to be significant.

Results

Myosin Isoform

Fig 2 shows the pyrophosphate gel electrophoresis of the native myosin from 3-week-old and hypothyroid rat hearts. In 3-week-old rats, left ventricles contained only the V1 type of cardiac myosin. On the other hand, in left ventricles of hypothyroid rats, cardiac myosin was exclusively the V3-type isomosin.

Fig 3 shows SDS–polyacrylamide gel electrophoresis of the purified myosin from 3-week-old rats (V,1) and hypothyroid rat (V3). We can confirm the purity of the myosin solutions that were used for in vitro motility assays and determination of ATPase activity.

The ATPase activity showed a linear relation with the relative content of V, myosin (Fig 4). This result was consistent with the previous findings15 and confirmed that the two isoforms were mixed in correct ratios. The Ca²⁺-activated ATPase activity of V, myosin was 1.24±0.19 μmol/L P1 per milligram per minute. The

![FIG 2. Pyrophosphate gel electrophoresis of the cardiac myosin from 3-week-old (3W) and hypothyroid (Hy) rats. Densitometric scan patterns are presented to the right of each gel.](http://circres.ahajournals.org/)

![FIG 3. Sodium dodecyl sulfate–polyacrylamide gels of purified myosins from 3-week-old (3W) and hypothyroid (Hy) rat hearts. Molecular weight markers are indicated. MHC indicates myosin heavy chain; LC1, myosin light chain 1; and LC2, myosin light chain 2.](http://circres.ahajournals.org/)

![FIG 4. Line graph showing the relation between Ca²⁺- activated ATPase activity and the relative content of V, (or V3) isomyosin. The activities are normalized to the value of the V, myosin (100%) sample. A linear relation was observed (r=.99, P<.01).](http://circres.ahajournals.org/)
difference between isomyosins was approximately two-fold \((V_3/V_1=0.49\pm0.02)\).

**In Vitro Motility Assays**

*Sliding actin filament assay.* Fig 5 shows the movement of an actin filament on a myosin-coated glass surface. Filaments were 1 to 5 \(\mu\)m in length. Each filament moved smoothly and unidirectionally at a constant velocity for each myosin solution. Within a given visual field, more than 90\% of the actin filaments moved. The mean velocity for \(V_1\) myosin (100\%) was \(4.8\pm0.4\ \mu\)m/s, and as the content of \(V_1\) was lowered, the velocity decreased in a sigmoid manner to \(2.4\pm0.3\ \mu\)m/s for \(V_3\) myosin (100\%) (Fig 6, left).

*Myosin-coated bead assay.* Fig 7 shows the electron micrographs of the beads coated with \(V_1\) and \(V_3\) isomyosins used for the in vitro motility assay. The beads were prepared in the same way as when they were used in the motility assay. They were applied to an electron microscope grid and negatively stained with 1\% uranyl acetate. We did not observe any appreciable difference in appearance between beads coated with \(V_1\) isomyosin and those coated with \(V_3\) isomyosin. Similarly, we did not find any difference among beads coated with myosin solutions with various mixing ratios used in the assay (photographs not shown).

When the myosin-coated beads were introduced into the tonoplast-free cell, beads or bead aggregates moved smoothly along the rows of actin bundles at a constant velocity. The nature of the movement was similar to that observed with skeletal muscle myosin,\(^{25-27}\) smooth muscle myosin,\(^{14,27}\) diactosteleum myosin,\(^{27}\) and rabbit cardiac myosin,\(^{28,29}\) except for the sliding velocities, which were proportional to their ATPase activities. The velocity for \(V_1\) myosin (100\%) was \(1.8\pm0.2\ \mu\)m/s, and that for \(V_3\) myosin (100\%) was \(1.0\pm0.1\ \mu\)m/s. The relation between the relative content of \(V_1\) and the velocity was also sigmoid (Fig 6, right), although there was a slight difference in the range of the plateau region from that observed in the sliding actin filament assay.

**Influence of Chemical Modification**

pPDM-treated \(V_1\) myosin was completely inactive both in the in vitro motility assay and in the ATPase activity assay; i.e., there was no movement and no catalytic activity. As the relative content of intact \(V_1\) increased, both sliding velocity and \(Ca^{2+}\)-activated ATPase activity increased in different manners. In Fig 8, the velocity measured in the sliding actin filament assay and the ATPase activity are normalized to the values of the intact \(V_1\) myosin sample and are plotted as a function of the relative content of unmodified \(V_1\) isomyosin. Similar to the case of the \(V_1/V_3\) mixture, a linear relation between the relative content of unmodified \(V_1\) isomyosin and the ATPase activity was observed. On the other hand, although the sliding velocity showed a nonlinear relation with the relative content of unmodified \(V_1\) isomyosin, as in the case of the \(V_1/V_3\) mixture, the shape was convex downward, suggesting an interaction of a different nature.
Discussion

Cardiac myosin shows heterogeneous isoform distribution that changes in response to various physiological and pathophysiological stimuli. Because this redistribution is considered to play an important role in long-term adaptation, its functional significance with cardiac function has often been discussed. A cardiac muscle with a high V sub 1 isomyosin content is known to have a high shortening velocity, which is attributed to the high ATPase activity of this isomyosin. On the other hand, the V sub 3 isomyosin has a low ATPase activity and a low shortening velocity. In this study, the ATPase activity and the actomyosin sliding velocity of V sub 1 isomyosin were approximately two times higher than that of V sub 3 isomyosin. These results are consistent with former studies that showed approximately a twofold difference between V sub 1 and V sub 3 isomyosins for ATPase activity and muscle shortening velocity.

When these two isomyosins coexist, the ATPase activity and the shortening velocity are expected to be proportional to the relative content of the isomyosin. In fact, several investigators have reported rough relations between the isoform distribution and the unloaded maximum shortening velocity of cardiac muscle. The difficulty, however, in controlling the myosin isoform distribution in vivo has hampered

FIG 6. Graphs show the relation between the sliding velocity and the relative content of V sub 1 (or V sub 3) isomyosin. Velocities were measured by the sliding actin filament assay (A) and the myosin-coated bead assay (B). Curves were fit by eye.

FIG 7. Electron micrographs of latex beads coated with V sub 1 (left) and V sub 3 (right) isomyosins. The electron microscope was operated at 75 kV. Bar=1 μm.
the establishment of a precise relation between isoform distribution and shortening velocity. Thus, the functional significance of myosin heterogeneity still remains obscure.30

In this study, using in vitro motility assay systems, we evaluated the relation between the velocity of actomyosin sliding and the biochemical property of cardiac myosin. The results suggested the existence of mechanical interaction between different myosin isoforms.

Nature of Interaction

When a muscle has a mosaic distribution of different myosins, fast-cycling and slow-cycling crossbridges should work coordinately during muscle contraction. Although some interaction seems to exist under such a condition, few studies have reported the functional significance of this heterogeneity. Josephson and Edman,35 using a bundle of frog skeletal muscle fibers with different shortening velocities, showed that heterogeneity modifies the shape of the force-velocity curve. They concluded that, when different fibers contract as one bundle, the performance of a fiber is influenced by the neighboring fibers.

We cannot, however, apply this observation to a discussion of the heterogeneity of cardiac myosin isoforms, because different types of myosins coexist in a single cell, the interaction between different myosins must take place at the molecular level, not at the cellular level. We cannot investigate this type of interaction with tissue or cell preparations.

Recently, in vitro motility assay techniques have been developed in which we can directly observe the actin-myosin sliding at the molecular level. With these techniques, several investigators studied the mechanical interaction under situations in which different types of myosins coexisted. Sellers et al.30 using the myosin-coated bead assay, showed that the sliding velocity has a nonlinear relation with the mixing ratio of phosphorylated and unphosphorylated smooth muscle myosins or with that of skeletal and smooth muscle myosins. Warshaw et al.15 reported a similar observation using the sliding actin filament assay. On the basis of these findings, they suggested the existence of mechanical interactions between different crossbridges. The present study also demonstrated a similar sigmoid relation between the sliding velocity and the mixing ratio, supporting the hypothesis that there exists a mechanical interaction between different cardiac isoforms. However, there still remains a question about the exact nature of myosin interaction at the molecular level.

To answer this question, Warshaw et al.15 proposed a model based on the following assumptions:

a) Cross-bridges act independently. b) The ability of a cycling cross-bridge to generate force and move an actin filament is described by a hyperbolic force versus velocity relationship similar to that observed in the whole-muscle. c) As fast-cycling cross-bridges propel an actin filament, the compression (i.e., negative strain) of attached slow-cycling or weakly-bound cross-bridges results in an internal load that opposes the faster cycling cross-bridges. d) The internal load-velocity relationship is described by extrapolation of the force-velocity curve to the negative force region.15

Using this model, they performed curve fitting to the experimental data with mixtures of different myosins. Their fitting was in good agreement with the actual data, but there was a problem in explaining the sigmoidal relation when they used an experimentally determined parameter set. Although we recognize that their model is useful in explaining the experimental data, a consideration of another factor may improve the theory.

Our data with pPDM-treated myosin provide a suggestion. Treatment of myosin with pPDM in the presence of Mg-ADP results in cross-linking of the two critical thiols of subfragment 1 (SH1 and SH2) and in concomitant trapping of Mg-ADP at the active site.36-38 According to Chalovich et al.39 pPDM-treated myosin binds to actin with an affinity similar to myosin · ATP and myosin · ADP · P, which are categorized as weakly bound states in the kinetic scheme of actomyosin-ATPase.40 Furthermore, pPDM-treated myosin remains in this state without splitting ATP (noncycling). Although this weakly bound and noncycling pPDM-treated myosin is in equilibrium between attached and detached states, the detachment and attachment cycle is so rapid that active myosin cannot find a sufficient load-free period to propel the actin filament. According to the Warshaw model,15 actively cycling crossbridges are required to generate more force against the load.

![Graph showing the sliding velocity of actin-myosin and the Ca²⁺-activated ATPase activity for the mixture of V₁ and p-N,N'-phenylenediaminamide (pPDM)-treated V₁ normalized to the values at unmodified V₁ (100%). A linear relation between the relative content of unmodified V₁ isomyosin and the ATPase activity was observed (r = .99, P < .01). Regarding the sliding velocity, the curve was fit by eye. △ indicates Ca²⁺-activated ATPase activity; □ velocity measured by the sliding actin filament assay.](http://circres.ahajournals.org/content/73/4/702.full)
and to reduce their velocity following the hyperbolic force-velocity relation. Using the mixtures of V₁ and pPDM-treated V₁ myosin, we obtained a hyperbolic relation between the sliding velocity of actin-myosin and the relative content of the intact V₁ myosin (Fig 8), which agreed with this prediction well. Then, why did the experimental data with the mixture of V₁ and V₃ show a sigmoid relation? Recent studies reported that in one ATP hydrolysis cycle the duration during which the myosin head binds the actin filament to generate force (“on-state”) is very short.¹⁴,¹⁵ If these results could be applied to the current situation, in which only a small number of actively cycling V₁ and V₃ crossbridges were working with a single actin filament, the actual time interval during which V₁ and V₃ myosins interact would be very short. When the relative content of V₁ isomyosin is small, V₁ myosin rarely interacts with V₃ myosin on-state, and the sliding velocity of actin-myosin is determined mainly by the property of the V₁ isofrom. On the other hand, when the V₁ myosin coexists with noncycling pPDM-treated myosin, modified V₁ myosin is always in a weakly bound state and exerts a constant internal load on the V₁ myosin, thus reducing the sliding velocity in proportion to its content. In other words, when actively cycling isomyosins coexist, they interact dynamically in contrast to the static interaction with an inactive myosin.

We consider that, in addition to the force-load relation proposed by Warshaw et al.,¹⁵ the dynamic nature of actively cycling (on-off) crossbridges could be the origin of the sigmoid relation between the sliding velocity and the relative content of different myosin isoforms.

Comparison of the Two Types of Motility Assay Systems

There are some differences between the two types of in vitro motility assay systems that we used in this study.⁴³,⁴⁴ First, in the sliding actin filament assay, myosin exists as a monomer, whereas the myosin-coated bead assay requires the presence of myosin filaments. Second, in the sliding actin filament assay, a single actin filament moves over the myosin-coated surface, whereas in the myosin-coated bead assay, myosin filaments interact with many actin filaments of an alga. Umemoto and Sellers⁴⁵ compared these two assay techniques by altering assay conditions such as ionic strength, concentration of calcium, magnesium, and ATP and by adding tropomyosin to the actin. They found no differences between the two assay systems except for the influence of MgCl₂. According to their results, differences in the myosin form or in the type of actin do not seem to be significant factors in determining the properties of actin-myosin sliding. Also, in this study, using two different types of in vitro motility assay systems, we obtained a similar relation between the sliding velocity of actin-myosin and the isoform content. The mechanical interaction between different myosin isoforms, which we proposed in this study, seems to take place regardless of the myosin form or the type of actin.

Energetic Implications

It is known that the V₁ isomyosin has a lower efficiency of energy utilization and a higher ATPase activity relative to the V₃ isomyosin.¹⁰ With a whole-heart preparation, Goto et al.⁴⁵ demonstrated decreased contractile efficiency and increased nonmechanical energy cost in a hyperthyroid rabbit heart. They wholly attributed this change in energy utilization to an increase in the ratio of V₁ to V₃ of the myosin isoform content. However, if the findings in this study could be applied to the in vivo situation, we must also consider another factor, “internal load,” when discussing the energy efficiency of myocardium containing heterogeneous myosin isoforms. When the slow-cycling crossbridges exert a load on the fast-cycling ones, a part of the energy released from the ATP hydrolysis by the fast myosin must be used as a waste to work coordinately with the slow myosin. To elucidate whether or not this is actually the case, further investigation is necessary.

Limitations of the Present Study

In the present study, we studied the active sliding between purified myosin and actin in vitro. However, we must consider several differences between in vitro and in vivo situations. First, in a myofibril of myocardium, myosins form “thick filaments” in regularly spaced sarcomeres. To the contrary, in motility assays in vitro, myosin molecules or myofilaments are arrayed at random. This structural difference may influence the mechanical property of actin-myosin interaction. Second, under the in vivo condition, the actomyosin interaction was shown to be regulated by other factors, such as regulatory proteins.⁴⁶ Furthermore, Winegrad and Weissberg⁴⁷ demonstrated that cAMP inhibits the ATPase activity of the V₁ isomyosin, whereas it increases that of the V₃ isomyosin, via histochemical detection of ATPase activity in a frozen section of the heart tissue. All of these factors must be examined before applying the current results to what is actually happening in the intact organism.

Conclusion

We studied the functional significance of cardiac myosin isoform heterogeneity using in vitro motility assay techniques. The results suggested that in actomyosin sliding different isomyosins mechanically interact when they coexist. Furthermore, the results with chemically modified myosin suggested that the interaction may be a dynamic one that cannot be explained by a simple load effect.

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References

39. Chalovich JM, Greene LE, Eisenberg E. Crosslinked myosin subfragment-1: a stable analogue of the subfragment-1
Dynamic interaction between cardiac myosin isoforms modifies velocity of actomyosin sliding in vitro.
M Sata, S Sugiura, H Yamashita, S Momomura and T Serizawa

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