Movement of scallop myosin on Nitella actin filaments: Regulation by calcium

(motility/calcium control/Nitella actin cables)

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ABSTRACT In order to determine if Ca$^{2+}$ regulates scallop myosin movement on actin, we have measured motility of scallop myosin along actin filaments using a direct visual assay. This procedure consists of covalently linking myosin to 1-µm beads and pipetting them onto a parallel array of actin filaments located on the cytoplasmic face of a Nitella internodal cell. In the absence of Ca$^{2+}$, scallop myosin-coated beads exhibit no directed motion; however, in the presence of pCa$^{2+}$ of greater than 5.84, these beads undergo linear translocations with average velocities of 2.0 µm/s. This Ca$^{2+}$-sensitive motility requires the presence of regulatory light chains on the scallop myosin. Removal of regulatory light chains with 10 mM EDTA produces a "desensitized" myosin, no longer sensitive to Ca$^{2+}$, which moves at rates of 0.09–0.3 µm/s in the presence or absence of Ca$^{2+}$. Readdition of regulatory light chains to preparations of desensitized myosin once again confers Ca$^{2+}$-sensitive motility. The Ca$^{2+}$ dependence of scallop-myosin motility shows a sharp transition, consistent with the Ca$^{2+}$ activation sensitivity of the actin-activated ATPase. Furthermore, relative rates of movement of calcium-regulated myosins from various molluscan species are consistent with their respective rates of ATP hydrolysis. Thus, myosin motility along actin filaments provides a sensitive and direct assay of myosin activity and is suitable for studying myosin regulation.

In all systems thus far examined, muscle contraction is tightly coupled to concentrations of intracellular Ca$^{2+}$. However, Ca$^{2+}$ regulation is mediated by a number of different means. In vertebrate striated and cardiac muscle, the tropomyosin–troponin complex controls the interaction of myosin with actin filaments (1). In smooth muscle, myosin ATPase activity is regulated by the phosphorylation of the light chains by a Ca$^{2+}$-sensitive protein kinase (2). Molluscan myosin, on the other hand, is directly regulated by Ca$^{2+}$ through the actions of regulatory light chains (3, 4). In scallop myosin, these regulatory light chains are required for Ca$^{2+}$ binding and Ca$^{2+}$ sensitivity (5–7). In the absence of Ca$^{2+}$, the light chains maintain the myosin in a low-activity state; binding of Ca$^{2+}$ to the molecule activates the myosin ATPase by 10– to 100-fold.

We have examined the Ca$^{2+}$-dependent regulation of scallop myosin using a method for assaying myosin activity described by Sheetz and Spudich (8). This method provides a direct visualization of myosin movement along actin filaments through the use of small polymer beads to which myosin molecules are covalently linked. The actin substrate is provided by the alga Nitella, which contains 0.1-µm actin cables, each consisting of 50–100 filaments. Cables run longitudinally on the cytoplasmic face of chloroplast rows which line the walls of these cells. The giant internodal cell of the Nitella can be opened by dissection, thus allowing access of myosin-coated beads to the actin cables. As described by Sheetz and Spudich (8), myosin-coated beads settle down onto these actin cables, attach, and then move continuously in one direction. The velocity of this movement can be determined easily, thus providing a quantitative assay for myosin motility. Rates of movement are similar to the velocities of movement of myosin along actin in the muscle sarcosome.

In this study we document a Ca$^{2+}$ dependence for the motility of scallop myosin along Nitella actin filaments. Velocities of movement correlate with actin-activated ATPase measurements. Ca$^{2+}$ dependence of motility is lost by removal of the regulatory light chains and can be restored upon their readdition.

MATERIALS AND METHODS

Materials. Aequipecten irradians, Placopecten magellanicus, and Loligo paele were obtained from the Marine Biological Laboratory (Woods Hole, MA). Myosin was obtained from scallop adductor muscle and squid siphon muscle by procedures as described (9). Chicken breast myosin was generously supplied by Susan Lowey from Brandeis University, MA. Nitella axillaris was propagated from a culture provided by Lincoln Taisz (University of California, Santa Cruz). Reactive beads (Covaspheres, 0.9 µm) were purchased from Covalent Technology (Ann Arbor, MI).

Motility Assay. Myosin (100 µg/ml, unless otherwise noted) was treated with a 10-fold dilution of Covaspheres in 50 mM KCl/1 mM MgCl$_2$/0.2 mM dithiothreitol/5 mM potassium phosphate, pH 6.5, for 30 min at 2°C. Reaction volumes were typically 50 µl.

An internodal Nitella cell (approximately 3 cm in length) was pinned at the ends to a silicone substrate in a Petri dish in 10 mM KCl/4 mM MgCl$_2$/50 mM sucrose/2 mM EGTA/1 mM ATP/5 mM imidazole, pH 7.1 (buffer A). The Nitella was cut longitudinally with microdissection scissors, and the sides were pinned down so that Nitella was flat with its cytoplasmic surface facing up. Beads treated with myosin were then diluted 1:1 with buffer A containing 500 mM sucrose and were applied to a localized region of Nitella substrate with a micropipette attached to a micromanipulator. Beads were viewed with a 50× (Leitz, 1.0 n.a.) water immersion objective on an Olympus BH-4 microscope, which was attached to a RCA color camera (TC5001) and RCA color monitor (TC5510AU). Recordings of bead motions were made with a Gyrr time-lapse recorder at 6× normal time.

To analyze linear bead motions and obtain velocities, Saran Wrap was placed over the monitor and a sequence was

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replayed. Positions of single beads or bead aggregates were marked as a function of time, and continuous bead motions of distances >5 μm (generally 25- to 50-μm distances were used) were analyzed to obtain velocity (the maximum velocity for >5 μm was taken when movements were irregular).

**Ca**²⁺-Dependent Motility. CaCl₂ was added to buffer A in varying amounts to obtain Ca**²⁺**/EGTA ratios from 0.33 to 1.4, and solutions were readjusted to pH 7.1. Free calcium concentrations were calculated as reported (6).

ATPase Assays. Assays for ATPase were performed as described (9) by following proton liberation in a pH-stat. The actin-activated Mg**²⁺**-ATPase was measured at 25°C in 20 mM NaCl/1 mM MgCl₂/2 mM MgATP/0.1 mM EGTA with or without 0.2 mM CaCl₂ in the presence of rabbit actin added to myosin in a weight ratio of 1:2.

**Preparation of Desensitized Aequipecten Myosin and Regulatory Light Chains.** Aequipecten myosin was desensitized by a 5-min treatment at 23°C with EDTA, and regulatory light chains were separated (10). Regulatory light chains were prepared as described (11).

**RESULTS**

**Ca**²⁺ Dependence of Scallop Myosin Motility. Beads, to which scallop myosin was covalently linked, were pipetted onto a Nitella actin substrate, and velocities of bead positions were recorded on video tape. In the absence of Ca**²⁺**, scallop myosin-coated beads exhibited no motion (Fig. 1 and Table 1). Many of the beads in fact appeared to be attached, but none underwent linear translocation. However, in the presence of concentrations of Ca**²⁺** > 1 μm, motility was observed. Single and aggregated beads moved at identical rates. Average velocities of 2.0 μm/s were achieved with a pCa**²⁺** of 5.84 at pH 7.1. The pCa**²⁺** dependence of scallop myosin motility showed a sharp transition. Motility increased from 0.23 to 2.1 μm/s between pCa**²⁺** of 6.19 and 5.84 and remained constant thereafter. In contrast, chicken breast myosin (Table 1) moved the beads at approximately 3.4 μm/s in the presence or absence of Ca**²⁺**. This result is expected since the actin-activated Mg**²⁺**-ATPase of vertebrate myosins is not directly regulated by Ca**²⁺**. Ca**²⁺** regulation of contraction in vertebrate skeletal muscles is conferred by the troponin–tropomyosin complex bound on actin (12). Thus, Ca**²⁺** dependence of motility is a function of the myosin and not the Nitella actin.

**Fig. 1.** Ca**²⁺** dependence of scallop myosin motility and comparison with ATPase activity and tension development by fiber bundles. Nitella were dissected in buffer A (pH 7.1) containing the indicated amount of Ca**²⁺** as described, and motility of Aequipecten myosin-coated beads was assayed (a). Error bars indicate SDs. ATPase values and tension measurements of Placopecten myosin at pH 7.0 (curve a) have been reproduced from figure 8 of ref. 12 for comparison with motility measurements. Aequipecten and Placopecten myosin Ca**²⁺**-dependence curves are superimposable (12). ATPase values of Placopecten myosin at pH 7.5 (curve b) are depicted from figure 7 of ref. 12, illustrating the shift of the curve at higher pH values.

The Ca**²⁺** dependence of myosin motility should correlate with ATPase activity and tension development in fiber bundles. Curves for actin-activated ATPase activity and tension development are superimposable (13) and, like myosin motility, are sigmoidal (see Fig. 1). The myosin motility curve was slightly shifted to the left of the ATPase and tension development curves at pH 7.0 but showed a similar shape. This displacement can be accounted for by the different pHs at which these experiments were conducted. As previously determined (13), a change in pH from 7 to 7.5 results in a 10-fold shift of the actin-activated ATPase curve to lower Ca**²⁺** concentrations (see Fig. 1). Motility assays were conducted at pH 7.1 and, thus, a slight displacement to lower Ca**²⁺** concentrations would be expected relative to the curves for ATPase activity and tension development, which were conducted at pH 7.0. Thus, the Ca**²⁺** dependence of scallop myosin motility is closely correlated with the enzymatic ATPase activity of the molecule.

The Ca**²⁺** sensitivity of scallop myosin motility was dependent upon the presence of regulatory light chains. These light chains, but not the essential light chains, can be dissociated from the heavy chains with 10 mM EDTA (6) and separated from the rest of the myosin molecule. As indicated in Table 1, >90% of the regulatory light chains were removed by this treatment. As a result of this procedure, whether or not Ca**²⁺** is present, the myosin molecule exhibited the same actin-activated ATPase activity and, hence, was termed "desensitized." However, ATPase activity was reduced by 50–70% after the removal of regulatory light chains. As predicted from ATPase measurements, desensitized Aequipecten myosin moved in the presence or absence of Ca**²⁺** at approximately equal rates (Table 1). Nonetheless, the velocity of movement was lower by a factor of 10–15 than normal Aequipecten myosin in a Ca**²⁺**-containing buffer and, thus, slower than would be anticipated on the basis of the ATPase values.

The motility of scallop myosin in a Ca**²⁺**-containing buffer was also dependent upon the concentration of myosin on the beads (Fig. 2). At myosin concentrations of 10 μg/ml or less in the bead reaction mixture, no motility was observed. However, velocity increased sharply as a function of myosin concentration above 10 μg/ml and changed insignificantly at concentrations of >20 μg/ml. At 100 μg of myosin per ml, the bead surface was saturated with myosin (unpublished results). The concentration of myosin on the bead surface required for motility did not appear to depend on the type of myosin used, since chicken breast and scallop myosin exhibited a similar concentration dependence for motility. The sharp concentration dependence is probably due to insufficient force generation but either to a weak attachment of beads to the actin cables or to a lack of myosin filament formation on the bead surface.

**Relationship Between ATPase Activity and Motility of Different Myosin Preparations.** As suggested by Fig. 1, the ability of a myosin-coated bead to move along actin filaments appears to be directly related to its capacity to hydrolyze ATP. Therefore, one would expect myosin preparations from different species to move at rates consistent with their relative actin-activated Mg**²⁺**-ATPase activities. Table 1 compares the rates of movement of myosins obtained from three mollusk species and from chicken. Aequipecten, Placopecten, and Loligo myosins did not move in the absence of Ca**²⁺**, which is consistent with their low actin-activated Mg**²⁺**-ATPase activity. In the presence of Ca**²⁺**, Loligo (4.37 μm/s) and Placopecten (3.57 μm/s) myosin moved at higher velocities than did Aequipecten myosin (1.98 μm/s) and also exhibited greater ATPase activity. Velocities, however, were not always proportional to ATPase values.

**Desensitization and Reconstitution of Scallop Myosin Attached to Beads.** Table 1 indicates that regulatory light chains
Table 1. ATPase activity and motility of different myosins

<table>
<thead>
<tr>
<th>Myosin source</th>
<th>Sample</th>
<th>ATPase*, μmol P_i/mg myosin/min</th>
<th>Motility†, μm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without Ca^{2+}</td>
<td>With Ca^{2+}</td>
</tr>
<tr>
<td>Aequípecten</td>
<td>Intact</td>
<td>0.035</td>
<td>0.465</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.016</td>
<td>0.51</td>
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<td>Desensitized</td>
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<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>Placópecten</td>
<td></td>
<td>0.024</td>
<td>1.56</td>
</tr>
<tr>
<td>Chicken</td>
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<td>ND</td>
</tr>
<tr>
<td>Loligo</td>
<td></td>
<td>0.023</td>
<td>0.83</td>
</tr>
</tbody>
</table>

ND, not determined.

*Actin-activated Mg^{2+}-ATPase was measured by the pH-stat (9).
†Velocities were derived from the analysis of at least 10 moving beads, and SDs were <20% of the given values.
§[1-ATPase EGTA/ATPase Ca^{2+}] x 100.
The regulatory light chain content of the samples in 1 and 2 was 10 and 5%, respectively.
\*

Ca^{2+} concentration was 0.1 mM in all cases.

are required for the Ca^{2+} sensitivity of Aequípecten myosin motility. Therefore, we sought to remove these light chains or add them back to myosin molecules already covalently attached to beads. Addition of 10 mM EDTA to normal scallop myosin on beads led to their desensitization (Fig. 3). In these experiments, dissociated light chains were not separated from the bead–myosin mixture, so excess EDTA was maintained up to the time of injection. However, the light chains may have been removed during injection, since re-binding of the regulatory light chains to myosin is a slow process requiring several minutes (14). Desensitized myosin-coated beads moved independently of Ca^{2+} at rates of approximately 0.3 μm/s. Since movement of the desensitized myosin-coated beads was not significantly higher in the presence of Ca^{2+} than in its absence, in marked contrast to the control, it is likely that this procedure dissociated virtually all of the regulatory light chains. Fig. 3 also shows that myosin desensitized on the beads moved at greater velocities (approximately 2-fold) than did desensitized myosin (light chains removed and separated away) that was later coupled to beads. Since desensitized myosin is less stable than normal scallop myosin (6), such a finding could be accounted for by a gradual loss of the ATPase activity of the desensitized myosin preparation over time.

Addition of purified regulatory light chains to beads containing desensitized scallop myosin restored Ca^{2+}-sensitive motility (Fig. 4). In a Ca^{2+}-containing buffer, addition of light chains to desensitized myosin-coated beads increased rates of movement by 10-fold. Velocities of the reconstituted myosin-coated beads were generally 50–70% of the normal scallop myosin. In the absence of Ca^{2+}, the reconstituted myosin-coated bead ceased to move. These experiments clearly indicate that Ca^{2+} sensitivity can be reconstituted and that scallop myosin is present in a conformation on the beads that allows the dissociation and re-binding of regulatory light chains.

DISCUSSION

We have documented Ca^{2+}-dependent motility of myosins from two scallop species and squid along actin filaments of Nitella. This movement presumably occurs by myosin binding to actin, followed by a conformational change of the myosin molecule, which produces a change of position of myosin relative to actin (15). Multiple cycles of this process enable the individual myosin molecules to "walk" along a stationary actin filament, in a process similar to the sliding of myosin filaments along actin in the muscle sarcomere (16, 17).

A central tenet of this hypothesis is that the motility of myosin is dependent upon its rate of ATP turnover. We have shown here that in the absence of Ca^{2+}, when the ATPase of molluscan myosin is effectively "turned off," no movement of Aequípecten, Placópecten, and Loligo myosin is ob-

![Fig. 2. Concenations of myosin required for bead motility. Myosin concentration was varied as indicated in the bead reaction mixture for chicken breast (A) or Aequípecten myosin (B) (Ca^{2+} concentration was 0.1 mM), and motility was subsequently assayed. Error bars indicate the SDs.](image)

![Fig. 3. Desensitization of scallop myosin attached to beads. Aequípecten myosin was allowed to react on beads as described. EDTA was then added to the bead mixture at a final concentration of 10 mM and incubated for 10 min at 23°C. Motility of the normal (N) scallop myosin, EDTA-treated (E) scallop myosin, and a preparation of previously desensitized (D) scallop myosin (light chains removed and separated) were determined in the presence (0.1 mM) (Right) and absence (Left) of Ca^{2+}. Bars indicate the SDs.](image)
The Ca\(^{2+}\)-sensitive regulation of scallop myosin motility is clearly dependent upon the presence of the regulatory light chains. Ca\(^{2+}\)-dependent motility is lost upon their removal with EDTA and can be restored by addition of regulatory light chains to desensitized myosin preparations. These experiments can be performed with myosin covalently linked to the beads, indicating that these molecules exhibit a conformation that readily allows the dissociation or binding of regulatory light chains. Reconstitution of Ca\(^{2+}\) sensitivity of ATPase activity of myosin (6) and tension development of fiber bundles (18) have been conducted previously on desensitized scallop preparations. It is also possible to reconstitute light chains from myosins of other species (19, 20) with desensitized Aequipecten myosin, but such experiments were not performed in our current study.

Since the Ca\(^{2+}\) dependence of scallop myosin motility is consistent with previously conducted experiments on the ATPase activity of this molecule, the motility assay of Sheetz and Spudich (8, 21) has proven to be a valuable and sensitive means of assaying myosin activity and regulation. Since motility is a measure of force generation of myosin, its usefulness in conjunction with ATPase determinations may provide important insights into the structure and function of various types of myosins.

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