Molecular mechanics of mouse cardiac myosin isoforms

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Alpert, Norman R., Christine Brosseau, Andrea Federico, Maike Krenz, Jeffrey Robbins, and David M. Warshaw. Molecular mechanics of mouse cardiac myosin isoforms. Am J Physiol Heart Circ Physiol 283: H1446–H1454, 2002.—Two myosin isoforms are expressed in myocardium, α-homodimers (V1) and β-homodimers (V3). V1 exhibits higher velocities and myofibrillar ATPase activities compared with V3. We also observed this for cardiac myosin from normal (V1) and propylthiouracil-treated (V3) mice. Actin velocity in a motility assay (Vactin) over V1 myosin was twice that of V3 as was the myofibrillar ATPase. Myosin's average force (Favg) was similar for V1 and V3. Comparing Vactin and Favg across species for both V1 and V3, our laboratory showed previously (VanBuren P, Harris DE, Alpert NR, and Warshaw DM. Circ Res 77: 439–444, 1995) that mouse V1 has greater Vactin and Favg compared with rabbit V1. Mouse V3 Vactin was twice that of rabbit Vactin. To understand myosin's molecular structure and function, we compared α- and β-cardiac myosin sequences from rodents and rabbits. The rabbit α- and β-cardiac myosin differed by eight and four amino acids, respectively, compared with rodents. These residues are localized to both the motor domain and the rod. These differences in sequence and mechanical performance may be an evolutionary attempt to match a myosin's mechanical behavior to the heart’s power requirements.

contractile proteins; heart; motility assay; molecular motor

THE POWER OUTPUT OF THE HEART is a key measure of ventricular performance, with power output being the rate at which the myocardium can perform work. At the cellular level, power output is a mechanical expression of the myocyte's force-velocity relationship (i.e., power = force × velocity), with the myocyte's ability to generate force and motion largely determined by the mechanoenzyme myosin. The myosin molecular motor interacts with actin to convert the energy from ATP hydrolysis into mechanical work.

Cardiac myosin is a dimeric protein, with each monomeric entity consisting of a myosin heavy chain (with hydrolytic and motor function) and two noncovalently bound light chains (53). Two myosin heavy chain isoforms (α and β) exist in heart muscle, with the α- and β-homodimers referred to as V1 and V3 myosin, respectively (13). The relative proportion of V1 and V3 expression depends on species, age, hormonal balance, and cardiovascular stress (8, 10, 17, 18, 22, 24). Specifically, small mammals such as adult rodents (mouse and rat) predominantly express the V1 isoform in the ventricle, whereas larger mammals (e.g., rabbits and humans) predominantly express the V3 isoform. This species-dependent difference in isoform expression may be an etiologic attempt to match the mechanical performance of the V1 and V3 isoforms to the power requirements of the heart in these various species.

The mechanical properties of cardiac tissue are well correlated to the level of V1 and V3 expression (see Table 1). For example, heart muscles consisting primarily of the V1 isoform have both higher maximum velocities of shortening (5, 19) and calcium-stimulated myofibrillar and actomyosin ATPase activities (31, 32, 46) than those containing primarily the V3 isoform. In contrast, rabbit cardiac muscle expressing the V3 isoform generates greater force-time integrals (12), suggesting that V3 myosin has greater force-generating potential. These isoform-dependent mechanical properties reflect the molecular mechanics of the individual myosin molecular motors. In an in vitro motility assay, which serves as a simplified model system for muscle contraction (49), individual actin filaments in contact with V1 myosin move two to three times faster than those in contact with the V3 isoform, regardless of the mammalian species (7, 36, 46). In addition, our laboratory showed (11, 46) that V3 myosin from rabbit hearts generates twice the average force of V1 myosin in the motility assay. However, Sugiura and coworkers reported (40, 41) that although rat V1 moves actin twice as fast as V3, there is no difference in their average force generation. This apparent discrepancy (Table 1) may be, as suggested above, the result of evolutionary pressure to match cardiac and molecular motor function across species. Therefore, apparent differences in force generation between rodents and larger mammals may be a natural adaptation. To address this question, we studied the molecular mechanics, i.e., the average force (Favg) and actin filament sliding velocity (Vactin) for mouse V1 and V3 myosin.

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Table 1. V₁ and V₃ mechanical properties

<table>
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<th>Species</th>
<th>Velocity</th>
<th>Average Force</th>
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<tr>
<td>Rabbit (11, 47)</td>
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<td>V₁ &lt; V₃</td>
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<tr>
<td>Rat (40, 41)</td>
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Nos. in parentheses indicate references.

The results suggest that in the mouse, the dependence of $V_{actin}$ and $F_{avg}$ on cardiac myosin isoform is similar to that previously observed in the rat (40, 41). We then took advantage of this inherent difference between rodent and rabbit cardiac myosins to begin probing how slight differences in amino acid sequence between these remarkably conserved myosin species relate to the functional performance of the molecular motor. In addition, the specific amino acid differences and their location pinpoint structural domains that are critical to myosin’s mechanics and kinetics.

MATERIALS AND METHODS

**Animal models and myosin preparation.** Mouse (FVB/N, 7–12 wk old, both sexes) were randomly separated into two groups. The nontreated mice had food and water ad libidum, whereas the treated animals had an iodine-deficient diet supplemented with 0.15% propylthiouracil (PTU) in drinking water for 8 wk before the experiment. The mice were treated with heparin (500 IU/kg ip) and then euthanized with CO₂. After thoracotomy, the heart was removed, placed in relaxing solution at 4°C [in mM: 5.37 ATP, 30 phosphocreatine, 5 EGTA, 20 Na₃bis(2-hydroxyethyl)-2-aminoethane sulfonic acid (BES), 7.33 MgCl₂, 0.12 CaCl₂, 10 DTT, and 32 potassium methane sulfonate, with 10 µg/ml leupeptin and 240 U/ml creatine phosphokinase, pH 7.0, ionic strength 175 mM]. Myosin used for isoform identification and motility assay was prepared as previously described (25, 44). The V₁ myosin sequences were from golden hamster (SWISSPROT:MYH6_MESAU; 1,939 amino acids), mouse (SWISSPROT:MYH6_MOUSE; 1,938 amino acids), rat (SWISSPROT:MYH6_RAT; 1,938 amino acids), New Zealand White rabbit (J. Gulick and J. Robbins, unpublished data; 1,939 amino acids), and human (SWISSPROT: MYH6_HUMAN; 1,939 amino acids). With ALIGN, any two sequences can be aligned and the residues at a given sequence location characterized as being identical, a conservative replacement, or a nonconserved substitution. All five α-cardiac myosin sequences (i.e., hamster, mouse, rat, rabbit, and human) were then aligned simultaneously with CLUSTALW algorithms on the San Diego Supercomputer Center Biology Workbench Website (http://workbench.sdsc.edu/).

**Primary sequence comparisons.** Alignment and comparison of all available complete mammalian V₁ (i.e., α-cardiac) amino acid sequences was performed with the ALIGN and CLUSTALW algorithms on the San Diego Supercomputer Center Biology Workbench Website (http://workbench.sdsc.edu/). The V₁ myosin sequences were from golden hamster (SWISSPROT:MYH6_MESAU; 1,939 amino acids), mouse (SWISSPROT:MYH6_MOUSE; 1,938 amino acids), rat (SWISSPROT:MYH6_RAT; 1,938 amino acids), New Zealand White rabbit (J. Gulick and J. Robbins, unpublished data; 1,939 amino acids), and human (SWISSPROT: MYH6_HUMAN; 1,939 amino acids). With ALIGN, any two sequences can be aligned and the residues at a given sequence location characterized as being identical, a conservative replacement, or a nonconserved substitution. All five α-cardiac myosin sequences (i.e., hamster, mouse, rat, rabbit, and human) were then aligned simultaneously with CLUSTALW. With this program, residues were characterized as identical, showing conservation of strong groups, showing conservation of weak groups, or showing no consensus. A similar comparison protocol was performed for the available complete mammalian V₃ (i.e., β-cardiac) myosin sequences from golden hamster (SWISSPROT:MYH7_MESAU; 1,934 amino acids), mouse (TrEMBL:Q9I283; 1,935 amino acids), rat (SWISSPROT:MYH7_RAT; 1,935 amino acids), New Zealand White rabbit (J. Gulick and J. Robbins, unpublished data; 1,935 amino acids), pig (SWISSPROT:MYH7_PIG; 1,935 amino acids), and human (SWISSPROT: MYH7_HUMAN; 1,935 amino acids). All programs were used with default settings for all user-defined parameters.

RESULTS

Myofibrillar ATPase and $V_{actin}$ for V₁ and V₃ mouse myosin. Myosin was isolated from normal and PTU-treated mice. The myosin expression in the ventricle of normal adult mice is 95–99% $V₁$ as estimated by gel electrophoresis (Fig. 1). In contrast, the PTU-treated animals experienced a shift in expression to predominantly the V₃ isoform (80–95%; see Fig. 1). This shift resulted in an approximately twofold reduction in the
myofibrillar ATPase activity at all pCa levels without any affect on the pCa for half-maximal activity (i.e., pCa 6.0; see Fig. 2, Table 2). The velocity of actin filament sliding as assessed in the in vitro motility assay was 5.5 $\pm$ 0.2 and 2.6 $\pm$ 0.4 $\mu$m/s ($P < 0.001$) for the V1 and V3 myosin isoforms, respectively (Table 2). The twofold-increased $V_{actin}$ for the V1 compared with the V3 myosin is similar to the ratio of the V1 versus V3 myofibrillar ATPase activities, suggesting that the myosin’s hydrolytic activity is correlated with its velocity of actin movement (45) as originally proposed by Barany (2) for whole muscle.

$F_{avg}$ for V1 and V3 myosin. $F_{avg}$ was determined with the myosin mixture assay (see MATERIALS AND METHODS). We initially mixed the V1 and V3 isoforms in varying proportions and determined the relationship for $V_{actin}$ as a function of the V1-V3 percentage mixture on the motility surface. $V_{actin}$ was proportional to the V1-V3 mixture (Fig. 3A). Fitting these data to the mixture model resulted in a relatively linear fit (Fig. 3A), with the model fit predicting a V3-to-V1 $F_{avg}$ ratio of 1.2 $\pm$ 0.1 (SE). This is in contrast to the two- to threefold difference we previously observed for rabbit myosin (11, 46).

To further substantiate this finding, we performed mixture assays in which the V1 and V3 myosins were mixed independently with the slower gizzard smooth muscle myosin (Fig. 3, B and C). We chose smooth muscle myosin because of its high $F_{avg}$ relative to all other muscle myosins (11, 47). If in fact smooth muscle myosin generates greater $F_{avg}$ compared with the mouse cardiac isoforms, the mixture assay should result in relationships between $V_{actin}$ and the percentage of V1 or V3 in the presence of smooth muscle myosin having significant curvature, with the curvature being concave downward. This was the case for the V1 versus smooth muscle myosin and V3 versus smooth muscle myosin mixtures (see Fig. 3, B and C). Fitting these mixture data to the model predicts that the smooth muscle myosin generates greater $F_{avg}$ than either mouse cardiac myosin isoform, with the fits to the model plotted as a solid curve in Fig. 3, B and C. The fits gave a smooth muscle-to-V3 $F_{avg}$ ratio of 2.7 $\pm$ 0.4 and a smooth muscle-to-V1 $F_{avg}$ ratio of 3.1 $\pm$ 0.3. On the basis of these two independent mixtures, one can calculate the V3-to-V1 $F_{avg}$ ratio to be 1.1 $\pm$ 0.1. This estimate is in agreement with the estimate obtained from the direct V1-V3 mixture experiment. Thus $F_{avg}$ values for V1 and V3 mouse cardiac myosin are similar, although the $V_{actin}$ values differ by twofold.

DISCUSSION

Marked differences exist in the hydrolytic and mechanical performance of the V1 and V3 cardiac myosin

Fig. 1. Nondenaturing gel of the cardiac myosin isoforms from the left ventricle of wild-type and propylthiouracil (PTU)-treated mice. Equal amounts of ventricular protein (0.5 mg) from control (left) and PTU-treated (right) mice were loaded onto a 5% glycerol gel under denaturing conditions and electrophoresed to separate the V1 and V3 isoforms. After electrophoresis, the gels were stained with silver and band intensities were determined with NIH Image (i.e., 100% V1, 0% V3 for control and 9% V1, 91% V3 for PTU-treated animals).

Fig. 2. Myofibrillar Mg$^{2+}$-ATPase activity graphed as a function of pCa. Ca$^{2+}$-stimulated Mg$^{2+}$-ATPase activity was determined by subtracting the activity at pCa 8 from the activity at all pCa. Myofibrils from PTU-treated mice showed significantly reduced Mg$^{2+}$-ATPase activity compared with myofibrils from control mice (Student’s t-test, $P < 0.05$).
isoforms across multiple mammalian species. In this study, we have determined that mouse cardiac V1 myosin both hydrolyzes MgATP and moves actin filaments in the motility assay approximately two times faster than the V3 isoform. However, in contrast to our earlier studies in the rabbit (11, 46), where the V3 myosin was found to generate twice the Favg of V1 myosin, mouse V1 and V3 myosins are comparable in their average force-generating capacity, as previously observed in the rat (40, 41). It is possible that rodent myosins may have shared similar evolutionary pressure to distinguish them from cardiac myosin obtained from larger mammals such as the rabbit. Can we begin to understand how the molecular mechanics and kinetics of the rodent cardiac myosins are altered to bring about the differences in V1 and V3 mechanical performance both within and across animal species?

**Myosin molecular mechanics.** At the molecular level, Vactin, is defined as Vactin = d/tat, where d is the unitary displacement generated by the myosin power stroke and tat is the attachment time after the power stroke (1, 14). Thus the faster Vactin associated with the V1 isoform can result from an increase in d, a decrease in tat, or a combination of the two. Single-molecule mechanical studies on cardiac myosin isoforms using the laser trap technique revealed that for both rat and rabbit V1 myosin, d was unchanged whereas tat was decreased relative to V3 myosin (26, 40). Although we have not measured d for the mouse V1 and V3 myosins in the present study, we previously determined (44) d to be ~10 nm for mouse V1 myosin, similar to the d for both rabbit V1 and V3 myosins (26). Thus it appears that the kinetics (i.e., tat) rather than the mechanics (i.e., d) of the myosin molecule accounts for the differences in Vactin for the V1 and V3 myosins. In our earlier laser trap studies of the rabbit myosin (26), we were able to relate the decrease in tat for the V1 compared with the V3 myosin to a twofold increase in the rate of MgADP release from the myosin active site with no difference in MgATP binding. The difference in kinetics without a change in the molecular mechanics may be a universal theme across all muscle myosins, because differences in kinetics, specifically differences in the MgADP release rate (38) without differences in d have been determined at the molecular level to account for the range of Vactin values that characterize skeletal, cardiac, and smooth muscle myosins (45).

Although differences in Vactin are uniformly reported for V1 and V3 myosins across all animal species studied to date, this is not the case for their force-generating capacity. Specifically, in this study the mouse cardiac V1 and V3 myosins exhibited similar Favg as previously reported for the rat (40, 41). These results are in contrast to reports from our own laboratory for the rabbit (11, 46), and if data from bovine V1 and V3 mixtures (7) are analyzed by our model, bovine V3 also generates significantly more force than the V1 isoform. In addition, VanBuren and coworkers recently observed greater Favg for human V3 versus V1 myosin (P. VanBuren, personal communication). Thus there appears to be a clear demarcation between rodents and larger mammals.

For the rabbit, we previously determined (46) that the higher Favg for V3 versus V1 myosin could be explained at the molecular level in the following manner: Favg = F × f, where F is the myosin unitary force and f is the duty ratio, or the fraction of the entire cross-bridge cycle time (tcycle) that the myosin is attached to actin and generating force (i.e., f = tat/tcycle) (see Fig. 2 in Ref. 46). Given that F was similar for the rabbit V1 and V3 myosins in the laser trap, we concluded that the duty ratio must be higher for the rabbit V3 myosin compared with the V1 myosin (26). Extending this logic to the mouse data, the lack of any difference between the V1 and V3 Favg estimates suggests that the duty ratio must be the same for the mouse V1 and V3 myosin, assuming that their unitary forces are similar. This in fact has been reported for the rat V1 and V3 myosins (40, 41). Thus, if we assume that the total cycle times under isometric conditions are different by a factor of two, based on the mouse V1 and V3 myofibrillar ATPase measurements (with the caveat that these are unloaded estimates), then the rate-limiting step for detachment under isometric conditions might be coupled to the overall cycle time to maintain a constant duty ratio for the two isoforms (45).

Given our laboratory’s previous studies of cardiac myosin from different mammalian species, it is instructive to compare their Vactin and Favg obtained from mixture assays. To facilitate this comparison, we have plotted the Favg for the various species relative to smooth muscle myosin (see Fig. 4) versus Vactin for the individual cardiac isoforms. With smooth muscle myosin generating greater Favg than either of the V1 and V3 isoforms, a ratio greater than 1 is expected and the higher the ratio, the lower the Favg for the cardiac isoform. After plotting these data (see Fig. 4), it is obvious that there is a significant range in Vactin for the cardiac myosins, with the mouse V1 and V3 myosins being faster than their respective rabbit and human isoforms. In contrast, it appears that all cardiac myosin isoforms generate the same Favg except for the rabbit.
V1 myosin, which generates significantly less F_avg. Although we have not measured F_avg for the bovine and human V1 myosins, based on data in the literature for the bovine species (Ref. 7; see above) and humans (P.

Fig. 4. F_avg for V1 and V3 cardiac compared with gizzard smooth muscle myosin from various species. All relative force measurements were obtained in the motility mixture assay and were determined previously in our laboratory in addition to the data presented here. The data are as follows: *rabbit α- and β-cardiac (11); †human β-cardiac (27). To obtain the smooth-cardiac value it was assumed that the relative F_avg for smooth-skeletal = 2.1 (11) because the mixture was performed as a cardiac-skeletal mixture in this study. ‡Mouse α-cardiac (44); ‡mouse α- and β-cardiac from the present study (see Fig. 3). A relative F_avg > 1 suggests that the smooth muscle myosin generates greater force than the cardiac isoform. Note that the α-rabbit myosin generates the least force given the high smooth-to-cardiac F_avg ratio. In addition, there is a wide range of V_actin for the various cardiac isoforms. The linear regression and its 95% confidence limits were fit to the data excluding the α-rabbit myosin. For the myosins studied in our laboratory to date, all cardiac myosins generate similar F_avg except for the α-rabbit myosin.

VanBurens, personal communication), we assume that the bovine and human V1 isoforms also generate significantly less F_avg than their V3 counterparts. Can we take advantage of this difference in both V_actin and F_avg across both myosin isoforms and species to help characterize the molecular structure and function relationships in these cardiac myosins?

Structural basis for differences in mechanical performance. Because of the high amino acid sequence identity (≈93%) between the V1 and V3 isoforms for many mammals (i.e., hamster, mouse, rabbit, and human) as originally reported for the rat (21), comparison of the
V₁ and V₃ primary sequence should be a choice model system to identify structural domains important to these myosins' functional differences. With only 131 (i.e., 7%) nonidentical amino acids out of a total of 1,938 amino acids, it is then possible to map these differences on the skeletal S₁ crystal structure (34), which is presumably similar to the cardiac myosin structure (Fig. 5). The majority of these amino acids are localized to five discrete regions of the molecule: 1) near the base of the catalytic domain and abutting the essential light chain, residues 32–36; 2) at the mouth and top of the nucleotide binding pocket, residues 210–214 (i.e., loop 1) and residues 349–351; 3) in surface loop 2 spanning the actin binding cleft, residues 619–641; 4) in the neck region or mechanical lever, residues 800–810; and 5) in the S₂ segment, residues 1088–1094. Because these are the only regions of difference between the V₁ and V₃ isoforms, either one or several regions in combination must underlie the hydrolytic and mechanical differences observed. Therefore, it is not surprising that two of these regions of difference are ones that Spudich (39) proposed might tune the ATPase activity and are ones that Spudich (39) proposed might tune the ATPase activity and Fₗᵦ(avg) for the V₁ and V₃ myosins will be linked to the same structural domain within the myosin molecule (45). It will be instructive to make chimeric myosin in which different regions from the β-cardiac isoform are individually or in combination introduced into the α-cardiac myosin backbone. Similar studies have been performed in which either the cardiac nucleotide binding or actin binding loops have been engineered into heterologous myosin backbones such as smooth muscle or Dictyostelium myosins (23, 42). Observed changes must be interpreted with caution given the heterologous nature of the myosin backbone.

Although there is significant sequence homology between the α- and β-cardiac isoforms within a mammalian species, there is even greater homology for similar isoforms across species (4, 29). For example, when comparing all available mammalian α-cardiac myosin sequences through a multiple-sequence alignment (see MATERIALS AND METHODS), the sequences for the hamster, mouse, rat, rabbit, and human are 95% identical, with only 102 amino acids being substituted in one or more of the species. In fact, 86 of these residues are conservative substitutions, with the 16 remaining residues being nonconservative substitutions (see Tables 3 and 4). We then took advantage of the fact that the mouse V₁ myosin has a faster Fₗᵦ(avg) and generates a greater Fₗᵦ(avg) than the rabbit V₁ (see Fig. 4) to help identify which of these 16 nonconservative substitutions may be responsible for the differences in mechanical performance between these two myosins. In fact, only eight nonconservative amino acid substitutions exist between the mouse and rabbit V₁ myosins (see Tables 3 and 4). Five of these differences are in the motor domain and neck region (residues 2, 210, 442, 452, and 801), whereas the remaining three are in the rod (residues 1092, 1637, and 1681).

A similar comparison was performed for the available mammalian β-cardiac myosin sequences, where the sequences for the hamster, mouse, rat, rabbit, pig, and human are 94% identical, with only 25 nonconservative substitutions (see Tables 5 and 6). Many of these

![Fig. 5. Atomic structure of myosin subfragment 1. The structural domains and the location of 7 amino acids within the motor domain and neck (i.e., residues 2, 210, 424, 442, 452, 573, 801) that differ between the mouse and rabbit are identified. Surface loops that span the actin-binding domain (loop 2) and the entrance to the nucleotide-binding pocket (loop 1) are not resolved in the crystal structure, but their approximate locations are shown. The neck or light chain (ELC) and regulatory light chain (RLC) would bind are depicted. The amino acid side chains for residues 424, 442, 452, 573, and 801 are shown in red.](http://ajpheart.physiology.org/)

Table 3. Mammalian α-cardiac myosin motor domain primary sequence comparison

<table>
<thead>
<tr>
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See text for details of comparison.

Table 4. Mammalian α-cardiac myosin rod domain primary sequence comparison

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See text for details of comparison.
The first nonconserved residue at position 2 in the amino terminus for the V1 rodent and rabbit myosins does not appear in any of the crystal structures, and so its relationship to the myosin structure is uncertain and thus it would be premature to speculate as to its importance. In contrast, residue 210 in these V1 myosins exists within the surface loop that spans the nucleotide binding pocket. Both the length and flexibility of this loop have been proposed to modulate the rate at which nucleotide enters and leaves the nucleotide pocket (16, 42) and thus may impact on myosin's enzymatic and mechanical activities. Because these loops are of the same length in the rodent and rabbit V1, it is of interest that a proline at residue 210 in the rodents, which may confer reduced flexibility, is replaced by a valine in the rabbit. If the proline reduces loop flexibility, then MgADP may not exit the active site as rapidly. This might prolong the myosin attachment time relative to the total cycle time and thus increase the duty ratio in the rodent V1 myosins relative to rabbit V1, accounting for the higher \( F_{\text{avg}} \) for the mouse V1. Residues 424, 442, and 452 are in the upper 50-kDa segment of the myosin molecule as part of a long \( \alpha \)-helix (residues 424, 442) and a linker strand (residue 452) that exits this helix. This helix borders the large cleft that separates the upper and lower 50-kDa segments. The extent to which this cleft opens and closes during the actomyosin cycle (9, 48) may play a crucial role in myosin functional performance. Thus having three residues that differ between the rodents and rabbit in this region of the myosin molecule as well as the nearby R453C mutation in FHC patients (52) further highlights the potential importance of this structural domain. The residue 573 substitution between the rodent and rabbit V3 exists in a surface loop that may extend to an adjacent actin monomer that is not the primary actomyosin binding site and thus may serve as a secondary actin binding loop (4). If so, it may then modulate the interaction kinetics between myosin and the actin filament. This could contribute to the twofold difference in \( V_{\text{act}} \) between the mouse and rabbit V3 myosins. Residue 801 in the rodent and rabbit V1 myosins resides within the essential light chain binding domain of the neck, i.e., myosin's mechanical lever (for review see Refs. 45, 51). The importance of this region has also been emphasized by FHC point mutations that exist in this region (30). Specifically, in vitro motility studies on these mutant myosins have demonstrated enhanced \( V_{\text{act}} \). Thus the isoleucine for rodent V1 being replaced by an alanine in the rabbit may account for the faster \( V_{\text{act}} \) in the rodents compared with the rabbit. Finally, the remaining amino acid differences exist within the rod (see Table 4). Although one might assume that differences in the coiled-coil rod segment should have little effect on the performance of the motor domain given their distance from the catalytic site, we are once again reminded

### Table 5. Mammalian \( \beta \)-cardiac myosin motor domain primary sequence comparison

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Solid line boxes, mouse vs. rabbit; dashed line boxes, rabbit vs. human. See text for details of comparison.

Amino acids were previously identified through similar sequence comparisons in the rat, pig, and human (4, 29). Once again, to help identify specific residues that might correlate with differences in myosin mechanical performance, we took advantage of our own data for the various V3 myosins (Fig. 4). Specifically, the mouse V3 myosin has a twofold faster \( V_{\text{act}} \) than the rabbit isoform. Interestingly, these two isoforms differ by only four nonconservative amino acid substitutions (residues 424, 573, 1210, and 1368). In fact, only two of the four nonconservative amino acid substitutions (residues 424, 573, 1210, and 1368). In fact, only two of the four residues may have any functional impact (i.e., 424 and 573), given that residues 1210 and 1368 are identical in the mouse and human V3 isoforms, where a twofold difference in \( V_{\text{act}} \) also exists, as with the rabbit. These amino acid substitutions, identified above, must account for the functional differences between V1 and V3 isoforms across species, and their localization within the molecule may confirm or potentially identify structural elements that are critical to myosin's functional performance. It is reasonable to assume that a few amino acid changes can have profound functional consequences. This fact is readily catalogued in the sarcomeric point mutations found in human familial hypertrophic cardiomyopathy (FHC) that have a profound effect on morbidity and mortality (33).

### Table 6. Mammalian \( \beta \)-cardiac myosin rod domain primary sequence comparison

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Solid line boxes, mouse vs. rabbit; dashed line boxes, rabbit vs. human. See text for details of comparison.
that FHC mutations in this region can have profound effects (33). For example, the L908V has been documented to have altered \( V_{actin} \) compared with myosin from normal patients (6, 27), with the alteration being related to changes in the myosin kinetics as determined in the laser trap assay (27). It is possible that the stability of the coiled coil is critical to proper functioning of the two myosin heads. For example, Lauzon et al. (15) demonstrated that by stabilizing the rod near the head-neck junction by inserting a stable leucine zipper, one observed profound effects on the ability of these expressed myosin mutants to generate a power stroke, the assumption being that some unwinding or breathing of the coiled coil may be required for normal cooperative communication between the two heads (43).

The extent to which one or any combination of these amino acids contributes to the differences in ATPase, \( V_{actin} \), and \( F_{avg} \) between the rodent and rabbit \( V_1 \) and \( V_2 \) myosins is a matter of speculation. A functional consequence of any or all of these differences will only be determined by generating chimeric myosin in either the mouse or rabbit transgenic models, with the highest priorities being given to the four amino acids that are localized to the motor domain and neck (i.e., residues 210, 442, 452, and 801) of the \( \alpha \)-cardiac backbone and the two amino acids (i.e., residues 424 and 573) in the \( \beta \)-cardiac backbone.

We thank Doug Swank for assistance in the sequence comparisons and Peter VanBuren for access to unpublished data and helpful conversations. In addition, we thank Guy Kennedy for instrumentation design and Steve Work for software development that allowed the motility data to be gathered and analyzed. We thank Jeff Moore, Neil Kad, and Josh Baker for critical reading of this manuscript and Neil Kad for rendering the myosin crystal structure.

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REFERENCES


