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. First published June 6, 2002; 10.1152/ajpheart.00274.2002.——Two myosin isoforms are expressed in myocardium, α- and β-homodimers (V 1) and V 3. Myosin's average force generation (Favg) was similar for V 1 and V 3. Comparing V actin and Favg across species for both V 1 and V 3, our laboratory showed previously (VanBuren P, Harris DE, Alpert NR, and Warshaw DM. Circ Res 77: 439–444, 1995) that mouse V 1 has greater V actin and Favg compared with rabbit V 3. Mouse V 1 myosin was twice that of V 3 as was the myofibrillar ATPase. Myosin's average force generation was similar for V 1 and V 3. In contrast, rabbit myosin is greater than in the various species. This species-dependent difference in isoform expression may be an attempt to match the mechanical performance of the V 1 and V 3 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 V 1 and V 3 expression (see Table 1). For example, heart muscles consisting primarily of the V 1 isoform have both higher maximum velocities of shortening and calcium-stimulated myofibrillar and actomyosin ATPase activities than those containing primarily the V 3 isoform. In contrast, rabbit cardiac muscle expressing the V 3 isoform generates greater force-time integrals, suggesting that V 3 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, individual actin filaments in contact with V 1 myosin move two to three times faster than those in contact with the V 3 isoform, regardless of the mammalian species. In addition, our laboratory showed (11, 46) that V 3 myosin from rabbit hearts generates twice the average force of V 1 myosin in the motility assay. However, Sugiuira and coworkers reported (40, 41) that although rat V 1 moves actin twice as fast as V 3, 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 sliding velocity (Vactin) for mouse V 1 and V 3 myosin.
MATERIALS AND METHODS

**Table 1. V₁ and V₃ mechanical properties**

<table>
<thead>
<tr>
<th>Species</th>
<th>Velocity</th>
<th>Average Force</th>
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<tbody>
<tr>
<td>Rabbit (11, 47)</td>
<td>V₁ &gt; V₃</td>
<td>V₁ &lt; V₃</td>
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<tr>
<td>Rat (40, 41)</td>
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</table>

Nos. in parentheses indicate references.

The results suggest that in the mouse, the dependence of \( V_{\text{act}} \) 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.** Mice (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 buffer with 1 mM MgATP; 5 mM MgCl₂, 10 DTT with oxygen scavengers, pH 7.4); and 32 potassium methane sulfonate, with 10 μM leupeptin and 240 μM creatine phosphokinase, pCa 8.0, 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 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/). 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 sequence 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:Q91Z83; 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 \( V_{\text{act}} \) 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
We initially mixed the \( V_1 \) and \( V_3 \) isoforms in varying proportions and determined the relationship for \( V_{\text{actin}} \) as a function of the \( V_1\text{-}V_3 \) percentage mixture on the motility surface. \( V_{\text{actin}} \) was proportional to the \( V_1\text{-}V_3 \) mixture (Fig. 3A). Fitting these data to the mixture model resulted in a relatively linear fit (Fig. 3A), with the model fit predicting a \( V_3\text{-}V_1 \) \( F_{\text{avg}} \) 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 \( V_1 \) and \( V_3 \) 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 \( F_{\text{avg}} \) relative to all other muscle myosins (11, 47). If in fact smooth muscle myosin generates greater \( F_{\text{avg}} \) compared with the mouse cardiac isoforms, the mixture assay should result in relationships between \( V_{\text{actin}} \) and the percentage of \( V_1 \) or \( V_3 \) in the presence of smooth muscle myosin having significant curvature, with the curvature being concave downward. This was the case for the \( V_1 \) versus smooth muscle myosin and \( V_3 \) 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 \( F_{\text{avg}} \) 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-\( V_3 \) \( F_{\text{avg}} \) ratio of 2.7 \( \pm \) 0.4 and a smooth muscle-to-\( V_1 \) \( F_{\text{avg}} \) ratio of 3.1 \( \pm \) 0.3. On the basis of these two independent mixtures, one can calculate the \( V_3\text{-}V_1 \) \( F_{\text{avg}} \) ratio to be 1.1 \( \pm \) 0.1. This estimate is in agreement with the estimate obtained from the direct \( V_1\text{-}V_3 \) mixture experiment. Thus \( F_{\text{avg}} \) values for \( V_1 \) and \( V_3 \) mouse cardiac myosin are similar, although the \( V_{\text{actin}} \) values differ by twofold.

**DISCUSSION**

Marked differences exist in the hydrolytic and mechanical performance of the \( V_1 \) and \( V_3 \) cardiac myosin...
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 $F_{\text{avg}}$ 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, $V_{\text{actin}}$ is defined as $V_{\text{actin}} = d/t_{\text{on}}$, where $d$ is the unitary displacement generated by the myosin power stroke and $t_{\text{on}}$ is the attachment time after the power stroke (1, 14). Thus the faster $V_{\text{actin}}$ associated with the V1 isoform can result from an increase in $d$, a decrease in $t_{\text{on}}$, 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 $t_{\text{on}}$ 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 $\sim 10$ 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., $t_{\text{on}}$) rather than the mechanics (i.e., $d$) of the myosin molecule accounts for the differences in $V_{\text{actin}}$ 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 $t_{\text{on}}$ for the V3 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 $V_{\text{actin}}$ values that characterize skeletal, cardiac, and smooth muscle myosins (45).

Although differences in $V_{\text{actin}}$ are uniformly reported for V1 and V3 myosin 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 $F_{\text{avg}}$ 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 $F_{\text{avg}}$ 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 $F_{\text{avg}}$ for V3 versus V1 myosin could be explained at the molecular level in the following manner: $F_{\text{avg}} = F \times f$, where $F$ is the myosin unitary force and $f$ is the duty ratio, or the fraction of the entire cross-bridge cycle time ($t_{\text{cycle}}$) that the myosin is attached to actin and generating force (i.e., $f = t_{\text{on}}/t_{\text{cycle}}$) (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 $F_{\text{avg}}$ 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 $V_{\text{actin}}$ and $F_{\text{avg}}$ obtained from mixture assays. To facilitate this comparison, we have plotted the $F_{\text{avg}}$ for the various species relative to smooth muscle myosin (see Fig. 4) versus $V_{\text{actin}}$ for the individual cardiac isoforms. With smooth muscle myosin generating greater $F_{\text{avg}}$ than either of the V1 and V3 isoforms, a ratio greater than 1 is expected and the higher the ratio, the lower the $F_{\text{avg}}$ for the cardiac isoform. After plotting these data (see Fig. 4), it is obvious that there is a significant range in $V_{\text{actin}}$ 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 $F_{\text{avg}}$ except for the rabbit

### Table 2. Myosin isoform hydrolytic and mechanical performance

<table>
<thead>
<tr>
<th>Mice</th>
<th>Myosin Isoform</th>
<th>Myofibrillar ATPase at pCa 5.0, nM P_i min^-1 mg protein^-1</th>
<th>$V_{\text{actin}}$ μm/s</th>
<th>$F_{\text{avg}}$ (normalized to V1)</th>
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<tbody>
<tr>
<td>Wild type</td>
<td>V1</td>
<td>207.1 ± 14.1</td>
<td>5.5 ± 0.2</td>
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<tr>
<td>PTU treated</td>
<td>V1</td>
<td>94.3 ± 9.7</td>
<td>2.6 ± 0.4*</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. $V_{\text{actin}}$, actin filament sliding velocity; $F_{\text{avg}}$, relative average force; PTU, propylthiouracil. $F_{\text{avg}}$ value was obtained from the V1–V3 mixture experiment. *$P < 0.01$. 

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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. VanBuren, 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 Vactin 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 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 Vactin 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.

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**Fig. 3**. In vitro motility mixture assay for estimating the relative average force ($F_{avg}$) for mouse cardiac V1 and V3 myosin. A: relationship between actin filament sliding velocity (Vactin) and % of mouse cardiac V1 and V3 within the myosin mixture that was applied to the motility surface. Solid line is the best fit to the mixtures model (11) and is linear, suggesting that the 2 myosins are of equal force-generating capacity. The fit resulted in a relative V1-to-V3 $F_{avg}$ ratio of 1.2 ± 0.1. B: relationship between Vactin and % of mouse cardiac V1 and gizzard smooth muscle myosin within the myosin mixture. The fit resulted in a smooth muscle-to-V1 $F_{avg}$ ratio of 2.5 ± 0.2. Note that the model fit is curvilinear and concave down, suggesting that smooth muscle myosin generates greater $F_{avg}$ than the mouse V1 myosin.

**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 Vactin 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.
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 functional differences. With only 131 amino acid side chains for residues 424, 442, 452, 573, and 801 are shown in red.

![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, and 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 domain (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.org/)

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

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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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Table 5. *Mammalian β-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_{act} than the rabbit isofrom. 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), which may confer reduced flexibility, are 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 the rabbit V1, accounting for the higher F_{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 α-helix (residues 424, 442, and 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 these 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_{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_{act} in the rabbit. Thus the isoleucine for rodent V1 being replaced by an alanine in the rabbit may account for the faster V_{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 of the importance of these differences.

The first nonconserved residue at position 2 at 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 the rabbit V1, accounting for the higher F_{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 α-helix (residues 424, 442, and 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 these 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_{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_{act} in the rabbit. Thus the isoleucine for rodent V1 being replaced by an alanine in the rabbit may account for the faster V_{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 of the importance of these differences.

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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 V1 and V2 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 α-cardiac backbone and the two amino acids (i.e., residues 424 and 573) in the β-cardiac backbone.

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REFERENCES


