Effects of ATP and Actin-Filament Binding on the Dynamics of the Myosin II S1 Domain

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ABSTRACT Actin and myosin interact with one another to perform a variety of cellular functions. Central to understanding the processive motion of myosin on actin is the characterization of the individual states along the mechanochemical cycle. We present an all-atom molecular dynamics simulation of the myosin II S1 domain in the rigor state interacting with an actin filament. We also study actin-free myosin in both rigor and post-rigor conformations. Using all-atom level and coarse-grained analysis methods, we investigate the effects of myosin binding on actin, and of actin binding on myosin. In particular, we determine the domains of actin and myosin that interact strongly with one another at the actomyosin interface using a highly coarse-grained level of resolution, and we identify a number of salt bridges and hydrogen bonds at the interface of myosin and actin. Applying coarse-grained analysis, we identify differences in myosin states dependent on actin-binding, or ATP binding. Our simulations also indicate that the actin propeller twist-angle and nucleotide cleft-angles are influenced by myosin at the actomyosin interface. The torsional rigidity of the myosin-bound filament is also calculated, and is found to be increased compared to previous simulations of the free filament.

INTRODUCTION

Myosin is a motor protein that is involved in a variety of cellular functions, including contraction of muscle cells and the transport of cargo along actin filaments (1–3). A great deal of understanding about myosin has been obtained via both experimental and computational methods, and there are extensive reviews on myosin and its interactions with actin in the literature (1–8). There are many different types of myosin, because the protein belongs to a diverse family of proteins (3). In particular, myosin II, also known as conventional or muscle myosin, has been very well characterized, including a structure of the actomyosin system in the rigor state (ATP-free state of myosin) which was determined using cryo-electron microscopy (cryo-EM) (9). More recently, a structure of myosin and tropomyosin on actin was solved using cryo-EM in combination with docking/ flexible fitting of crystal structures into the map (10). To perform its variety of functions, myosin interacts with actin filaments in a mechanochemical cycle (1,4,5) (referred to as the Lymn-Taylor cycle (11)).

Computer simulation has offered valuable insight into the functioning of isolated myosin protein (not including actin) in a variety of its nucleotide states (using both all-atom and simplified representations of myosin) (12–18). For example, the process of ATP hydrolysis in myosin has been investigated using QM/MM methods (19–22), and elastic network models at an α-carbon level of resolution of myosin II and myosin V bound to actin have been studied to elucidate how actin binding can lead to phenomena such as phosphate release in myosin, and other protein conformational changes (23,24). However, all-atom simulation of myosin on a fully periodic actin filament, including both the myosin lever arm and light chains, has not yet been performed. Previous simulation work on actin interacting with myosin at the all-atom level has been limited to small systems (25,26). For example, the presence of an important electrostatic interaction between actin and myosin was established over the course of 15-ns simulations in a study of myosin interacting with an actin trimer (25), and simulation of myosin interacting with an actin dimer in an implicit solvent was used to study interactions at the actin/myosin interface (26). Our all-atom molecular dynamics (MD) simulations are, to our knowledge, the first of their kind for the myosin S1 domain, including the lever-arm binding light chains, interacting with a fully periodic actin filament. By including a fully periodic actin filament we are able to improve upon these previous simulations by ensuring that each actin monomer has appropriate contacts within the filament environment, and also gain the new capability to calculate filament-level properties such as the torsional rigidity of the actin filament from all-atom and coarse-grained (CG) analysis, which cannot be calculated from actin dimer and trimer systems.

In this study we simulate rigor actomyosin, consisting of a periodic actin filament (built from an actin 13-mer) and the myosin II S1 domain from squid muscle (27). The S1 domain of myosin consists of the motor domain (where actin-binding and ATP hydrolysis occur), and the lever arm (7). In addition to our simulation of rigor actomyosin, we simulate myosin in the rigor state in the absence of actin, as well as myosin in the post-rigor state (the state with ATP present in the myosin nucleotide cleft). All-atom simulations of the actomyosin filament can shed light on the presence of stable interactions between actin and myosin.
at the interface of the two proteins. All-atom MD in explicit solvent has an advantage in that it can be used to gain information about the dynamical interactions between actin and myosin—information that, necessarily, cannot be provided by static structures. By making comparisons of the simulations of the myosin rigor state bound to actin to the rigor state free of actin and the post-rigor state, we can also understand the influence of actin binding (and ATP binding) on myosin dynamics. Furthermore, because extensive simulations of the free actin filament have been previously carried out (28–32), comparisons can be made to our simulation of actomyosin to assist understanding of how myosin modulates actin filament dynamics. For example, it has been demonstrated experimentally that myosin increases the torsional rigidity of actin filaments (33–35). This can have important functional consequences for actomyosin systems. It has been suggested that actin’s ability to act as a torsional spring could help to strain the flexible connection of the myosin motor to the thick filament (36), and that a larger torsional rigidity might aid in establishing cooperativity between actin monomers in the filament (34).

In addition to all-atom level analysis of interactions between actin and myosin, we also make use of coarse-grained (CG) methods to analyze the dynamics and interactions in the actomyosin system and the myosin rigor and post-rigor states. For large proteins and protein complexes, CG representation and analysis allows one to reduce an all-atom system to a smaller number of domains and interactions (37). In this study, we used the essential dynamics coarse-graining (ED-CG) (38) and the heterogeneous elastic network model (hENM) (39) methods to perform a CG analysis. These methods have previously been applied by our group to assist understanding of the nature of heterogeneity in myosin-free actin filaments (29), interactions in the large ribosomal subunit (40), and NBAR domain proteins (41). ED-CG allows us to investigate how the definitions of dynamic domains in myosin and actin are influenced by their interactions with one another in the actomyosin rigor state. In contrast to other approaches for coarse-graining proteins, which may use an α-carbon level of resolution as in the Gō model (42) or a few residues per CG site as in the MARTINI model (43,44), methods like ED-CG can be used to group amino acids into CG sites at a highly coarse-grained level of resolution where tens of amino acids are assigned to each CG domain (29,40,41). Specifically, the ED-CG method demonstrates that the N-terminal region of the myosin-bound actin monomer is influenced by myosin binding, and that the various squid muscle myosin states have relatively similar regions of collective motion along the myosin primary sequence. Furthermore, the hENM method allows the determination of the regions of myosin that most strongly interact with actin, and reveals differences between domain couplings in myosin dependent on whether it is actin-bound or has ATP in its nucleotide cleft. For example, we find that the two domains comprising the actin-binding cleft of myosin have a larger effective coupling in the bound-rigor compared to the free-rigor and post-rigor states, and that switch I and the P-loop are much more strongly coupled in the post-rigor state than in either the bound-rigor or free-rigor states. This provides further evidence for the reciprocal coupling of the actin binding cleft and the nucleotide clefts of myosin. We also observe, by calculating the actin propeller twist and nucleotide cleft angles, that myosin has an influence on both of these properties at the monomer with the largest myosin interface. From our simulations, we also create hENM-EDCG models of the actomyosin system that can reproduce the torsional rigidity, which we calculate from the all-atom myosin-bound actin filament. The values that we obtain are consistent with the observation that the torsional rigidity of actin filaments bound by myosin S1 domain in the rigor state is increased in comparison to the free filament (33–35).

METHODS

All-atom MD simulations

All-atom MD simulations were run using the software NAMD (45) with the CHARMM27 force field (46), including CMAP corrections. For myosin, we used the myosin II S1 domain (including the lever-arm and lever-arm binding light chains) rigor (PDB:315G) and post-rigor (PDB:315F) states from squid muscle (27). Missing loop regions were modeled using the software MODELER (47) and the Falc-Loop server (48,49). The structure for the actin 13-mer filament was built the same way as has been previously discussed in the literature (30–32), with the monomer structure based on the Oda structure (PDB:2ZWH) (50). The starting structure for the filament includes Mg2+ and ADP in the nucleotide cleft, as well as active site waters coordinating the Mg2+ ion (30). Periodic boundary conditions were enforced so that the actin filament is continuous across the cell boundaries, leading to a filament of infinite length, as in previous simulations of the periodic actin filament (30–32).

To build the actomyosin complex, the molecular-dynamics flexible-fitting (MDFD) refined actomyosin structure (51) was used as a template for positioning of the squid muscle myosin II rigor state (27) onto the actin 13-mer. The middle five monomers of the 13-mer filament were aligned with the five monomers from the MDFD model by root mean-square deviation (RMSD) fitting the backbone α-carbons (51). The squid rigor state was then aligned to the myosin II protein from the aligned MDFD structure using the built-in MULTISEQ tool (52) in the program VMD (53) by using the STAMP structural alignment algorithm (54) on the residues in the myosin II motor domain. The final system is shown in Fig. 1. Systems were solvated with sufficient water to provide padding between the protein and its periodic image (15 Å of padding for isolated myosin states along each box direction, and 8 Å of padding for the filament system perpendicular to the filament axis, with no water caps along the filament axis, as in previous work). All residues were modeled in their standard protonation states at a pH of 7. In addition, the N-terminus of the actin monomers was acetylated. Neutralization with 0.180 M KCl was performed (with number of K/Cl ions equal to 238/220, 297/277, and 1378/1178 for free-rigor, post-rigor, and bound-rigor, respectively). Full solvation and neutralization of the system were accomplished with the VMD SOLVATE and AUTOIONIZE plug-ins (55), respectively.

Systems were energy-minimized with constraints released in a stepwise fashion. For the isolated myosin II proteins a long, restrained equilibration phase was performed, with harmonic restraints gradually released on the protein backbone. To not disturb the initial configuration of the magnesium and crystal waters used in building the filament, the actomyosin system was
gradually heated while constraining the Mg$^{2+}$ ion and coordinated waters before a long restrained equilibration phase. Unrestrained dynamics were then run for all systems for 100 ns. Numerical calculations of the electrostatic interactions were performed using the particle-mesh Ewald method (55), employing a 12 Å distance cutoff. Dynamics were run using a 2-fs timestep, which required constraining all hydrogen bonds in the simulation. Production simulations were run in the constant NPT ensemble, with the pressure maintained at 1 atm using the Langevin piston method in the software NAMD (45), and the system temperature was maintained at 310 K using the Langevin thermostat with the damping coefficient set to 5 ps$^{-1}$. Data used for ED-CG and hENM analysis consisted of the portion of the simulations after the RMSD for the $\alpha$-carbons in the myosin II motor domain (for free myosin simulations) and the RMSD for the actomyosin system (motor domain and 13-mer filament) was stabilized (which occurred for the last 20 ns of each simulation, respectively; see Fig. S1 in the Supporting Material). We used the convergence of the RMSD as an indication that the simulated structures had equilibrated. The myosin states will generally be referred to as “free-rigor” (rigor myosin not bound to actin), “bound-rigor” (rigor myosin bound to actin), and “post-rigor” (the myosin post-rigor state).

Coarse-grained analysis of MD simulations

The bound-rigor, free-rigor, and post-rigor simulations were analyzed at a coarse-grained (CG) level of detail using the ED-CG method to place CG sites (38) and the hENM method to measure effective interactions between CG sites (39,40). Further detail on these methods can be found in the Supporting Material.

RESULTS AND DISCUSSION

Electrostatic interactions are important for actomyosin binding

The actin-binding interface of myosin is highly populated with amino acids possessing charged side chains (56). Electrostatic interactions between actin and myosin have been suggested based on cryo-EM structural models of actomyosin (9,56,57) as well as recent hydroxyl radical footprinting experiments (58). Predictions of such interactions at the interface between chicken skeletal myosin II and actin have been made based on an MDFF model of actomyosin (51). A specific electrostatic interaction between the actin N-terminus and a region on myosin referred to as the “activation loop” has been previously observed based on the results of protein-protein docking and MD simulation refinement of the docked structures for Dictyostelium myosin II (25). The importance of this interaction for efficient muscle contraction was confirmed experimentally in the same study (25).

In Table S3 in the Supporting Material we show a list of the initial contacts between actin and myosin, and contacts present near the end of the bound-rigor simulation. Most contacts are with the U50, L50, and loop 2 domains of myosin. The very flexible (and highly positively charged) loop 2 develops several contacts with actin over the course of the simulation (see Table S3). In our MD simulation of actomyosin, we observe a number of salt-bridge interactions at the actomyosin interface that form during the course of the simulation and are not present in the initial structure. In particular, residues in loop 2 and the lower 50 kDa (L50) domain of myosin participate in salt-bridge interactions with the N-terminus of actin monomer m7 (Fig. 2). These interactions lead to at least one salt bridge to form nearly continuously with the actin m7 N-terminus. At late times in the simulation, we observed the formation of a salt bridge between D1 of actin monomer m7 with K528 on myosin (Fig. 2 and Fig. 3 a). K528 in squid myosin II

![FIGURE 1 The actomyosin system, comprised of a 13-mer actin filament and the myosin S1 domain, in the rigor state, along with the two lever-arm binding light chains. Actin monomers are labeled m1–m13. To see this figure in color, go online.](image1)

![FIGURE 2 (a) Number of salt bridges between the N-terminal cluster of residues in actin monomer m7 and residues of myosin II S1. (b) Residues involved in salt-bridging interactions with the N-terminus of monomer m7 during the 100-ns actomyosin simulation. Myosin (dark blue), and actin monomer m7 (dark pink) are shown. The residues shown are the four negatively charged N-terminal residues of actin monomer m7 (yellow), R486 in the myosin relay helix (orange), lysine residues 635, 636, 638, and 640 in loop 2 of myosin (red), residue K528 of the myosin activation loop (pink), and lysine residues 652, 657, and 660 located in the L50 domain of myosin (cyan). To see this figure in color, go online.](image2)
is homologous to R520 in *Dictyostelium* myosin (the activation-loop residue).

In another study, the salt bridge between R520 and the N-terminal cluster was formed from the beginning of the protein-protein docking phase (25). Our observation that this salt bridge forms over the course of 100-ns MD simulations suggests this interaction is indeed important for the definition of the actomyosin interface, and not specific to a single isoform of myosin II. The initial distance between any of the N-terminal actin residues and the activation-loop residue K528 is 20–30 Å, falling quickly below 20 Å within the next 20 ns, and during the last 20 ns maintaining distances of <15 Å (including the close approach within our salt bridge cutoff of 3.2 Å by D1 in actin near the end of the simulation; Fig. 3 a). Similar to a recent study in which the effect observed upon mutation of R520 in *Dictyostelium discoideum* myosin II (25), we would expect, based on our simulations, that mutation of K528 to a neutral or negatively charged amino acid in squid muscle myosin could influence the efficiency of myosin.

We also observe particularly stable salt-bridge interactions between actin monomer m5 (Fig. 3, b and d) and a region of myosin referred to as “loop 3” (squid myosin II residues 567–580). This loop has been previously hypothesized to interact with actin (51,56). It has also been suggested that myosin II has interactions with the actin W-loop (residues 165–172) via a salt-bridge interaction between actin residue E167 and myosin residue K544 (51). Interaction between these residues was observed experimentally for rabbit skeletal muscle myosin (58). We observe a salt-bridge interaction (and hydrogen-bonding interaction, Table 1) between myosin and the W-loop in our simulation, as well as between another upper 50 kDa (U50) domain residue and another SD3 residue in actin (Fig. 3, c and d).

Because the myosin salt bridge to the W-loop was predicted for the chicken skeletal myosin II (51) and observed experimentally for rabbit skeletal muscle (58), that we also observe this for squid muscle myosin suggests that this interaction between myosin and the W-loop is important for the various myosin isoforms in defining the actomyosin interface. To confirm the presence of this interaction between squid muscle myosin and actin (which would help to demonstrate that the actin/myosin interface has many common features across different myosin isoforms), cysteine-mutant-plus-fluorescence-labeling experiments similar to those performed for the rabbit skeletal myosin (58) could be carried out.

Several regions of myosin participate in hydrogen bonding with actin monomers m5 and m7 (Table 1). The hydrogen bonds listed in Table 1 were formed over the course of the simulation. A long-lived hydrogen bond is formed between the side chain of E538 in the L50 domain of myosin and the side chain of T351 in the C-terminus of actin (Table 1). This is the most stable hydrogen bond observed, having an occupancy of 75.4% over the last 20 ns of simulation. Likewise, in the cardiomyopathy (CM) loop—a region of myosin in which mutation has been related to the condition familial hypertrophic CM (59,60)—it has been proposed that the actin residues P332–E334 could interact with myosin CM loop residues (56). Supporting the idea that these regions interact in the actomyosin complex, we observe a hydrogen bond between

<table>
<thead>
<tr>
<th>Myosin residue</th>
<th>Actin residue</th>
<th>Occupancy percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>E538 (side chain, L50)</td>
<td>T351 (side chain, C-terminus)</td>
<td>75.4</td>
</tr>
<tr>
<td>E538 (side chain, L50)</td>
<td>T351 (main chain, C-terminus)</td>
<td>50.4</td>
</tr>
<tr>
<td>K569 (side chain, L50)</td>
<td>E99 (side chain, SD1)</td>
<td>43.4</td>
</tr>
<tr>
<td>Y412 (side chain, CM-loop)</td>
<td>E334 (side chain, SD1)</td>
<td>39.4</td>
</tr>
<tr>
<td>K572 (side chain, L50)</td>
<td>E100 (side chain, SD1)</td>
<td>38.3</td>
</tr>
<tr>
<td>K635 (main chain, Loop 2)</td>
<td>E4 (side chain, N-terminus)</td>
<td>37.9</td>
</tr>
<tr>
<td>E373 (side chain, U50)</td>
<td>K328 (side chain, SD3)</td>
<td>36.4</td>
</tr>
<tr>
<td>K636 (side chain, Loop 2)</td>
<td>E100 (side chain, SD1)</td>
<td>30.9</td>
</tr>
<tr>
<td>K569 (side chain, L50)</td>
<td>E100 (side chain, SD1)</td>
<td>30.7</td>
</tr>
<tr>
<td>K544 (side chain, L50)</td>
<td>E167 (side chain, SD3)</td>
<td>28.0</td>
</tr>
<tr>
<td>K636 (main chain, Loop 2)</td>
<td>E4 (side chain, N-terminus)</td>
<td>26.6</td>
</tr>
</tbody>
</table>
Y412 in myosin with E334 in actin monomer m7, which is located on the edge of actin SD1 and SD3. This hydrogen bond has an occupancy of 39.4% over the last 20 ns of the 100-ns simulation (Table 1).

**Binding of myosin to actin affects the motions of the N-terminus of actin relative to the rest of the actin molecule, but does not significantly affect the collective regions in myosin**

ED-CG analysis was carried out on the myosin bound-rigor, free-rigor and post-rigor states, as well as on the actin monomers to assign collective domains of motion. The collectively moving domains can, in principle, be influenced by a protein’s environment (i.e., presence of other binding proteins), or the presence of small ligands. For example, collective domain assignment could be different for actin monomers in the actomyosin system that are close to the myosin binding site, versus those that are farther away. We use the ED-CG method to compare the dynamic domains between the actin monomers in the actomyosin filament and between different myosin states (free-rigor, bound-rigor, and post-rigor), and how the definitions change as the number of CG beads increases. Interactions between CG domains will be addressed in the next section, based on the results of the hENM analysis, and ultimately hENM-ED-CG models of actomyosin will be simulated to determine their effectiveness at describing the torsional rigidity of a myosin-bound actin filament.

The most distinct difference between the actin monomers that interact with myosin in the filament (monomers m5 and m7) and the rest of the actin monomers was observed in the N-terminus of monomer m7. Actin monomer m7 (Fig. 1), the monomer with the largest binding interface with myosin, behaves differently at its N-terminus than other monomers in the filament (Fig. 4). Specifically, the actin N-terminus is placed into a separate CG site in each of the actin monomers, except for monomer m7 (Fig. 4). In m7, at the six-site level of CG resolution, the first 52 amino acids are assigned to a single CG site, whereas for the other monomers the first 4–6 amino acids are placed into the first CG site. This trend continues at the eight-site level of CG resolution, with monomer m7 having the first 33 amino acids grouped together, unlike the other actin monomers for which the first few N-terminal residues belong to the first site. At the 12-site level of CG resolution, the N-terminus is identified as a separate CG site in each of the actin monomers, including m7 (Fig. 4). By the 12-site level of resolution, the ED-CG sites are placed in a more homogeneous fashion across the actin monomers (Fig. 4 d). It should be noted that although the N-terminus may be identified as a separate site in each monomer, this does not mean that the N-terminus moves in the same way in each monomer. It only means that at a given level of CG-resolution, the optimal way to minimize the ED-CG residual is to group the N-terminal residues into a CG domain. The increased collectivity of the N-terminus with other regions of actin in monomer m7 is likely influenced by the salt bridges and hydrogen bonds with the myosin loop 2 region and the activation loop, as was previously noted in our discussion of all-atom data. The grouping of the N-terminus of actin monomer m7 into a combined CG site with other actin residues at lower levels of CG resolution demonstrates that the m7 N-terminus is less flexible due to the interactions it has with the myosin S1 domain compared to the other actin monomers. However, although the N-terminus of actin monomer m7 has increased collectivity in its motion with other residues in the N-terminal region of actin and its flexibility is reduced compared to the other actin N-termini, these four residues still retain some mobility, which we demonstrate by calculating the average RMSD of the N-terminus and its solvent accessibility are reduced in the strong myosin binding state of actomyosin, while still remaining flexible in the rigor state (61).

Differences in the ED-CG assignments between myosin states are less pronounced, with the free-rigor, bound-rigor, and post-rigor states having ED-CG site assignments at various levels of resolution that are relatively similar (see Fig. S2). This result suggests that the regions of myosin behaving as collective domains along the primary sequence are not greatly dependent on the interactions of myosin with actin, or on whether the ATP-induced transition to
the post-rigor state has occurred. Because the ED-CG method places sites along the primary sequence to preserve chemical connectivity within the CG site, a direct comparison to the structural domains, for example in Houdusse et al. (62), cannot be made because the structural domains are not sequence-continuous (see Table S1). However, hENM analysis can be used to measure the effective couplings between the myosin structural domains, allowing us to determine how strongly various structural elements of myosin interact with one another, and the differences between states. Analysis of myosin with the hENM method is discussed in the next section.

Analysis of interactions between CG sites reveals that both filament binding and ATP binding affect dynamics in myosin

The hENM method can be used to describe the effective interactions between the CG sites within a protein or protein complex (29,39–41). To evaluate the differences between myosin states, and to probe interactions between myosin and actin, we have utilized the hENM method as a CG-level analysis tool. Two CG sites that are coupled by a large spring constant in an hENM model interact more strongly with one another than those with smaller spring constants. Because ED-CG does not assign the same dynamic domains in every myosin state or between every actin monomer (nor should it, because ED-CG is designed to capture the differences in dynamic motions between states), we do not use the ED-CG site assignments for this analysis. Instead, a single set of CG sites based on the functional domains of myosin (or actin) is chosen, so that there is a consistent set of CG site definitions for which interactions can be compared effectively (see Table S1 and Table S2). The site definitions are chosen to represent specific structural elements in myosin and actin, as has been done previously to compare interactions between actin sites in the free filament (29).

Several differences are observed in the hENM profile between myosin states at the CG level. For example, it has been proposed that the actin-binding cleft of myosin, between the U50 and L50 domains of the protein, is tightly closed in the rigor state, and upon binding of ATP into the myosin nucleotide cleft the myosin actin-binding cleft opens as myosin transitions to the post-rigor state and releases the actin filament (27). The interaction strength between the U50 CG site and the L50 CG site was measured using the hENM analysis method. We observed for the three myosin states that the U50-L50 coupling has strength in the order of

\[ k_{U50-L50-BOUND-RIGOR} > k_{U50-L50-FREE-RIGOR} > k_{U50-L50-POST-RIGOR} \]

The values of the effective spring constants between the U50 and L50 domains for these three states are 5.8 kcal/mol/Å², 4.1 kcal/mol/Å², and 1.0 kcal/mol/Å², respectively. Therefore, in the post-rigor state the U50-L50 coupling is much weaker than in either the free-rigor or bound-rigor state.

The spring constant for the post-rigor state being the smallest is consistent with weaker interactions between the U50 and L50 domains being associated with the presence of ATP in the nucleotide cleft (27). It also demonstrates that myosin being bound to the actin filament increases the strength of the coupling between the two domains compared to the free-rigor state. Therefore, our hENM analysis suggests that binding of myosin to actin acts to stabilize the bound-rigor-state, actin-binding cleft relative to the free-rigor state as evidenced by the increased strength of the coupling between the U50 and L50 domains. This is further supported by the small outer-cleft width in the bound-rigor state of myosin (Fig. 5, a and b). In the squid rigor crystal structure, the far outer cleft has a width of ~13.2 Å, whereas...
the Dictyostelium myosin II rigor state has a width of ~20.3 Å (27). Relative to the crystal structure values, we observe widening of this cleft in both the free-rigor and bound-rigor states, with less widening in the bound-rigor state. Specifically, in our simulations, the free-rigor state and post-rigor states sample the largest outer-cleft widths in the last 20 ns of the simulation, whereas the bound-rigor state samples a smaller outer-cleft width than either of the two simulations (Fig. 5). Therefore, although actin binding is not required for the closed myosin actin-binding cleft, as has been demonstrated by the ability to produce closed-cleft structures in the crystal environment (27) and by spin-labeling and electron-paramagnetic resonance experiments (63), actin binding does help to stabilize the more closed outer-cleft state of the myosin protein (63).

Two of the myosin sites that sense the nucleotide state in myosin also have greatly differing interactions as probed by the hENM analysis method. The P-loop and switch I both interact with nucleotide, and the sensing of ATP in this site has been suggested to be related to the widening of the actin-binding cleft of myosin (5) (see Fig. S3). In the post-rigor state the coupling between these two elements is measured to be

\[ k_{P\text{-}LOOP-SWI\text{-}POST\text{-}RIGOR} = 18.3 \text{ kcal/mol/Å}^2, \]

which is the largest spring constant observed between any domains in myosin. The ATP molecule present in the post-rigor cleft leads to the very strong coupling between these regions of myosin. Comparatively, in the free-rigor state the coupling between these elements is only

\[ k_{P\text{-}LOOP-SWI\text{-}FREE\text{-}RIGOR} = 0.05 \text{ kcal/mol/Å}^2, \]

a factor of ~360-smaller than in the post-rigor state. In the bound-rigor state, the value of the coupling is

\[ k_{P\text{-}LOOP-SWI\text{-}BOUND\text{-}RIGOR} = 1.6 \text{ kcal/mol/Å}^2, \]

which is a factor-of-30 larger than in the free-rigor state (and a factor of ~10-smaller than the post-rigor state). This hierarchy of scales for the P-loopswitch I coupling,

\[ k_{P\text{-}LOOP-SWI\text{-}POST\text{-}RIGOR} > k_{P\text{-}LOOP-SWI\text{-}BOUND\text{-}RIGOR} > k_{P\text{-}LOOP-SWI\text{-}FREE\text{-}RIGOR}, \]

suggests that there is an influence of both actin binding and ATP binding on the dynamics of the nucleotide-sensing cleft. The large difference between the couplings in the post-rigor state compared to the rigor state is also consistent with electron-paramagnetic resonance measurements using spin-labeled nucleotides, which demonstrated a more open nucleotide pocket in the strong actin-binding state of myosin compared to the ATP state (64).

In Fig. 5, c and d, we show that the P-loopswitch I distance distribution (and absolute distances) are smallest for the post-rigor state, and largest for the free-rigor state. It is possible that the ability of the P-loopswitch I distance to probe larger average separations in the bound-rigor state compared to the post-rigor state could be important for the nucleotide cleft to be able to bind ATP, initiating the transition to the post-rigor conformation. Furthermore, the smaller average separation in bound-rigor compared to free-rigor suggests that actin-binding could aid in the process of the recruitment of switch I to the P-loop to bind ATP in the myosin nucleotide cleft. Fig. S4 shows that the P-loopswitch I distance and the outer-cleft width are both stabilized toward smaller average separations by the presence of actin (comparing bound-rigor to free-rigor). In the absence of actin (free-rigor), the P-loopswitch I and outer-cleft widths can assume larger average values. The presence of ATP in the myosin cleft more tightly couples the P-loop and switch I elements. In the bound-rigor simulation, a hydrogen-bonding interaction between S178 (P-loop) and N244 (switch I) appears to be preferentially stabilized compared to the same hydrogen bond in the free-rigor state (43 and 1% occupancy respectively, over the last 20 ns of simulation). This difference in hydrogen bonding likely contributes to the difference in effective couplings between the P-loop and switch I in these two simulations. Taken together, our result for the hierarchy of P-loopswitch I couplings and the difference in the effective couplings between the U50/L50 domains of myosin in the rigor versus post-rigor state, provide additional support for the reciprocal coupling between the actin-binding cleft and the nucleotide cleft in myosin.

We have also analyzed effective couplings among switch II, the relay helixloop, the SH3 motif in the N-terminal domain of myosin, and the converterlever domains. Table S5 shows the values of the hENM springs between these elements for the three myosin states. Both the switch II/relay helix and relay loopconverter effective couplings are smaller in post-rigor compared to the bound-rigor and free-rigor states (see Table S5). However, the couplings are still quite large in each instance (none are close to zero), indicating that there is a continuous mechanical pathway from the nucleotide cleft of myosin (switch II) to the converter, as expected for communication between these sites. We also observed a stronger coupling between the SH3 domain and the converter in bound-rigor compared to post-rigor and free-rigor. The SH3 domain has been implicated in helping to establish an interaction between the N-terminal extension of the essential light chain (ELC) and an actin N-terminus (65). Although our simulations did not include the ELC N-terminal extension, the enhanced coupling of the interaction between the SH3 domain and the converter (comparing our bound-rigor to free-rigor result) may be important for SH3 to communicate the ELC/actin contact back to the converter domain in the strongly bound state.
We also find that there is an influence of actin binding (comparing bound-rigor to free-rigor) on the strength of the interactions between the converter domain and the myosin lever arm (see Table S5). The converter is coupled to the lever more strongly by ~20% in bound-rigor versus free-rigor. Additionally, the presence of ATP in the nucleotide cleft increases the converter/lever coupling by ~30% compared to free-rigor. This is consistent with the increased coupling of the converter into the neck domain of myosin V as observed in Cecchini et al. (66). An increased effective coupling between these domains in post-rigor is likely important for coupling the converter rotation to the lever arm during the recovery stroke. It is also interesting to note that none of the states have a converter/lever coupling ~0, indicating that the converter is not disengaged from the lever in either rigor or post-rigor. In Cecchini et al. (66), it was also observed that for myosin V the converter was more coupled to the motor domain in rigor than in post-rigor. The increased coupling of the converter to the relay loop in the rigor state compared to post-rigor is also consistent with the results for myosin V, which indicated that the converter is more coupled into the motor domain in rigor than in post-rigor (66).

U50/L50 domains and loop 2 interact most strongly with actin monomers m5 and m7 in the periodic filament

The hENM analysis was also used to probe interactions between myosin and actin domains. Large spring constants indicate that two domains in the protein complex are strongly interacting. As shown in Table S3, most contacts between myosin and actin are with the U50/L50 domains and loop 2 of myosin (in addition to the activation-loop contact between actin and the myosin relay helix). Therefore, we examine the couplings among myosin and actin for the U50 domain, the L50 domain, and the loop-2 regions of myosin, which are all near the actomyosin interface. The regions of the U50 and L50 domains that are near to the surface of actin remain largely unchanged during the simulation, and residues on loop 2 orient to form favorable electrostatic contacts with the actin filament (see the Supporting Material for a discussion of structural changes at the actomyosin interface due to the MD relaxation, as well as several structural figures, Fig. S5, Fig. S6, Fig. S7, and Fig. S8). The U50 domain of myosin has strong interactions with SD2 of actin monomer m5 ($k_{U50-SD2(m5)} = 0.61\, \text{kcal/mol/Å}^2$), with SD3 of actin monomer m7 ($k_{U50-SD3(m7)} = 0.18\, \text{kcal/mol/Å}^2$), and with the N-terminal residues of actin monomer m7 ($k_{U50-NTERM(m7)} = 0.36\, \text{kcal/mol/Å}^2$). On the other hand, the L50 domain of myosin has strong interactions with SD2 of actin monomer m5 ($k_{L50-SD2(m5)} = 0.52\, \text{kcal/mol/Å}^2$), the C-terminal domain of actin monomer m7 ($k_{L50-CTERM(m7)} = 0.64\, \text{kcal/mol/Å}^2$), and its two strongest interactions with the SD1 and SD3 domains of actin monomer m7, which have $k$ values of 1.8 and 1.3 kcal/mol/Å², respectively. The myosin loop 2 region is observed to have interactions with the N-terminus of actin monomer m7 ($k_{LOOP2-NTERM(m7)} = 0.26\, \text{kcal/mol/Å}^2$), as well as with the C-terminus, SD1, SD2, and SD3 of actin monomer m7 ($k$ values of 0.57, 0.80, 0.13, and 1.23 kcal/mol/Å², respectively, for these domains). Hence, hENM suggests that the strongest interactions between myosin and actin occur between myosin loop 2 and SD3 of actin monomer m7, and between the L50 domain and SD1 and SD3 of actin monomer m7.

Also, both the U50 and loop 2 domains of myosin interact with the N-terminus of actin monomer m7, which, based on the all-atom analysis, is reduced in its flexibility compared to the N-terminus of the other actin monomers (see Table S4). Interactions between myosin and actin monomer m5 are approximately a factor of 2 smaller than the interactions with monomer m7. Because a majority of the interface between myosin and actin is the overlap of myosin with monomer m7 and not with m5, stronger interactions with monomer m7 are expected. However, interactions between the myosin U50 and L50 domains with both actin monomers m5 and m7 are important for defining the strong actin-binding rigor state (51). The interactions of myosin with actin also affect the actin monomers near the myosin binding site. In particular, the dihedral angle related to the actin-propeller twist (SD2-SD1-SD3-SD4 dihedral) is flatter for monomers m7 and m6 (see Fig. S9 and Table S6), and the actin SD2-SD1-SD3 angle, which is related to the actin nucleotide cleft (28), is smaller for monomer m7 (see Table S6). These results suggest that myosin S1 has the ability to regulate the extent of the propeller twist motion of individual actin monomers, and access to the nucleotide cleft of actin. Experimental studies have previously suggested that myosin S1 has an effect on the dynamics of the actin nucleotide cleft (58,67,68). Our result suggests that the effect on the nucleotide cleft may result from myosin S1’s ability to influence both the propeller twist- and cleft-angles. Furthermore, the interactions of loop 2 with SD3, and L50 with SD1 and SD3 of m7 found by hENM, suggest that those interactions might be leading to the propeller-twist and cleft-angle effects.

Torsional rigidity of the myosin-bound actin filament increases based on the results of all-atom and hENM-ED-CG simulations of actomyosin

The torsional rigidity of an actin filament is an important property for muscle contraction, as it has been demonstrated that the myosin power stroke can produce axial rotations in the thin filament (69), and that myosin can increase the torsional rigidity of the actin filament (33–35). With regard to the functional role of actin torsional rigidity in relation to interactions of actin with myosin, it has been suggested that the flexible joint connecting myosin to the thick filament
should become taut during the powerstroke and that the ability for actin to behave as a torsional-spring could assist in straining these joints because myosin pulls on the actin filament (36). A more torsionally stiff filament may also be able to assist in promoting cooperativity between actin monomers by not allowing the influence of myosin binding at one location to dissipate quickly (34). Other actin-binding proteins also influence the torsional rigidity of the filament. For example, the protein coflin is an actin-severing protein that binds along the actin filament, greatly reducing the stiffness compared to the free filament (70). Several CG level simulations of the actomyosin system were carried out using the software GROMACS (71). The CG sites were obtained using the ED-CG method (38), and the interactions between sites were determined using the hENM method (39). Data from the hENM-ED-CG simulations were used in the analysis of the torsional rigidity of the CG actin filaments. Our calculations of the torsional rigidity of the myosin-bound actin filament from the hENM-EDCG simulations agree well with those based upon the all-atom data, and are described in the Supporting Material. The all-atom filament gave a result of $0.87 \times 10^{-26}$ N m$^2$/rad ± $0.12 \times 10^{-26}$ N m$^2$/rad. For the torsional rigidity of the CG filaments, we obtained values consistent with the result of the all-atom calculation for each level of CG resolution used (see Table S7). These results are a factor of ~2 larger than the ADP-bound bare actin filament that was described in Fan et al. (29). We find that the value of the torsional rigidity is practically independent of the number of CG sites that are used in the model (see Table S7). This is consistent with the result found for the free actin filament in Fan et al. (29). In that study it was found that heterogeneous interactions between CG sites were important for the construction of a model that can describe filament properties such as the torsional rigidity (29). This study demonstrates that heterogeneous interactions between CG sites can also correctly reproduce the all-atom simulations measurement of this filament property in the presence of actin-binding proteins (in this case, myosin) at various levels of CG resolution. Our results for the torsional rigidity of the myosin-bound actin filament are also in qualitative agreement with experimental observations that the binding of myosin S1 in the rigor state to actin filaments leads to an increase in filament torsional stiffness (33–35).

CONCLUSIONS

In this study we have presented, to our knowledge, the first all-atom simulations of myosin II S1 interacting with an actin filament. At the all-atom level of resolution we identified a number of salt-bridge and hydrogen-bond interactions between actin and myosin. Regions of the filament that were found to be involved in such interactions included the N-terminal region of actin monomer m7, which salt-bridges with residues in the myosin loop-2 region as well as in other regions, and also with the loop-3 region of myosin (which is involved in the previously predicted Milligan contact (56)). Hydrogen bonding was observed between a residue in the cardiomyopathy loop of myosin II, and a residue on the border of the SD1/SD3 domains of the actin filament monomer m7.

The ED-CG analysis performed on the actin filament demonstrated that actin monomer m7 behaves differently in its N-terminal region compared to the other actin monomers in the filament, because its N-terminus is not identified as a single CG site until the 12-site level of resolution. The N-terminus was identified as its own CG site at the lowest level of resolution (six-site EDCG model) for each of the other monomers in the filament. ED-CG analysis assigned similar CG sites for the myosin states. Analysis with the hENM method distinguishes between the various myosin states in many of the key interactions. For example, the U50 and L50 domains are most strongly coupled in the bound-rigor state, and most weakly coupled in the post-rigor state. There is also a large difference in hENM couplings for CG sites in the myosin nucleotide cleft, especially between the P-loop and switch I, which are very tightly coupled in the post-rigor myosin state. Taken together with the result for the U50/L50 coupling, these two results provide further support for the existence of a reciprocal coupling between the actin-binding cleft and nucleotide-binding cleft of myosin. The hENM analysis also demonstrates that the mechanical channel from switch II to the converter is intact in both rigor and post-rigor states, and that the SH3 domain is coupled to the converter of myosin most strongly in bound-rigor. Communication between the SH3 and converter domains may be important when the ELC N-terminal extension is guided by the SH3 domain toward the actin filament. This method also identified regions of strong interaction between myosin and the actin filament, and these regions agree with those that interact through various electrostatic interactions based on the all-atom data.

At the actin/myosin interface, we find that actin monomer m7 has both its propeller twist and its actin nucleotide cleft angle influenced by the presence of myosin. To our knowledge, this is the first observation of such a modification of these properties of the actin monomer by myosin, and the ability of myosin to regulate these angles can explain how myosin can influence the actin nucleotide cleft without directly binding into the cleft region itself. Based on the hENM analysis, it appears that loop 2/SD3, and L50/SD1 plus L50/SD3 interactions, may be responsible for the regulation of those angles. Finally, we were able to calculate a large-scale property of the actin filament with myosin bound, namely the torsional stiffness of the filament. We calculated this property both from the all-atom data, and from CG simulations based upon an hENM-ED-CG model, as has been previously carried out in our group for free actin filaments (29). These calculations demonstrate that the actin filament with myosin bound has an increase in
its torsional rigidity by approximately a factor of two over the value previously calculated for the free actin filament. This increase is qualitatively consistent with experimental results that indicate a stiffening of actin filaments upon myosin II binding (33–35).

The creation of a CG model of actomyosin that is able to capture the complete mechanochemical cycle is an ambitious task, especially at the highly CG level. Here we have taken a first step toward understanding how a CG model of just a single state along the Lynn-Taylor cycle can be characterized, and whether available CG methods are able to lead to models that can correctly reproduce properties consistent with all-atom data and experimental measurements of the actomyosin system. Much future work remains to be done, including understanding how to coarse-grain the process of ATP hydrolysis, and to build multistate models that can correctly capture the main features of the iterative, force-producing cycle of this system.

SUPPLEMENTARY MATERIAL

Nine figures, seven tables, supplemental information and further analysis are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(13)00970-3.

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