Monoclonal Antibodies Detect and Stabilize Conformational States of Smooth Muscle Myosin

Kathleen M. Trybus and Larry Henry
Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254-9110

Abstract. Antibodies with epitopes near the heavy meromyosin/light meromyosin junction distinguish the folded from the extended conformational states of smooth muscle myosin. Antibody 10S.1 has 100-fold higher avidity for folded than for extended myosin, while antibody S2.2 binds preferentially to the extended state. The properties of these antibodies provide direct evidence that the conformation of the rod is different in the folded than the extended monomeric state, and suggest that this perturbation may extend into the subfragment 2 region of the rod. Two antihead antibodies with epitopes on the heavy chain map at or near the head/rod junction. Magnesium greatly enhances the binding of these antibodies to myosin, showing that the conformation of the heavy chain in the neck region changes upon divalent cation binding to the regulatory light chain. Myosin assembly is also altered by antibody binding. Antibodies that bind to the central region of the rod block disassembly of filaments upon MgATP addition. Antibodies with epitopes near the COOH terminus of the rod, in contrast, promote filament depolymerization, suggesting that this region of the tail is important for assembly. The monoclonal antibodies described here are therefore useful both for detecting and altering conformational states of smooth muscle myosin.

The assembly of smooth muscle myosin in vitro is regulated by light chain phosphorylation. In the presence of MgATP, dephosphorylated filaments are disassembled to a monomer in which the tail is folded into thirds. This assembly-incompetent conformation unfolds and immediately reassembles into filaments upon phosphorylation of the regulatory light chain (Suzuki et al., 1978; Trybus et al., 1982; Onishi and Wakabayashi, 1982; Trybus and Lowey, 1984). Although the dephosphorylated folded form of myosin is prevalent in vitro at salt concentrations and pH that approximate physiological conditions, it is not known if this conformation of myosin exists in the cell. A goal of the present study was to produce an antibody that would react preferentially with the folded conformation of myosin. Of the many monoclonal antibodies produced, two conformation-specific antirod antibodies were identified. One binds with high avidity to folded myosin, while a second binds preferentially to extended myosin. By reacting fixed muscle tissue with these antibodies it may be possible to determine, by techniques such as immunogold labeling, if the soluble folded form of myosin exists in a smooth muscle cell.

Another region of myosin that undergoes large changes upon folding is the head/rod junction. In the bent form, the myosin heads are constrained in a downward orientation toward the tail (Onishi and Wakabayashi, 1982; Trybus and Lowey, 1984). The regulatory light chain, which plays a key role in the folded-to-extended conformational transition (Trybus and Lowey, 1988), has been localized to this region in both skeletal and scallop myosin (Winkelmann et al., 1983; Flicker et al., 1983). Changes in the neck region may also be critical for determining the enzymatic activity of the smooth muscle myosin molecule, which is controlled by light chain phosphorylation. Two antihead antibodies, which bind to the heavy chain in this region, are sensitive to changes induced in the nearby phosphorylatable regulatory light chain.

Monoclonal antibodies can also be used to stabilize myosin conformational states that would otherwise be inaccessible for study in a particular solvent. To compare the actin-activated activity of filamentous and monomeric myosin, for example, antibodies have been used to depolymerize filaments formed from Acanthamoeba (Kiehart and Pollard, 1984) and Dictyostelium (Pagh and Gerisch, 1986) myosins. Dephosphorylated filaments formed from smooth muscle myosin, in contrast, are unusually labile and disassemble to a folded monomer under conditions normally used to measure actin-activated ATPases. Even copolymerizing dephosphorylated myosin with phosphorylated myosin is insufficient to stabilize the dephosphorylated species in the filamentous form (Trybus and Lowey, 1987). Several antirod antibodies are described that stabilize dephosphorylated myosin in the filamentous form. These antibodies have made it possible to show that light chain phosphorylation is essential for actin activation of the enzymatic activity of myosin filaments (Trybus, 1989).

Materials and Methods

Immunization Protocol and Production of Monoclonal Antibodies

(C.B20 X Balb.K)F1 mice were immunized with 100 μg turkey gizzard myosin emulsified in complete Freund’s adjuvant, which was administered both intraperitoneally and injected into the foot pads. The myosin was cross-linked to stabilize it in the folded monomeric conformation (Trybus
Lowey, 1988). Mice were reared for 35 or 128 d before the secondary i.p. boost with 50 µg of cross-linked folded myosin in PBS. The mice were killed for fusion 4 d after the secondary boost. The spleen cells were fused with the hybrid myeloma cell line Sp2/0-Ag 14 (Shulman et al., 1978). The procedures used for fusion, subcloning of cell lines, and ascites production are described in detail in Winkelmann et al. (1983).

The antibody designated as 10S.1 is the only antibody described in this paper in which a pool of subclones was injected into mice for ascites production. This was necessary because of the extremely slow growth properties of this cell line. Prolonged growth in culture, which was necessary to generate enough cells for injection into mice, was inevitably accompanied by a change in the cells which made them stop secreting anti-myosin antibody and grow faster. Thus the strategy used for ascites production was to grow positive subclones in 96-well plates instead of in large volume flasks, screen the wells by ELISA to identify and remove hybridomas that had stopped secreting the antibody of interest, and the same day inject cells from the positive wells into pristane-primed mice. It is likely, however, that the subclones are identical. This antibody had two properties that made it easy to identify; i.e., an unusual isotype (IgG2a), and a much higher affinity for the folded conformation of myosin.

Antibody Purification

Antibodies were purified from ascitic fluid on an Affi-Gel Protein A MAPS II kit (Bio-Rad Laboratories, Richmond, CA) essentially as described by the manufacturer. IgG1 was eluted from the column at pH 6.0, IgG2a at pH 4.5, and IgG2b at pH 3.5. Concentration of purified antibody was determined using E1%(280 nm) = 14.5.

Direct Binding ELISA

Antibody binding to myosin or its fragments was determined by solid-phase ELISA. The adsorption of antigen to the plate and the washing procedure between steps was as described in Winkelmann et al. (1983). Antibody binding to antigen was detected with biotinylated anti-mouse IgG, followed by streptavidin-β-galactosidase, and the substrate p-nitrophenyl-β-D-galactopyranoside. For screening of hybridomas, the secondary antibody was digoxigenin-labeled mouse antihuman immunoglobulin (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Solution Competition ELISA

A solution competition method is needed to quantitate relative affinities of the antibodies for different conformational states of myosin. A constant amount of antibody was mixed with twofold serial dilutions of myosin, starting with >0.2 mg/ml antigen. Folded myosin was prepared by adding 1 mM MgATP to myosin in 10 mM KPi, pH 7.5, 50 mM NaCl, 1 mM EGTA, 1 mM DTT, 1 mM NaN3. Any filaments that did not depolymerize were removed by centrifugation (100,000 g, 30 min). KCi was added to this soluble myosin to generate myosin in different conformational states at the same concentration. The antibody concentration in the competition solution was that which gave half-maximal color by direct solid-phase ELISA, and was typically on the order of 0.1 µg/ml. The competition solution also contained 0.5–1% BSA. The amount of free antibody in the antibody-antigen mixtures was determined by binding to the solid-phase antigen as a direct ELISA. The solid-phase antigen was cross-linked folded myosin, because this species does not change conformation as a function of salt concentration. The relative affinities of antibody for the antigen could be determined from the amount of myosin in solution required to inhibit binding to the antigen by 50%. Myosin concentration, determined using E1%(280 nm) = 5.0, was expressed as concentration of active sites; i.e., 1 mg/ml myosin is 4 µM myosin.

Immunoblots

SDS gels were run according to Laemmli (1970). Myosin and its fragments were electrophoretically transferred onto nitrocellulose (Towbin et al., 1979). Antibody binding to antigen was detected with an anti-mouse antibody coupled to horseradish peroxidase or alkaline phosphatase (Bio-Rad Laboratories). Primary antibody concentration was >5 µg/ml.

Immunoelectron Microscopy

1 mg/ml myosin in 10 mM NaPi, pH 7.5, 0.4 M NaCl, was incubated with 0.3 mg/ml antibody (equimolar ratio) for 15-30 min at room temperature. The myosin was diluted 100-fold into 0.5 M ammonium acetate, pH 7.2, 65% glycerol, and rotary shadowed with platinum by the method of Tyler and Branton (1980) as described by Trybus and Lowey (1984). Electron microscopy was performed on an electron microscope operated at 60 kV (EM 30); Philips Electronic Instruments, Inc., Mahwah, NJ). For localization of 10S.1, 1 mg/ml folded myosin and 0.3 mg/ml antibody were cross-linked together with 6 mg/ml dimethylsuberimidate for 3 h on ice before dilution into the shadowing buffer. The reaction was done in 20 mM KP, pH 7.5, 0.15 M KCl, 1 mM EGTA, and stopped by removing excess cross-linker on a spun gel filtration column (Neal and Florini, 1973).

Filament Pelleting Assay

Myosin filaments at 0.5 mg/ml (10 mM imidazole, pH 7, 0.15 M NaCl, 5 mM MgCl2, 1 mM EGTA) were incubated with 0.15 mg/ml antibody (equimolar ratio) for 10 min at room temperature. Antibody-myosin mixtures in the presence or absence of 1 mM MgATP were pelleted in an airfuge (Beckman Instruments, Inc., Fullerton, CA) for 10 min at 23 psi (130,000 g). Equal amounts of the supernatant and pellet were applied to SDS–polyacrylamide gels.

Protein Preparation

Turkey gizzard myosin was prepared as described by Sellers et al. (1981). Rod and S1 were prepared by papain digestion (6 µg/ml, 15 min, room temperature) of 7 mg/ml myosin in 0.2 M ammonium acetate. Heavy meromyosin (HMM) and light meromyosin (LMM) were prepared by α-chymotryptic digestion (10–50 µg/ml, 15 min, room temperature) of 5 mg/ml myosin in 10 mM NaPi, pH 7.5, 0.5 M NaCl, 1 mM EGTA. Rod and LMM were purified by ethanol precipitation. Papain S1 (2 mg/ml in 10 mM imidazole, pH 7.5, 40 mM NaCl, 1 mM DTT) was cleared with trypsin-TPCK (10 µg/ml, 3 min, room temperature) to generate the 29-, 50-, and 25-kD tryptic head fragments (Marianne-Pepin et al., 1983).

Results

Antithead Monoclonal Antibodies Detect Changes at the Head/Rod Junction

Of the 11 monoclonal antibodies produced against the heavy chain of gizzard smooth muscle myosin, 4 were directed against the head region of myosin (Table I and Fig. 1). Solid-phase ELISA showed that SI.1 through SI.4 reacted with myosin, HMM, and subfragment 1 (S1), but not with rod, the 20-kD regulatory light chain, or the 17-kD light chain. A representative example of solid-phase binding for two of these antibodies, SI.2 and SI.4, is shown in Fig. 2.

An unusual feature of two of the antihead antibodies (SI.3 and SI.4) was that they reacted with HMM and dephosphorylated or phosphorylated myosin only in the presence of magnesium (Fig. 2, A and B). S1, in contrast, bound equally well under all conditions. The regulatory light chain is presumably the site of divalent cation binding, but based on ELISAs, the epitopes for these antibodies reside on the heavy chain. The conformationally sensitive nature of the site to which these antibodies bind is consistent with the observation that these antibodies do not react with myosin or its tryptic fragments in immunoblots, despite a high affinity for native myosin in solution (Table I). Electron microscopy of metal-shadowed antibody–myosin complexes showed that both antibodies SI.3 and SI.4 mapped to the head/rod junction, yet had distinct patterns of binding and were therefore unique antibodies (Fig. 3, A and B).

The two antihead antibodies that do not show divalent cation sensitivity (SI.1 and SI.2) bind to more distal regions of the head (Fig. 3, C and D). The epitope recognized by SI.1
Table 1. Characteristics of Anti-gizzard Myosin Monoclonal Antibodies

| Antibody | Clone designation | Immunoglobulin isotype | Folded dimer | Folded monomer | Extended monomer | Relative affinities* |
|----------|-------------------|------------------------|--------------|----------------|-----------------|----------------------|
| S1.1     | 4F6.1             | IgGl                   | 2            | 1              | 2               | $10^4 M^{-1}$        |
| S1.2     | 1A1.3             | IgGl                   | 90           | 70             | 180             | $10^5 M^{-1}$        |
| S1.3     | 5D1.1             | IgGl                   | －           | －              | －               | $10^5 M^{-1}$        |
| S1.4     | 5E9.1             | IgGl                   | 36           | 10             | 8               | $10^5 M^{-1}$        |
| S1.5     | 6A3.4             | IgGl                   | 4            | 3              | 3               | $10^5 M^{-1}$        |
| S2.2     | 6C11.2            | IgGl                   | 0.4          | 3              | 42              | $10^5 M^{-1}$        |
| LMM.1    | 5D11              | IgG2a                  | 14           | 10             | ＜0.1           | $10^5 M^{-1}$        |
| LMM.2    | 3H9.1             | IgG2b                  | 5            | 5              | 5               | $10^5 M^{-1}$        |
| LMM.3    | 1A6.1             | IgGl                   | 8            | 8              | 18              | $10^5 M^{-1}$        |
| LMM.4    | 5D10.3            | IgGl                   | －           | －              | －               | $10^5 M^{-1}$        |
| LMM.41   | 2E8.1             | IgGl                   | 17           | 17             | 17              | $10^5 M^{-1}$        |

The antibodies are designated by their fragment specificity, followed by a number which indicates the relative position of an epitope within a fragment. Antibody 10S.1 binds at the HMM/LMM hinge. The epitope for antibody S1.1 was further localized to the NH2-terminal 29-kD region of the myosin head. All of the antibodies react with both turkey and chicken gizzard myosin, but none cross react with chicken pectoralis skeletal myosin. All of the antibodies have κ light chains.

* The relative affinities listed in this table are the reciprocals of the myosin concentrations required to inhibit binding to the solid-phase antigen, cross-linked folded myosin, by 50% in a solution competition ELISA.

$ \equiv 50 \text{ mM KCl.} \\
\equiv 150 \text{ mM KCl.} \\
\equiv 600 \text{ mM KCl.} \\
* \text{Cross reacts with bovine aorta smooth muscle myosin.}

The affinity of these antibodies for myosin was high, as could be seen from the high percentage of myosin molecules that bound antibody in fields of metal-shadowed molecules, even at the low concentrations required for metal shadowing (10 $\mu$g/ml). Solution competition ELISAs provided a more quantitative measure of the relative affinities of antibody for myosin (Table 1), but in general the two methods agreed qualitatively.

Trybus and Henry Antibodies Detect and Stabilize Myosin Conformations
Figure 2. Binding of antihcnd antibodies to myosin and its fragments in the presence or absence of magnesium. (A and B) Antihcnd antibody SI.4 reacted with myosin (●), HMM (○), and SI (△), but not rod (◆), the 20-kD regulatory light chain (□), or the 17-kD light chain (△) by direct ELISA. An unusual property of antibody SI.4 is that binding to myosin and HMM is seen in the presence of magnesium (B), but not in its absence (A). Antibody SI.3 shows a similar metal ion dependency (data not shown). (C) SI.2 binds to myosin, HMM, and SI independent of divalent cations (same symbols as in A and B). SI.1 shows a similar pattern of reactivity (data not shown).

All of the S2 region is not altered upon folding. Similarly, none of the other antihcnd or antirod antibodies showed a marked difference in affinity for myosin at low or high salt (Table I).

Solution competition ELISAs were done over a much wider range of ionic strengths to determine the extent to which the antibodies affected the folded-to-extended conformational transition (Fig. 6). The arrows at the top of the figure show the ionic strengths at which the 6S extended monomer, the 10S folded monomer, or the 15S folded dimer are formed (Trybus and Lowey, 1984). In the absence of antibody, myosin starts to unfold at >0.2 M KCl, and the transition is nearly complete by 0.25 M KCl. The affinity of 10S.1 for myosin continued to decrease between 0.25 and 0.3 M KCl. This shift of the conformational transition to higher salt in the presence of antibody indicates that antibody binding has to some extent stabilized the folded conformation.

The affinity of S2.2 for extended myosin was constant and high above 0.3 M KCl (Fig. 6). The affinity decreased below this salt concentration as the myosin started to fold, reaching a minimum of 100-fold lower affinity at 50 mM KCl. Based only on this curve, however, it is difficult to determine the relative affinities of S2.2 for the folded dimer and the folded monomer.

**Antirod Antibodies Affect Myosin Assembly**

Airfuge pelleting experiments were used to determine if the antirod antibodies could bind to preformed myosin filaments, and if their binding prevented filament disassembly in the presence of MgATP. Without antibody, myosin filaments pelleted in the absence of nucleotide, and were solubilized to the folded conformation upon addition of MgATP (Fig. 7 B). In the presence of antibodies that mapped to the central portion of the rod (S2.1, S2.2, LMM.1, and LMM.2), antibody and myosin were found in the pelleted fraction both in the presence or absence of MgATP (Fig. 7 C). These antibodies not only bind tightly to myosin filaments, but block their disassembly to the folded conformation. Electron microscopy confirmed that the antibody–myosin complex remained filamentous in the presence of MgATP (Trybus, 1989).

Filament depolymerization was promoted by antibodies that bind close to the COOH terminus of the rod (LMM.3 and LMM.4). In the absence of MgATP, binding of LMM.4 solubilized approximately half the myosin (Fig. 7 A). Antibody binding to the pelleted filaments was minimal. Metal-shadowed images showed that myosin with LMM.4 bound to the tail was still able to assume the folded conformation. In the presence of antibody 10S.1, some filaments also disassembled. Depolymerization probably occurs via antibody binding to the monomer in equilibrium with polymer, followed by filament disassembly to reestablish the monomer pool of antibody-free molecules.

**Discussion**

Among the monoclonal antibodies described here, two are especially unusual in their ability to distinguish between conformational states of smooth muscle myosin. Antibody 10S.1, localized at the hairpin bend of the folded myosin monomer, has a much higher affinity for folded than extended myosin. The ability to generate an antibody against this structural state provides direct evidence that the conformation of the rod is different in the folded and extended forms of myosin. If a hingelike region that underwent constant bending existed in the rod, it would be unlikely that an antibody with this specificity could have been produced. In
agreement with these findings, secondary structure calculations based on the gizzard rod sequence show no obvious interruptions in the α-helix in the regions where the myosin rod folds (Yanagisawa et al., 1987).

Antibody S2.2, which binds at the COOH-terminal end of S2, strongly favors the extended form of myosin, suggesting that the conformation of this region may also be perturbed upon formation of the nearby bend in the rod. The change must be quite localized, however, because antibodies LMM.1 and S2.1, which also map near the HMM/LMM hinge, have equal affinities for folded and extended myosin.

Conformation-specific antibodies are uncommon. One well-known example is the collection of antibodies specific for left-handed Z-DNA, which have been useful in determining the natural occurrence of this form of the double helix (Lafer et al., 1981). Another example is a monoclonal antibody that binds to the calcium/calmodulin/phosphodiesterase complex, but not to phosphodiesterase or calmodulin alone (Hansen and Beavo, 1982). A number of metal ion–dependent antibody–antigen reactions have also been reported, including antibodies against the prothrombin–calcium complex (Tai et al., 1980), and against skeletal myosin light chain 2 (Shimizu et al., 1985).

Metal ion–dependent antibody–antigen reactions were also observed here: both dephosphorylated and phosphorylated myosin required divalent cations to bind to antibody S1.3 or S1.4. Changes induced in the neck region by regulatory light chain phosphorylation thus appear to be distinct from those associated with metal binding to the light chain. Consistent with this observation, the actin-activated ATPase of phosphorylated myosin can be further increased in the presence of calcium, presumably due to metal ion binding to the non-specific calcium–magnesium sites on the regulatory light chain (Chacko and Rosenfeld, 1982). Only myosin species
Figure 4. Localization of antibody 10S.1 to the HMM/LMM hinge. (A) Antibody 10S.1 reacted with cross-linked folded myosin (○), rod (△), and extended myosin (●), but not with HMM (●), LMM (●), or SI (●). Reaction with neither HMM nor LMM suggested that the epitope for this antibody was located at the hinge region between these two subfragments. The degree to which the rod reacted with antibody 10S.1 by direct ELISA was variable. By solution competition ELISA, however, rod and extended myosin showed similar weak reactions. (B) Reactivity of antibody 10S.1 with myosin was progressively lost as whole myosin was digested with chymotrypsin.

with an intact neck region required metal ions for antibody binding; binding of SI to these antibodies was divalent cation independent. In a similar fashion, light-chain phosphorylation only regulates the activity of those species that contain an intact head/rod junction.

In addition to detecting changes in myosin conformations, the antirod antibodies could also stabilize myosin structural states. Four antibodies with epitopes in the central portion of the rod (S2.1, S2.2, LMM.1, and LMM.2) bind tightly to synthetic filaments and block their disassembly to the folded monomer in the presence of MgATP. The stabilization is probably due to a physical cross-linking between myosin molecules in the filament. Alternatively, antibody binding could induce a change in the myosin that mimics phosphorylation, although only modifications to the head have been observed to have this effect (Nath et al., 1986).

LMM.3 and LMM.4, whose epitopes are located within the terminal one-third of the rod, depolymerize smooth muscle myosin filaments. The effect of these antibodies on polymerization is consistent with the observation that the COOH-terminal region of the rod is important for filament assembly in a number of myosins. The aggregation of skeletal LMM at low ionic strength is due to a small region located ~15–20 nm from the COOH terminus of the skeletal muscle rod (Nyitray et al., 1983). A larger, 20-nm COOH-terminal segment, which overlaps the region responsible for insolubility in skeletal myosin, appears to play a role in intermolecular interactions between smooth muscle myosin rods (Cross and Vandekerckhove, 1986). The antibody with the greatest disassembling effect, LMM.4, binds within this region of the smooth muscle myosin tail. Although Dictyostelium myosin has a longer tail than smooth or skeletal myosin, the region responsible for aggregation was mapped to a similar region.
Figure 6. Relative affinities of conformation-specific antibodies for myosin as a function of salt. Relative affinities were determined from midpoints of competition curves such as those shown in Fig. 5. The arrows at the top of the figure indicate salt concentrations where myosin is a folded dimer (15S), a folded monomer (10S), or an extended monomer (6S) (see Trybus and Lowey [1984] for the complete curve of how conformation varies with salt). The binding of 10S.1 (●) to folded myosin is high at low salt, and gets much weaker in the range of salt concentrations where the myosin unfolds. Antibody S2.2 (▲) binds appreciably only at >0.3 M KCl, where myosin is extended. The binding of LMM.1 (○) to myosin is conformation and salt independent. Conditions: as in legend to Fig. 5.

Figure 7. Effect of antirod antibodies on myosin filament stability. Antibody–filament complexes were pelleted in the presence or absence of MgATP. In each panel, the lanes show starting material, supernatant, and pellet in the absence of MgATP, and supernatant and pellet in the presence of MgATP. (A) LMM.4 and LMM.3 (data not shown) depolymerize filaments; myosin is found in the supernatant even in the absence of nucleotide. (B) Without antibody, myosin is filamentous and pellets. Upon addition of MgATP, the myosin is solubilized to the folded form and is found in the supernatant. (C) LMM.1, as well as S2.1, S2.2, and LMM.2 (data not shown), bind to myosin filaments and block their disassembly in the presence of nucleotide. Conditions: 10 mM imidazole, pH 7, 0.15 M KCl, 5 mM MgCl₂, 1 mM EGTA.

with respect to the head/rod junction (Pagh et al., 1984). Antibodies against this region also inhibit polymerization, while an antibody against the very tip of the long tail only abolished antiparallel interactions, and unipolar filaments were formed (Peltz et al., 1985; Pagh and Gerisch, 1986). In Acanthamoeba myosin II, which has a short, 89-nm rod, proteolytic removal of 12–13 nm of the tail abolished assembly (Kuznicki et al., 1985), as did antibody binding to the tip of the tail (Kiehart and Pollard, 1984).

The epitope for antibody LMM.4, which binds at the tip of the gizzard myosin tail, must be near the nonhelical, proline-containing tailpiece. Smooth muscle myosins from gizzard (Yanagisawa et al., 1987), rabbit uterus (Nagai et al., 1988), and the mollusc Mytilus edulis (Castellani et al., 1988) all contain such a region. Only with Acanthamoeba myosin II (Hammer et al., 1987), however, has a functional role been assigned to this nonhelical region. Phosphorylation of three serines per chain in the tailpiece inhibits actin-activated ATPase activity, but does not change the state of assembly of the myosin (Collins et al., 1982; Kuznicki et al., 1983; Atkinson and Korn, 1987). Phosphorylation of the tailpiece of the molluscan smooth muscle myosin, in contrast, favors filament disassembly to the folded monomer (Castellani and Cohen, 1987). The heavy chain of vertebrate smooth muscle myosin can be phosphorylated, but the effect of this modification on assembly and the location of the phosphorylation site have not been determined (Kawamoto and Adelstein, 1988).

The ability of the monoclonal antibodies described here to alter the state of myosin without changing solvents allows the relationship between enzymatic activity, light chain phosphorylation, and smooth muscle myosin conformation to be further investigated. In the accompanying paper (Trybus, 1989), the actin-activated activity of dephosphorylated filaments, a state that is present in relaxed smooth muscle cells but difficult to stabilize in vitro, was measured in a more physiological solvent than was previously possible.

I thank Susan Lowey for support and helpful discussions during the course of this work, and Donald Winkelman for advice on the preparation of monoclonal antibodies.

This work was supported by a National Institutes of Health (NIH) grant HL38113 to K. Trybus and NIH (AR17350), National Science Foundation, and Muscular Dystrophy Association grants to S. Lowey.

Received for publication 10 April 1989 and in revised form 11 August 1989.

References

Atkinson, M. A. L., and E. D. Korn. 1987. Appendix. A model for the polymerization of Acanthamoeba myosin II and the regulation of its actin-activated Mg²⁺-ATPase activity. J. Biol. Chem. 262:15809--15811.

Castellani, L., and C. Cohen. 1987. Rod phosphorylation favors folding in a catch muscle myosin. Proc. Natl. Acad. Sci. USA. 84:4058--4062.

Collins, L., B. W. Elliott, Jr., and C. Cohen. 1988. Phosphorylatable serine residues are located in a non-helical tailpiece of a catch muscle myosin. J. Muscle Res. Cell Motil. 9:533--540.

Chacko, S., and A. Rosenfeld. 1982. Regulation of actin-activated ATP hydrolysis by arterial myosin. Proc. Natl. Acad. Sci. USA. 79:292--296.

Cross, R. A., and J. Vandenbergh. 1986. Solubility-determining domain of smooth muscle myosin rod. FEBS (Fed. Eur. Biochem. Soc.) Lett. 200: 355--360.

Flicker, P. F., T. Wallimann, and P. Vibert. 1983. Electron microscopy of scallop myosin: location of regulatory light chains. J. Mol. Biol. 169:723--741.

Hammer, J. A. II, B. Bowers, B. M. Paterson, and E. D. Korn. 1987. Com-
Onishi, H., and T. Wakabayashi. 1982. Purification of two calcium/calmodulin-dependent forms of cyclic nucleotide phosphodiesterase by using conformation-specific monoclonal antibody chromatography. Proc. Natl. Acad. Sci. USA. 79:2788–2792.

Kawamoto, S., and R. S. Adelstein. 1988. The heavy chain of smooth muscle myosin is phosphorylated in aorta cells. J. Biol. Chem. 263:1099–1102.

Kiehart, D. P., and T. D. Pollard. 1984. Stimulation of Acanthamoeba actinomyosin ATPase activity by myosin II polymerization. Nature (Lond.). 308:864–866.

Kuznicki, J., J. P. Albanesi, G. P. Cote, and E. D. Korn. 1983. Supramolecular regulation of the actin-activated ATPase activity of filaments of Acanthamoeba myosin II. J. Biol. Chem. 258:6011–6014.

Kuznicki, J., G. P. Cote, B. Bowers, and E. D. Korn. 1985. Filament formation and actin-activated ATPase activity are abolished by proteolytic removal of a small peptide from the tip of the tail of the heavy chain of Acanthamoeba myosin II. J. Biol. Chem. 260:1967–1972.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 227:680–685.

Lafer, E. M., A. Muller, A. Nordheim, B. D. Stollar, and A. Rich. 1981. Antibodies specific for left-handed Z-DNA. Proc. Natl. Acad. Sci. USA. 78:3546–3550.

Marianne-Pepin, T., D. Mornet, E. Audemard, and R. Kasab. 1983. Structural and actin-binding properties of the trypsin-produced HMM and S1 from gizzard smooth muscle myosin. FEMS (Fed. Eur. Biochem. Soc.) Lett. 159:211–216.

Nagai, R., D. M. Larson, and M. Periasamy. 1988. Characterization of a mammalian smooth muscle myosin heavy chain cDNA clone and its expression in various smooth muscle types. Proc. Natl. Acad. Sci. USA. 85:1047–1051.

Nath, N., S. Nag, and J. C. Seidel. 1986. Location of the sites of reaction of N-ethylmaleimide in papain and chymotryptic fragments of the gizzard myosin heavy chain. Biochemistry. 25:6169–6176.

Neal, M. W., and J. R. Flornini. 1973. A rapid method for desalting small volumes of solution. Anal. Biochem. 55:328–330.

Nyitray, L., G. Mocz, I. Sziagyi, M. Balint, R. C. Lu, A. Wong, and J. Gergely. 1983. The proteolytic substructure of light meromyosin: localization of a region responsible for the low ionic strength insolubility of myosin. J. Biol. Chem. 258:13213–13220.

Onishi, H., and T. Wakabayashi. 1982. Electron microscopic studies of myosin molecules from chicken gizzard muscle. I. The formation of the intramolecular loop in the myosin tail. J. Biochem. (Tokyo). 92:871–879.

Pagh, K., and G. Gerisch. 1986. Monoclonal antibodies binding to the tail of Dictyostelium discoideum myosin: their effects on antiparallel and parallel assembly and actin-activated ATPase activity. J. Cell Biol. 103:1527–1538.

Pagh, K., H. Martuta, M. Claviez, and G. Gerisch. 1984. Localization of two phosphorylation sites adjacent to a region important for polymerization on the tail of Dictyostelium myosin. EMBO (Eur. Mol. Biol. Organ.) J. 3:3271–3278.

Pelz, G., J. A. Spudich, and P. Parham. 1985. Monoclonal antibodies against seven sites on the head and tail of Dictyostelium myosin. J. Cell Biol. 100:1016–1023.

Sellers, J. R., M. D. Pato, and R. S. Adelstein. 1981. Reversible phosphorylation of smooth muscle myosin, heavy meromyosin, and platelet myosin. J. Biol. Chem. 256:13137–13142.

Shimizu, T., F. C. Reinach, T. Masaki, and D. A. Fischman. 1985. Analysis of the metal-induced conformational change in myosin with a monoclonal antibody to light chain two. J. Mol. Biol. 183:271–282.

Shulman, M., C. D. Wilde, and G. Kohler. 1978. A better cell line for making hybridomas secreting specific antibodies. Nature (Lond.). 276:269–270.

Suzuki, H., H. Onishi, K. Takahashi, and S. Watanabe. 1978. Structure and function of chicken gizzard myosin. J. Biochem. (Tokyo). 84:1529–1542.

Tak, M. M., B. C. Puric, and B. Puric. 1980. Conformation-specific antibodies directed against the bovine prothrombin-calcium complex. J. Biol. Chem. 255:2790–2795.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. 76:4350–4354.

Trybus, K. M. 1989. Filamentous smooth muscle myosin is regulated by phosphorylation. J. Cell Biol. 109:2887–2894.

Trybus, K. M., and S. Lowey. 1984. Conformational states of smooth muscle myosin: effects of light chain phosphorylation and ionic strength. J. Biol. Chem. 259:8564–8571.

Trybus, K. M., and S. Lowey. 1987. Subunit exchange between smooth muscle myosin filaments. J. Cell Biol. 105:3021–3030.

Trybus, K. M., and S. Lowey. 1988. The regulatory light chain is required for folding of smooth muscle myosin. J. Biol. Chem. 263:16485–16492.

Trybus, K. M., T. W. Huiatt, and S. Lowey. 1982. A bent monomeric conformation of myosin from smooth muscle. Proc. Natl. Acad. Sci. USA. 79:6151–6155.

Tyler, J. M., and D. Branton. 1980. Rotary shadowing of extended molecules dried from glycerol. J. Ultrastruct. Res. 71:95–102.

Winkelmann, D. A., S. Lowey, and J. L. Press. 1983. Monoclonal antibodies localize changes on myosin heavy chain isozymes during avian myogenesis. Cell. 34:295–306.

Yanagisawa, M., Y. Hamada, Y. Katsuragawa, M. Inamura, T. Mikawa, and T. Masaki. 1987. Complete primary sequence of vertebrate smooth muscle myosin heavy chain deduced from its complementary DNA sequence: implications on topography and function of myosin. J. Mol. Biol. 198:143–157.