The Effects of Smooth Muscle Caldesmon on Actin Filament Motility*

(Received for publication, April 21, 1992)

Joe R. Haeberle‡§, Kathleen M. Trybust§, Mark E. Hemric‡, and David M. Warshaw‡

From the Department of Physiology and Biophysics, Given Building, University of Vermont, Burlington, Vermont 05405 and the Rosenthal Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254

The movement of reconstituted thin filaments over an immobilized surface of thiophosphorylated smooth muscle myosin was examined using an in vitro motility assay. Reconstituted thin filaments contained actin, tropomyosin, and either purified chicken gizzard caldesmon or the purified COOH-terminal actin-binding fragment of caldesmon. Control actin-tropomyosin filaments moved at a velocity of 2.3 ± 0.5 μm/s. Neither intact caldesmon nor the COOH-terminal fragment, when maintained in the monomeric form by treatment with 10 mM dithiothreitol, had any effect on filament velocity; yet both were potent inhibitors of actin-activated myosin ATPase activity, indicating that caldesmon primarily inhibits myosin binding as reported by Chalovich et al. (1). The inhibition of filament motion was, however, observed under conditions where cross-linking of caldesmon via disulfide bridges was present. To determine if monomeric caldesmon could “tether” actin filaments to the myosin surface by forming an actin-caldesmon-myosin complex as suggested by Chalovich et al., we looked for caldesmon-dependent filament binding and motility under conditions (80 mM KCl) where filament binding to myosin is weak and motility is not normally seen. At caldesmon concentrations ≥0.26 μM, actin filament binding was increased and filament motion (2.6 ± 0.6 μm/s) was observed. The enhanced motility seen with intact caldesmon was not observed with the addition of up to 26 μM COOH-terminal fragment. Moreover, a molar excess of the COOH-terminal fragment competitively reversed the enhanced binding seen with intact caldesmon. These results show that tethering of actin filaments to myosin by the formation of an actin-caldesmon-myosin complex enhanced productive actomyosin interaction without placing a significant mechanical load on the moving filaments.

Caldesmon is a component of thin filaments in smooth muscles and is particularly abundant in phasic smooth muscles (2). Caldesmon was first described as an actin- and calmodulin-binding protein that inhibits actin-activated myosin ATPase activity of both smooth and skeletal muscle myosins (3). The molecular weight of chicken smooth muscle caldesmon is 87,000-89,000, depending on the tissue (4-6). The actin- and calmodulin-binding domains have both been localized to the carboxyl-terminal end of caldesmon (7-9). The amino-terminal end contains a specific myosin-binding domain (10-13) that binds to the S1/S2 junction of myosin (10) and possibly a second calmodulin-binding domain (14, 15). The myosin ATPase inhibitory activity of caldesmon is localized to the COOH-terminal actin-binding region of caldesmon (6-9). It has been reported that caldesmon inhibits actin-activated myosin ATPase activity by blocking the release of ATP hydrolysis products from myosin (16); however, more recent studies suggest that caldesmon may inhibit by competing with myosin for a common binding site on actin (17-20). Caldesmon increases the binding of myosin to actin (21) by forming an actin-caldesmon-myosin complex (1). A role for this complex in the maintenance of force in tonically contracted smooth muscle has been suggested (11, 21-24).

Little physiological data exist to suggest how caldesmon contributes to the regulation of smooth muscle contraction. The addition of caldesmon to chemically skinned skeletal muscle fibers produces partial relaxation that is reversed by calcium/calmodulin (25). The stiffness of relaxed skinned skeletal muscle fibers is decreased following the addition of caldesmon to the muscle bath (26). The addition of caldesmon to skinned smooth muscle accelerates relaxation in response to reduced calcium (27). In general, these observations are consistent with the suggestion that caldesmon competitively inhibits the binding of myosin to actin. A previous study from our laboratory has shown that caldesmon is more abundant in phasic smooth muscles such as the uterus and taenia coli (1 mol of caldesmon/22 mol of actin) than in tonic vascular muscles, which contain relatively little caldesmon (1 mol of caldesmon/205 mol of actin) (2). The abundance of caldesmon in phasic muscles suggests that caldesmon may contribute to the contractile phenotype of phasic muscle.

In this study, we have investigated the effects of caldesmon on the movement of unloaded actin filaments over a surface of immobilized myosin. We were interested in answering three basic questions. Does caldesmon inhibit actin-activated myosin ATPase activity by an effect on Vmax or Kbind? Does caldesmon cross-link or “tether” actin filaments to myosin? Is the actin-caldesmon-myosin complex a load-bearing structure? Our findings agree with a study previously published by Chalovich et al. (1), which showed that caldesmon competitively blocks myosin binding to actin and also causes tethering of myosin to actin by formation of an actin-caldesmon-myosin complex; however, we found no evidence that the actin-caldesmon-myosin complex is a load-bearing structure.

EXPERIMENTAL PROCEDURES

Materials—SDS was purchased from Bio-Rad. Polycrylamide was purchased from BDH Chemicals Ltd. N,N'-Methylenebisacrylamide was purchased in accordance with 18 U.S.C. Section 1734 in accordance with 18 U.S.C. Section 1734.
Caldesmon and Actin Filament Motility

Fig. 1. Purified proteins used for motility assay and for reconstituted thin filaments. Lanes A and H, molecular weight standards (myosin, 200,000; β-galactosidase, 116,250; phosphorylase b, 97,400; bovine serum albumin, 66,200; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; and lysozyme, 14,400); lane B, rabbit skeletal muscle myosin; lane C, turkey gizzard smooth muscle myosin; lane D, rabbit skeletal muscle actin; lane E, turkey gizzard tropomyosin; lane F, chicken gizzard caldesmon; lane G, COOH-terminal 2-nitro-5-thiocyanobenzoic acid fragment of caldesmon. Proteins were processed on a 7.5% SDS-polyacrylamide gel as described under "Experimental Procedures."

and acetic acid were purchased from Mallinckrodt Chemical Works. Ammonium persulfate, bromphenol blue, and Coomassie Blue R-250 were purchased from Schwarz/Mann. All other chemicals and reagents were purchased from Sigma.

Gel Electrophoresis—Caldesmon was processed on 5% SDS-polyacrylamide gels using the buffer system of Porzio and Pearson (28). Proteins were solubilized by incubation for 2 min at 100 °C in buffer containing 65.5 mM Tris base, 3% SDS, 20% glycerol, and 40 mM DTT. To examine the extent of disulfide cross-linking, proteins were solubilized in the same buffer without DTT and without heat treatment. Gels were stained for 2 h in a stain containing 0.1% Coomassie Blue R-250, 25% isopropyl alcohol, and 10% acetic acid and destained overnight in destaining solution containing 10% acetic acid and 30% methanol. Stained bands were quantified by densiometric gel scanning (protein+DNA ImageWare Systems).

Protein Preparation—Caldesmon was prepared from frozen chicken gizzards according to the method of Lynch and Bretcher (29). The COOH-terminal fragment of caldesmon was prepared by treatment with 2-nitro-5-thiocyanobenzoic acid as described by Sutherland and Walsh (12). Actin was prepared from rabbit skeletal muscle using the method of Pardee and Spudich (30). Tropomyosin was isolated from an ethanol/acetone gizzard powder (31). Myosin was purified from turkey gizzard smooth muscle according to Sellers et al. (32) and stored in monomeric form at −20 °C in a solution containing 50% glycerol. Protein concentration was determined by the method of Lowry et al. (33) or by absorbance using the following optical extinction coefficients and/or molecular masses: caldesmon, 1.18 cm−1 at 3.3 and 88 kDa; COOH-terminal fragment of caldesmon, 18.8 kDa (4); G-actin, A1/2 [nm] = 6.3 and 42 kDa; myosin, A1/2 [nm] = 5.0; and tropomyosin, A1/2 [nm] = 2.9 and 70 kDa. Smooth muscle myosin was phosphorylated to >1.95 mol of PO4/mol of myosin, as determined by glycerol-polyacrylamide gels (34), by the addition of myosin light chain kinase, calmodulin, calcium, and ATPγS (35). Fig. 1 shows a 7.5% SDS-polyacrylamide gel of the purified proteins used in this study.

Motility Assay—Details of the motility assay have been previously described (36). Briefly, a nitrocellulose-coated coverslip was supported on a glass microscope slide by two pieces of coverslip to form a 30-μl flow-through chamber. Monomeric myosin (250 μg/ml, 300 mM KCl) was added to the flow cell for 1 min, and the flow cell was then washed with buffer containing bovine serum albumin (300 mM KCl) to remove unbound myosin and to block exposed nitrocellulose surfaces. F-actin labeled with tetramethylrhodamine isothiocyanate-phalloidin was then added to the flow cell at a concentration of 0.45 μg/ml in an ATP-free buffer and allowed to bind for 1 min. After washing out unbound actin, motility buffer was added. Motility buffer contained 25 mM imidazole (pH 7.4), 1 mM ATP, 4 mM MgCl2, 1 mM EGTA, 80 mM KCl, 0.1 mg/ml glucose oxidase, 0.018 mg/ml catalase, 2.3 mg/ml glucose, and 10 mM DTT; in some instances, 0.5% methylocellulose was included in the motility buffer to enhance filament binding. Caldesmon and/or tropomyosin was added to the motility buffer prior to perfusion through the flow cell. The slide was placed on the stage of an inverted microscope (Zeiss IM) equipped for epifluorescence. To maintain the flow cell at 32 °C, the objective lens (Zeiss, Plan Apo, 63×/1.4NA) was heated by a servomechanism-controlled resistive heating element (Vermont Technologies, Burlington, VT). Filaments were tracked using a computer-assisted video imaging system (36, 37). To calculate filament velocity, video images were digitized at a rate of five/s. A sequence of 8–10 digitized images was manually scanned to identify filaments that moved continuously and in a straight line for 0.8–1.0 s (four to five images). The total distance moved was divided by the elapsed time to determine the filament velocity.

RESULTS AND DISCUSSION

Sulfhydryl-dependent Cross-linking of Caldesmon—The interaction of caldesmon with actin and myosin is complex. One of the earliest reported effects of adding caldesmon to actin is the formation of bundles of parallel actin filaments (38). A subsequent study demonstrated that caldesmon-dependent bundling requires disulfide cross-linking of caldesmon (39). During our analysis of actin filament motility, we observed evidence for what appeared to be actin filament cross-linking when high concentrations of caldesmon were added to motility buffer containing 1 mM DTT. Clusters of actin filaments were routinely observed (Fig. 2, A and B); and on numerous occasions, we observed sliding filaments that suddenly stopped when they came into close proximity with a stationary filament (Fig. 2, C–F). On the assumption that this represented cross-linking of actin filaments by disulfide-cross-linked oligomers of caldesmon, we preincubated caldesmon with 100 mM DTT and increased the concentration of DTT in the assay buffer to 10 mM; this completely eliminated filament clustering and any signs of filament-filament interaction.

Lynch et al. (39) have previously shown that actin filament bundling requires formation of disulfide-cross-linked oligomeric species of caldesmon that could be visualized by non-reducing SDS-polyacrylamide gel electrophoresis. We used a similar technique to analyze our own preparations of caldesmon and with storage conditions (data not shown). Incubation with 10 mM DTT for 16 h at 0 °C eliminated (<0.5% total protein) the oligomeric forms as well as the cross-linked monomer (lane B). To determine the extent of cross-linking that would be present at the time velocity measurements were made (4–6 min after increasing temperature to 32 °C), caldesmon from this same preparation was added to fresh motility buffer with either 1 or 10 mM DTT and incubated for 5 min at 30 °C. With 1 mM DTT in the motility buffer (lane C), 8.1% of the total protein was dimeric and 0.9% was internally cross-linked. In contrast, 10 mM DTT reduced the caldesmon to a single monomeric form. Consequently, for all experiments reported in this study, an aliquot of the purified caldesmon stock solution was incubated

1 The abbreviations used are: DTT, dithiothreitol; ATPγS, adenosine 5′-O-(thiotriphosphate).
overnight with fresh 100 mM DTT at 0 °C prior to the experiment, and 10 mM DTT was included in the motility buffer, unless otherwise indicated. Concentrations of DTT in the motility buffer as high as 100 mM had no effect on actin-tropomyosin filament velocity.

Effect of COOH-terminal Actin-binding Fragment of Caldesmon on Filament Velocity—The COOH-terminal fragment of caldesmon, prepared by treatment with 2-nitro-5-thiocyanobenzoic acid, was used to gain insight into the mechanism by which caldesmon inhibits actin-activated myosin ATPase activity. Because actin filaments do not bind to the myosin-coated surface at 80 mM, methylcellulose was normally included in the motility buffer to restrict the diffusion of actin filaments. The use of methylcellulose was first described by Uyeda et al. (40), who utilized it to enhance filament binding when the myosin density on the coverslip was very low. They found that 0.5–0.9% methylcellulose had no effect on filament velocity, but dramatically reduced the rate of dissociation of filaments from the myosin surface. When they measured the random motion of actin filaments in solution, they observed that methylcellulose reduced motion perpendicular to the filament long axis, but had no effect on the axial motion of the filaments (40). In all the experiments reported in this study, 1.5 μM tropomyosin was also present in the motility buffer. Tropomyosin alone increased filament velocity from 1.7 ± 0.5 μm/s (mean ± S.D., n = 37 velocity measurements) to 2.3 ± 0.5 μm/s. As shown in Fig. 4, the addition of the COOH-terminal fragment had no effect on velocity at concentrations up to 26 μM in spite of the fact that, under identical

Fig. 2. Actin filament cross-linking by caldesmon. The motility buffer contained 25 mM imidazole (pH 7.4), 1 mM ATP, 4 mM MgCl₂, 1 mM EGTA, 80 mM KCl, 0.1 mg/ml glucose oxidase, 0.018 mg/ml catalase, 2.3 mg/ml glucose, 1 mM DTT, 1.6 μM tropomyosin, 2.6 μM intact caldesmon, and no methylcellulose. A, arrowheads, points where actin filaments overlap. None of these filaments moved in the presence of motility buffer during the observation period. B, arrow, two or more parallel cross-linked actin filaments; arrowheads, small actin filaments cross-linked to a larger filament. C, arrow, direction of motion of the filament located immediately behind the arrow. D, arrowhead, the front end of the moving filament shown in C stopped moving when it encountered the stationary filament. The filament got shorter and brighter as the back end of the filament continued to move after the front end had stopped. E, arrow, the leading end of the filament began to move after a few seconds in the direction indicated. F, arrowhead, a fragment from the trailing end of the moving filament remained attached to the stationary filament while the leading end of the filament continued to move in the direction indicated by the arrow. The calibration scale (5 μm) showed in E applies to A and C-F. A 10-μm calibration scale is shown in the lower right-hand corner of B.

Fig. 3. Disulfide cross-linking of caldesmon. Purified caldesmon was solubilized and processed on 5% polyacrylamide gels as described under “Experimental Procedures,” except that DTT was not added to the protein solubilization buffer and the samples were not heated at 100 °C. Gels were pre-electrophoresed at 5 °C for 2 h at 100 V prior to loading the solubilized proteins. The gel was then run at 5 °C for 16 h at 100 V. Lane A, purified caldesmon dialyzed against buffer containing 1 mM DTT and stored on ice for 2 weeks; lane B, caldesmon incubated with 1 mM DTT for 60 min at 30 °C; lane C, caldesmon incubated with 1 mM DTT for 5 min at 30 °C; lane D, caldesmon incubated with 10 mM DTT for 5 min at 30 °C. Monomeric caldesmon (CaD), internally cross-linked monomeric caldesmon (M), dimeric caldesmon (D), and trimeric caldesmon (T) are indicated as described by Lynch et al. (39). Molecular weight standards were myosin (200,000), β-galactosidase (116,250), and phosphorylase b (97,400).

Fig. 4. Effect of caldesmon COOH-terminal fragment on filament velocity. Motility measurements were made as described under “Experimental Procedures” with 0.5% methylcellulose. The COOH-terminal fragment was prepared by treatment of purified caldesmon with 2-nitro-5-thiocyanobenzoic acid as described under “Experimental Procedures.” Data points are means ± 95% confidence interval. The number of measurements made at each point is indicated.
conditions, the COOH-terminal fragment was a potent inhibitor of actin-activated myosin ATPase activity, as shown in Fig. 5. These findings are consistent with the reports by Chalovich and co-workers (41) and Horiuchi and Chacko (42) showing that the COOH-terminal region of caldesmon reduces \( K_{\text{binding}} \) while having little or no effect on \( V_{\text{max}} \) for actin-activated myosin ATPase activity of smooth muscle heavy meromyosin. The absence of any effect on filament velocity in the face of reduced myosin binding is consistent with reports that filament motion is independent of the density of myosin on the surface of the coverslip over a wide range of myosin concentrations (36). Others have shown that filament velocity is not affected by a reduction in the number of interacting cross-bridges except at very low myosin concentrations, where continuous interaction with myosin no longer occurs (40). As shown in Fig. 6, we have confirmed this observation under the conditions used for this study; a 5-fold reduction in the concentration of myosin used to coat the coverslip had no effect on filament velocity.

Inhibition of myosin binding by the COOH-terminal fragment was readily apparent when the salt concentration of the motility buffer was reduced to 25 mM and methylcellulose was not included in the motility buffer. At this lower ionic strength, binding and motility were observed in the absence of methylcellulose. The addition of 2.6 \( \mu \text{M} \) COOH-terminal fragment resulted in 100% reversal of filament binding under these conditions (data not shown).

**Effect of Intact Caldesmon on Filament Velocity**—Fig. 7 illustrates the effects of intact caldesmon on filament velocity. As shown by the upper curve (open squares), there was no effect of intact caldesmon on velocity compared to control actin-tropomyosin (2.3 \( \pm \) 0.5 \( \mu \text{m/s} \), \( n = 144 \)). The lower curve (open circles) illustrates the dramatic inhibition of velocity that was seen with caldesmon when the motility buffer contained only 1 mM DTT. The concentration at which full inhibition of motility occurred (1 mM DTT) varied from one

![Fig. 5. Inhibition of actin-activated myosin ATPase activity by caldesmon. The assay buffer contained 80 mM KCl, 10 mM DTT, 1 mM ATP, 3 mM MgCl\(_2\), 10 mM imidazole (pH 7.0), 25 \( \mu \text{M} \) skeletal muscle actin, 5 \( \mu \text{M} \) chicken gizzard tropomyosin, 0.2 \( \mu \text{M} \) thiotoprophosphorylated chicken gizzard myosin, and 0.5–25 \( \mu \text{M} \) caldesmon (CaD) (O) or COOH-terminal fragment (\( \bullet \)). The liberation of \(^{32}\text{P}\) from \(^{32}\text{P}\)ATP was measured at 25 °C (47). Rates were corrected for the rate of hydrolysis by myosin in the absence of actin (0.02 s\(^{-1}\)) and normalized to the control value determined in the absence of caldesmon (0.23 s\(^{-1}\)).](image)

![Fig. 7. Effect of intact caldesmon on filament motility in presence of methylcellulose. Conditions were the same as those described for Fig. 4, except that intact caldesmon was added and the DTT concentration in the motility buffer was either 1 (O) or 10 (\( \bullet \)) mM.](image)

![Fig. 8. Effect of intact caldesmon on filament motility in absence of methylcellulose. Conditions were the same as those described for Fig. 7, except that methylcellulose was not added to the motility buffer. O, 10 mM DTT; \( \bullet \), 1 mM DTT.](image)
caldesmon preparation to another; but, in general, freshly prepared caldesmon required a higher concentration for full inhibition than preparations that had been stored at 0 °C or in 50% glycerol at −70 °C. These findings indicate that cross-linking of caldesmon was required for inhibition of filament velocity. The extreme conditions required to prevent oxidation of purified caldesmon raise the question of what the state of caldesmon is in situ. The dramatic effect of DTT on filament motility in vitro suggests a potentially interesting regulatory mechanism in which the effects of caldesmon on muscle contraction would be governed by the redox state of the tissue. Our preliminary studies, however, have shown no evidence of cross-linked oligomers of caldesmon associated with native thin filaments isolated from smooth muscle (data not shown).

A recent report by Okagaki et al. (43) describes the inhibition of actin filament velocity by caldesmon using a motility assay similar to that described in the present study. These authors saw that, with smooth muscle myosin, velocity decreased from 0.65 μm/s (27 °C) or 0.35 μm/s (24 °C) to 0 μm/s in the presence of a 5-fold molar excess of caldesmon over actin. The significantly higher control filament velocity observed in this study (1.9 μm/s) was due to at least three factors. Our experiments were conducted at 32 °C. Linear extrapolation of the velocities obtained by Okagaki et al. (43) gives a calculated velocity of 1.15 μm/s at 32 °C. The ionic strength of our solutions was slightly higher (80 to 50 mM KC1), which would result in an ~50% increase in velocity (36). We measured velocities only during periods of continuous filament motion that were sustained for 0.5–1.0 s, whereas Okagaki et al. (43) measured the displacement over a period of 20 s. We reanalyzed some of our control actin filament recordings to determine the average velocity over a 20-s period using a sampling rate of one frame/s and found it to be significantly lower (1.2 ± 0.2 μm/s; mean ± S.D., n = 28) than our previous determination of 1.9 μm/s (sampling rate = five frames/s). This apparent reduction in velocity was clearly the result of averaging intermittent filament motion when longer sampling intervals were used (37). The combined effects of temperature, ionic strength, and sampling interval account for the observed differences in control actin filament velocity in these two studies. The velocity reduction in the presence of caldesmon that these authors report may reflect, based on our own observations, the presence of covalently cross-linked oligomers of caldesmon. This would be consistent with their observation that actin filament bundling does occur in the presence of caldesmon (43) and with the observation of Lynch et al. (39) that actin filament bundling does not occur with fully reduced caldesmon.

Effects of Intact Caldesmon on Filament Binding and Velocity in the Absence of Methylcellulose—To determine if caldesmon can tether actin filaments to myosin as suggested by Hemric and Chalovich (23), we looked at filament motility in the absence of methylcellulose. We found that actin-tropomyosin filaments quickly dissociated from the myosin surface when ATP-containing motility buffer was added without methylcellulose and thereafter showed only brief periods of reversible binding. However, when 0.3–10 μM caldesmon was present, filament binding was increased and motility was evident. Filaments attached to the myosin surface only briefly with 0.3 μM caldesmon, but remained continuously attached with 10 μM caldesmon. The overall effect of caldesmon-dependent tethering was similar to that of methylcellulose; and presumably, caldesmon also promoted binding of actin to myosin and filament motion by restricting diffusion of the filaments away from the myosin surface. As shown in Fig. 8 (filled squares), the velocity of filament motion over the range of caldesmon concentrations where motility was evident (i.e. 0.3–10 μM) was not different from that determined in the presence of methylcellulose (Fig. 7, open squares), demonstrating that methylcellulose has no direct effect on filament velocity. Preliminary studies showing that an NH₂-terminal fragment of caldesmon competitively blocks filament binding at 30 mM KCl in the absence of methylcellulose (44, 45) further support the idea that the enhancement of filament binding by intact caldesmon involves the formation of an actin-caldesmon-myosin complex as originally described by Hemric and Chalovich (10).

The results of the motility studies also address the question of whether the actin-caldesmon-myosin complex can function as a load-bearing structure in smooth muscle, as has been proposed by others (11, 21–24, 46). Since the filaments are moving under unloaded conditions, velocity should be a very sensitive indicator of imposed load, as predicted by the minimal velocity curve for muscle. Evidence that small loads can affect filament velocity comes from a study by Warshaw et al. (36), who demonstrated that actin filament velocity with either smooth or skeletal muscle myosin is reduced presumably by the small load created by the presence of “weak-binding” cross-bridges. The actin-caldesmon-myosin complex is able to restrict diffusional motion of the filaments, but apparently imposes a relatively small load compared to the forces generated by cycling cross-bridges.

Conclusions—The effects of caldesmon on thin filament motility agree with the binding and kinetic studies previously reported by Chalovich et al. (1, 41). Our results demonstrate that binding of the COOH-terminal domain of caldesmon to actin competitively displaces myosin. Binding of actin filaments to myosin was increased in the presence of high concentrations of intact caldesmon, but was decreased by the actin-binding fragment of caldesmon, suggesting that formation of an actin-caldesmon-myosin complex can tether myosin to actin. This conclusion was further supported by a study that a molar excess of the COOH- or NH₂-terminal myosin-binding fragment competitively blocks the tethering of filaments to myosin by caldesmon (44). The observation that monomeric caldesmon and the actin-binding fragment had no effect on filament velocity argues that binding of caldesmon to actin does not alter the rate of cross-bridge cycling and that the actin-caldesmon-myosin complex is not a load-bearing structure at normal filament velocities. Therefore, it is unlikely that the actin-caldesmon-myosin complex contributes directly to the maintenance of force during the “latch state” in smooth muscle as proposed by others (11, 21–24) unless caldesmon forms cross-linked oligomers in situ. Preliminary studies have shown no evidence of covalently cross-linked caldesmon in native thin filament preparations. Further evidence that caldesmon is not required for the formation of latch bridges comes from our previous finding that smooth muscles that show latch, such as the porcine carotid artery, have a caldesmon:myosin:actin molar ratio of 1:4.5:240, compared to several phasic smooth muscles (e.g. guinea pig taenia coli, rat uterus, and rabbit ileum) in which the caldesmon:myosin:actin ratio is 1:1.22. If actin-caldesmon-myosin complexes are present in situ, it seems more plausible that they play a cytostructural role in maintaining the alignment of actin and myosin filaments or possibly in promoting polymerization or stabilizing the filamentous state of myosin in smooth muscle by providing a template for the localization of monomeric myosin. In this regard, it is inter-
cesting that the molar ratio of caldesmon to myosin is ~1:1 in rat uterus and guinea pig taenia coli. It remains to be determined if the inhibition of myosin binding to actin by the COOH-terminal domain of caldesmon has a regulatory role in smooth muscle.

Acknowledgments—We thank Christina Smith and Janet Desrosiers for technical assistance and Steven Work for helpful discussions about filament velocity measurements and for assistance with the computerized filament-tracking system.

REFERENCES