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Fluorescence Polarization from Isomers of Tetramethylrhodamine at SH-1 in Rabbit Psoas Muscle Fibers

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ABSTRACT We have used fluorescence polarization to examine orientational changes of the 5- and 6-isomers of acetamidotetramethylrhodamine (ATR) covalently bound to SH-1 (Cys-707 of the myosin heavy chain) in single, skinned fibers from rabbit *psoas* muscle after rapid length steps or photolysis of caged nucleotides. Similar results were obtained with both the 5- and 6-isomers of ATR. After the photolysis of caged ATP, large and rapid changes in the fluorescence polarization signals were observed and were complete well before appreciable force had been generated. Changes in the fluorescence polarization signals after the photolysis of caged ADP were similar to those after the photolysis of caged ATP, despite an almost negligible change in force. The fluorescence polarization signals remained almost constant after rapid length steps in both rigor and active muscle fibers. These results suggest that structural changes at SH-1 monitored by 5- or 6-ATR are not associated directly with the force-generating event of muscle contraction, but may be involved in the communication pathway between the nucleotide and actin-binding sites of myosin.

INTRODUCTION

A complete description of the molecular mechanism of muscle contraction must include an understanding of the conformational changes in actin and myosin during force generation and myofilament sliding. The tilting cross-bridge theory is the most commonly accepted explanation for force generation at the molecular level, but direct evidence of myosin undergoing such a powerstroke has remained elusive. Fluorescent and paramagnetic spectroscopic techniques have been used widely to study the orientation and/or mobility of probes attached to myosin, with the predominant site of labeling being the fast reacting sulfhydryl of myosin (Cys-707) known as SH-1 (reviewed by Thomas, 1987). Despite an intensive effort to do so, few studies have been able to correlate changes in the orientation or mobility of these spectroscopic probes at SH-1 directly with the force-generating events of muscle contraction. In fact, Tanner et al. (1992) reported that changes in the orientation of rhodamine probes at SH-1 in single rabbit psoas muscle fibers preceded the development of force after activation by the photolysis of caged ATP. However, the rhodamine dye used in the Tanner study was a commercial preparation containing unknown proportions of the 5- and 6-isomers of iodoacetamidotetramethylrhodamine. Because the presence of two isomers could lead to artifacts in the orientational distribution of rhodamine dyes covalently bound at SH-1, and it has been reported that these isomers have different reactivities within the myofilament lattice (Ajtai et al., 1992), we have synthesized and characterized pure samples of each of the two isomers (Corrie and Craik, 1994). In this study, we have simultaneously recorded fluorescence polarization ratios ($Q_\parallel$ and $Q_\perp$) and tension transients with high time resolution, after the photolysis of caged nucleotides or rapid length steps, to examine the relationship between force generation and changes in the orientation of 5- and 6-ATR covalently bound to SH-1 in single rabbit psoas muscle fibers.

MATERIALS AND METHODS

Bundles of rabbit psoas muscle fibers were dissected, skinned with glycerol, and stored as described previously (Goldman et al., 1984). The 5- and 6-isomers of iodo-ATR were synthesized and characterized as described previously (Corrie and Craik, 1994). Fibers were labeled with the 5- or 6-isomer of iodo-ATR as described by Tanner et al. (1992). The apparatus for mechanical measurements on single, skinned muscle fibers after photolysis of caged nucleotides or rapid length changes was similar to that described previously by Goldman et al. (1984).

Fluorescence polarization data were acquired essentially as described by Tanner et al. (1992), except the emitted fluorescence was split into two components, parallel and perpendicular to the muscle fiber axis, by a Wollaston prism and collected by two photomultiplier tubes. Fluorescence polarization ratios were defined as

$$Q_\parallel = \frac{{}_\parallel I_\parallel - {}_\perp I_\parallel}{{}_\parallel I_\parallel + {}_\perp I_\parallel} \qquad Q_\perp = \frac{{}_\perp I_\perp - {}_\parallel I_\perp}{{}_\perp I_\perp + {}_\parallel I_\perp}$$

where I is fluorescence intensity and the left and right subscripts denote the polarization of the excitation and emission polarizers, respectively. The fluorescence polarization ratios can be used in a model-independent manner to assess qualitatively the orientational distribution of rhodamine probes with respect to the muscle fiber axis. An anisotropic distribution of probes relative to the muscle fiber axis, in most cases, will produce a difference between the values of $Q_\parallel$ and $Q_\perp$, the magnitude of which will depend on the degree of anisotropy.

RESULTS

In the absence of nucleotide (rigor), for both the 5-ATR- and 6-ATR-labeled fibers, $Q_\parallel > Q_\perp$, indicating that the probes are

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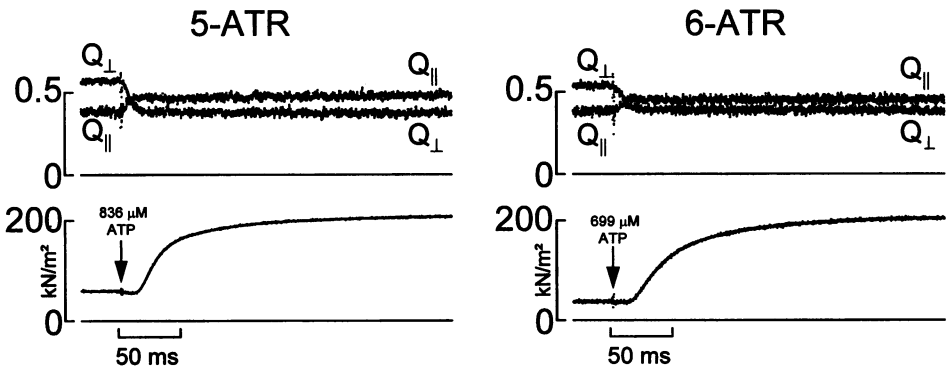

FIGURE 1 Changes in fluorescence polarization (*top*) and force (*bottom*) after photolysis of caged ATP (*arrow*) in 5-ATR (*left*)- or 6-ATR (*right*)-labeled single muscle fibers (200 mM ionic strength, pH 7.1, 20°C).

preferentially oriented perpendicular to the muscle fiber axis under these conditions. Upon the addition of nucleotide (5 mM MgADP or 5 mM MgATP), in the absence or presence of ≈30 μM calcium, the 5- and 6-ATR probes in SH-1-labeled fibers reorient substantially toward the muscle fiber axis ($Q_\parallel > Q_\perp$).

To determine whether the reorientation of rhodamine probes at SH-1 is related to force generation (i.e., the powerstroke), we simultaneously recorded force transients and fluorescence polarization ratios upon photolysis of caged nucleotides in the presence of ≈30 μM calcium. In both the 5-ATR- or 6-ATR-labeled fibers, photolysis of caged ATP induces large and very rapid changes in the fluorescence polarization ratios that are complete well before appreciable force has been generated (Fig. 1). The crossing of the two Q traces indicates a large decrease in the average probe angle with respect to the muscle fiber axis.

The time course and magnitude of the changes in $Q_\parallel$ and $Q_\perp$ after the photolysis of caged ADP are similar to those observed after the photolysis of caged ATP for both isomers, despite an opposite and much smaller change in force (Fig. 2). The greater separation between the Q traces in the presence of ADP than ATP (compare Figs. 1 and 2) suggests a greater degree of anisotropy of the probes in the presence of ADP. Because the changes in the fluorescence polarization of rhodamine probes at SH-1 occur either in the absence of force generation (e.g., after photolysis of caged ADP) or much earlier than force generation (e.g., after photolysis of caged ATP), this suggests that the observed structural changes at SH-1 are not associated directly with the force-generating event of the contractile cycle. This conclusion is supported further by the result that no significant change in $Q_\parallel$ or $Q_\perp$ is observed after rapid length changes in active or rigor muscle fibers (data not shown).

The rate of cross-bridge detachment, estimated by fitting a simple kinetic model (scheme IV of Dantzig et al., 1991) to the tension transients, was compared with the rate constant for change in fluorescence polarization signals after the photolysis of caged ATP. Changes in $Q_\parallel$ and $Q_\perp$ seem to occur even faster than cross-bridge detachment.

DISCUSSION

The recently reported atomic structure of the myosin head (Rayment et al., 1993) indicates that SH-1 is located on a short α-helix that lies near both the nucleotide-binding pocket and the base of the large cleft that separates the upper and lower domains of the 50-kDa segment. Thus, it seems likely that conformational changes at the nucleotide-binding site are propagated over a large distance to the actin-binding sites through the SH-1 region and 50-kDa cleft of myosin. Previous cross-linking studies (Huston et al., 1988) have demonstrated the movement of SH-1 and SH-2 (Cys-697) some 10 Å toward each other upon nucleotide binding to S1. Such a large conformational change must involve a melting or breaking of the helices containing SH-1 and SH-2 that may be part of the communication pathway between the nucleotide-binding pocket and actin-binding sites of myosin.

Our current work appears to support this hypothesis, with the structural changes at SH-1 occurring very rapidly after the photolysis of caged nucleotides, well before force generation and even preceding cross-bridge detachment. It may be that the observed structural changes at SH-1 are related to the

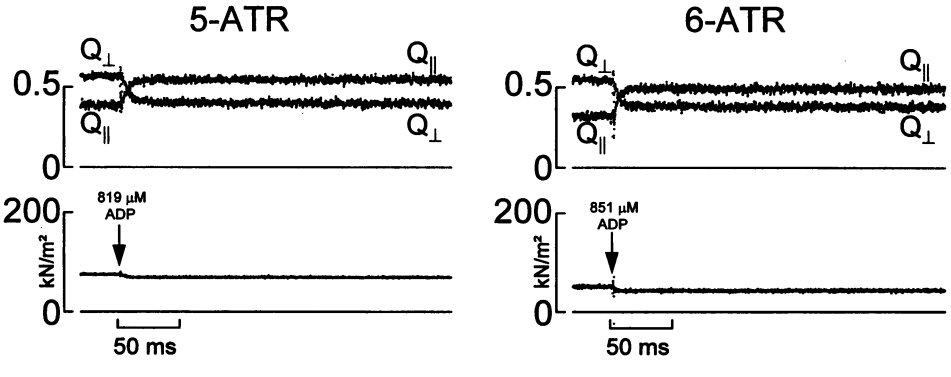

FIGURE 2 Changes in fluorescence polarization (*top*) and force (*bottom*) after photolysis of caged ADP (*arrow*) in 5-ATR (*left*)- or 6-ATR (right)-labeled single muscle fibers (200 mM ionic strength, pH 7.1, 20°C).

weakening of myosin's affinity for actin upon nucleotide binding. The combination of atomic level structural information about actin and myosin, and high time resolution spectroscopic and mechanical experiments, is a powerful approach that should lead to an even more detailed picture of the molecular mechanism of muscle contraction in the future.

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DISCUSSION

Session Chairperson: Ivan Rayment
Scribe:: Jennifer Olney

IVAN RAYMENT: Could the tension be due to unlabeled heads?

CHRISTOPHER BERGER: These fibers are labeled greater than 75%. That would argue against the tension being a combination of labeled heads that aren't doing anything and unlabeled heads. Marcus Bell has a poster here that looks at the effect of labeling SH-1 on both the kinetics and force-generating capabilities of the heads. They find that there are changes in the kinetics, and that the average force that a labeled cross-bridge can produce is knocked down, but it is still actively generating force.

DAVE THOMAS: You have to treat the modified system accurately and see if it still follows similar rules. The other important point is that it is not necessarily a theorem that any probe attached to this site is going to be reporting only these local conformational changes; in fact, there are probes that don't. You have to do control experiments where you immobilize the S1 artificially and decide whether there are internal motions or not. Some do and some don't. I think it's not necessarily that these cysteines are moving, it's that the environment around them is changing and some probes are going to find ways to move around and others aren't.

DONALD MARTYN: In an earlier similar slide, it seemed to me that the change in $Q_{\perp}$ was greater than the $Q_{\parallel}$.

BERGER: What do you mean by greater?

MARTYN: It seemed to drop more. It's probably just the way I'm looking at it. Do they have to be reciprocal?

BERGER: No, they don't have to be reciprocal.

MASATAKA KAWAI: Which step is rate-limiting?

BERGER: The hydrolysis step for the labeled fibers, at least for the iodoacetamide spin labels which have been better characterized, seems to become much slower. That's certainly going to contribute to what's happening early in the cycle.

KAWAI: How much does ADP release contribute to that early phase?

BERGER: The modeling we did was not for the biochemical cycle, it was for a simple mechanical cycle. The only states were rigor, detached, and actin.

KAWAI: But ATP release may be rate-limiting. You may be just seeing the ATP release time course.

BERGER: You mean the ATP released from the caged. . . .

KAWAI: Yes.

BERGER: The rate for that is well known, and that was taken into account in the modeling we did. The rate is 118/s.

KAWAI: Is that much faster than that, or is that comparable?

BERGER: That's comparable.

ROGER COOKE: If you model the angular change that would produce this change in your $Q_{\perp}$-$Q_{\parallel}$ parallel, how large is it?

BERGER: If we use a simple Tregear-Mendelson model it's on the order of 30°.

COOKE: So a large change.

BERGER: Yes.