Activation of Regulated Actin by SH1-Modified Myosin Subfragment 1†

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ABSTRACT: The reactive SH1 (Cys-707) group of the myosin subfragment 1 (S1) has been used frequently as an attachment site for fluorescent and spin probes in solution and muscle fiber experiments. In this study we examined (i) the motor function of SH1 spin-labeled heavy meromyosin (HMM) in the *in vitro* motility assays and (ii) the effect of SH1-modified S1 on the motility of regulated actin, i.e., actin complexed with tropomyosin and troponin. *N*-ethylmaleimide (NEM), *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-iodacetamide (IASL), *N*-[[(iodoacetyl)amino]ethyl]1-sulfo-5-naphthylamine (IAEDANS), and iodoacetamide (IAA) were used to selectively modify the SH1 group on S1; the SH1 group on HMM was labeled with IASL. In the *in vitro motility* assays, 10−20% of unregulated actin filaments moved at a speed of ∼1 μm/s over a surface coated with 90−95% modified IASL-HMM. Actin sliding was not observed with 95−98% modified IASL-HMM. The sliding of regulated actin over unmodified HMM was activated by the addition of S1 modified with any of the SH1 reagents to the *in vitro* motility assay solutions; both the speeds and the percentage of the moving filaments increased at pCa 5, 7, and 8. To shed light on the activation of regulated actin sliding by SH1-modifed S1, acto-S1 ATPase and the binding to actin were determined for IASL-S1. While the binding affinities to actin were similar for IASL-S1 and unmodified S1 in the presence and absence of ADP and ATP, the K_m and V_{max} values were approximately 10-fold lower for the modified protein. It is concluded that the activation of regulated actin by SH1-modifed S1 facilitates the interaction of unmodified HMM heads with actin and thus can increase the sliding speeds and the percentage of regulated actin filaments that move in the *in vitro* motility assays.

The reactive SH1 (Cys-707) group of the myosin subfragment 1 (S1),[1] which can be selectively labeled with thiol reagents, has been a frequent target for attachment of fluorescent and spin probes in solution and muscle fiber experiments. Although the properties of SH1-modified S1 in solution have been studied extensively, the possible effects of SH1 modification on the interaction of myosin with regulated actin, as it occurs in muscle fibers, are known less well.

Earlier observations demonstrated that the SH1 modification altered strongly the Mg-ATPase cycle of S1, mostly accelerating the phosphate release step (Sleep et al., 1981; Ostap et al., 1993). Also, as indicated by the K_m and V_{max} values, the modification of the SH1 group drastically alters the actin-activated ATPase of S1 (Mulhern & Eisenberg, 1978). Furthermore, the motor function of such modified myosin appeared to be blocked in the *in vitro* motility assays (Root & Reisler, 1992). A similar conclusion was reached using a caged reagent to block the SH1 group on HMM. The labeled HMM did not propel actin filaments, but the movement was restored when the caged reagent was removed from the SH1 group by photolysis (Marriott & Heidecker, 1996). These and other solution studies demonstrated that the SH1 modification strongly affects the kinetic properties of S1 and disrupts its motor functions.

In contrast, fiber experiments revealed that SH1 spin-labeling of up to 50% of myosin heads in the muscle fibers with *N*-(1-oxy-2,2,6,6-tetramethyl-4-piperidinyl)iodacetamide (IASL) did not affect significantly the velocity of fiber shortening and force production (Crowder & Cooke, 1984). In another report, fibers with up to 95% IASL-modified myosin heads could still produce about 25% of the force generated by unmodified fibers (Bell et al., 1995). This suggests either that the modification of the SH1 group with IASL is less disruptive to the myosin motor functions than the labeling with some other SH reagents or that SH1 modification has additional effects on the systems containing regulated actin; i.e., actin complexed with tropomyosin and troponin.

Such additional effects can be predicted on the basis of experiments with S1 extensively modified by NEM (NEM-S1). NEM-S1 and *N,N′-p*-phenylenedimaleimide cross-linked S1 (pPDM-S1) activated the ATPase of S1-regulated actin complex (Williams et al., 1988; Greene et al., 1987). Also, it was shown that NEM-S1 accelerated fiber shortening and activated force production at submaximal calcium concentrations (Swartz & Moss, 1992). These observations were made on S1 modified at more than one thiol group (SH1). However, the SH1 modification was shown to abolish the Ca^{2+} sensitivity of regulated actomyosin (Titus

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[1] Abbreviations: HMM, heavy meromyosin; IAA, iodoacetamide; IAEDANS, *N*-[[(iodoacetyl)amino]ethyl]-1-sulfo-5-naphthylamine; IASL, *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)iodacetamide; NEM, *N*-ethylmaleimide; S1, myosin subfragment 1; SH1, the reactive SH1 group (Cys-707) on myosin.

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et al., 1989). We hypothesize that the SH1-modified S1 may also activate the regulated actin. If this is true, then in partially labeled muscle fibers the modified heads could activate regulated actin more efficiently than unmodified heads, facilitating, perhaps, the contractile function of unmodified heads.

In this work the interaction of SH1-modified myosin heads with regulated and unregulated actin was studied by using *in vitro* motility assays. Because IASL labeling of SH1 was used in the previous fiber studies, most of our experiments were performed on IASL-modified S1 and HMM. The main observations made on IASL-S1 were also confirmed using other SH1 reagents.

MATERIALS AND METHODS

Reagents. *N*-Ethylmaleimide (NEM) and iodoacetamide (IAA) were purchased from Sigma, *N*-(1-oxy-2,2,6,6-tetramethyl-4-piperidinyl)iodacetamide (IASL) was from Aldrich (Milwaukee, WI), and *N*-[[(iodoacetyl)amino]ethyl]-1-sulfo-5-naphthylamine (IAEDANS) was obtained from Molecular Probes (Junction City, OR).

Proteins. Myosin and actin from back and leg muscles of rabbits were prepared according to Godfrey and Harrington (1970) and Spudich and Watt (1971), respectively. S1 from rabbit myosin was prepared by digestion of myosin filaments with α-chymotrypsin (Weeds & Pope, 1997). Heavy meromyosin was prepared according to Margossian and Lowey (1982). The preparation of pyrene-labeled actin (pyrenyl-actin) was carried out as previously described (Cooper et al., 1983). The concentrations of S1, HMM, and actin were determined spectrophotometrically by using extinction coefficients of $E_{280}^{1\%} = 7.5\ \text{cm}^{-1}$, $E_{280}^{1\%} = 6.0\ \text{cm}^{-1}$, and $E_{290}^{1\%} = 11.5\ \text{cm}^{-1}$, respectively. The concentrations of SH1-modified S1 and pyrenyl-actin were determined by using the Bradford protein assay (1976).

SH1 modifications were carried out according to Reisler (1982) in solutions containing between 10 and 20 μM S1 or HMM, a 20× molar excess of IASL or IAEDANS, a 40× molar excess of iodacetamide, and a 2× molar excess of NEM over S1. The reactions were carried out in 30 mM KCl and 20 mM Tris-HCl at pH 7.5, at 0 °C, over 30−60 min (4 h for iodacetamide). The degree of SH1 modification was determined by measuring the K^+-EDTA and Ca^{2+}-ATPase activities (Reisler, 1982). Typically, 90−98% modified S1 and HMM were used in our experiments unless stated otherwise. More extensive modifications of myosin heads were avoided because they required higher reagent concentrations and longer modification times which could increase the probability of nonspecific modifications.

ATPase Activities. The ATPase activities of S1 and HMM were measured at 37 °C (Ca^{2+}- and K^+-EDTA-ATPase) and 20 °C (Mg^{2+}-ATPase) under steady-state conditions using the Malachite Green phosphate determination assay (Kodama et al., 1986). The Ca^{2+}-ATPase and K^+-EDTA-ATPase assay solutions contained 30 mM Tris-HCl (pH 7.5), 0.5 M KCl, and either 5.0 mM $CaCl_2$ or 5.0 mM EDTA. Mg^{2+}-ATPase measurements were done in solutions containing 10 mM PIPES (pH 7.0), 10 mM KCl, 3.0 mM $MgCl_2$, and 3.0 mM ATP, in the presence and absence of F-actin (between 5 and 100 μM).

In vitro motility assays were performed at 25 °C as described elsewhere (Homsher et al., 1996). In the assays with unregulated actin, IASL-HMM or mixtures of IASL-HMM and unmodified HMM were used with a total HMM concentration (IASL-HMM + HMM) set at 0.3 mg/mL. Concentrations between 0.02 and 0.3 mg/mL of unmodified HMM were used in the control experiments. Movement of filaments was initiated with solutions containing 0.4% methyl cellulose, 25 mM MOPS (pH 7.4), 25 mM KCl, 2.0 mM $MgCl_2$, 1.0 mM EGTA, 10 mM DTT, 1.0 mM ATP, and an oxygen-scavenging system. Unmodified HMM and SH1-modified S1 and HMM were prespun before experiments with actin and ATP to remove damaged heads as described before (Homsher et al., 1996). However, it should be noted that this procedure had no effect on the activation of regulated actin by SH1-modified S1 in the motility assays. Similar activation was observed with prespun and unspun SH1-modified S1.

The assays with regulated actin were performed using unmodified HMM (between 0.04 and 0.4 mg/mL). Reconstitution of thin filaments was carried out by mixing 2.0 μM rhodamine−phalloidin-labeled F-actin, 0.5 μM bovine cardiac or skeletal tropomyosin, and 0.5 μM bovine cardiac troponin in a buffer containing 4.0 mM imidazole hydrochloride at pH 7.1, 2.0 mM $MgCl_2$, and 1.0 mM DTT, followed by incubation on ice overnight. Tropomyosin and troponin were a generous gift of Dr. Larry S. Tobacman. SH1-modified S1 (1.0 μM) was added to this solution in the activation experiments. Movement of filaments was initiated with solutions containing the same components as for the assays with unregulated actin except for the addition of calcium to pCa 5, 7, and 8 levels, 0.1 μM tropomyosin, 0.1 μM troponin, and, in some experiments, 1.0 μM SH1-modified S1. Tropomyosin and troponin were included in the assay solution to stabilize the regulated actin at the low protein concentration used in these experiments (Homsher et al., 1996).

In the analysis of filament motility a filament was considered to be moving using criteria previously described (Homsher, et al., 1996). The reported filament sliding speed was the mean of the speeds of all moving filaments regardless of the uniformity of their movement.

F-Actin Binding. The binding of S1 and SH1-modified S1 to pyrenyl-F-actin was measured according to Geeves and Jeffries (1988) by a titration of 0.5 μM pyrenyl-F-actin with S1 or SH1-modified S1 at 20 °C in a solution containing 10 mM PIPES at pH 7.0, either 10 or 100 mM KCl, and 3.0 mM $MgCl_2$ in the presence and absence of 1.0 mM ADP. Pyrenyl-actin fluorescence was measured with excitation and emission wavelengths set at 365 and 407 nm, respectively. The K_d values were obtained by fitting the data to

$$(F_0 - F)/(F_0 - F_\infty) = \{(A + S + K_d) - [(A + S + K_d)^2 - 4AS]^{1/2}\}/2A \quad (1)$$

where F is the observed fluorescence, F_0 is the fluorescence in the absence of S1, and F_∞ is the fluorescence of pyrenyl-actin completely saturated with S1. A and S are pyrenyl-actin and S1 concentrations, respectively.

Cosedimentation Assays. The weak binding of S1 and SH1-modified S1 to F-actin was measured using cosedimentation assays as described before (Miller & Reisler, 1995). The solutions contained S1, between 5.0 and 30 μM, 4.0 μM phalloidin-stabilized F-actin, 3 mM ATP, 3 mM

Table 1: *In Vitro* Motility of Unregulated Actin over Unmodified HMM and Over IASL-Modified HMM[a]

	degree of modification (%)	moving filaments (%)	sliding speed (μm/s)
HMM	n/a	80−100	4.0−5.5
IASL-HMM	50−60	50−70	1.5−2.0
IASL-HMM	90−95	10−20	∼1.0
IASL-HMM	95−98	0	0

[a] Between 200 and 300 actin filaments were taken into analysis for each HMM preparation. The motilities of actin over unmodified HMM represent data from six separate preparations of HMM, of the same age as that of IASL-HMM. The IASL-HMM results refer to measurements on three separate preparations of HMM.

$MgCl_2$, 10 mM KCl, and 10 mM PIPES (pH 7.0). The samples were centrifuged at room temperature in a Beckman airfuge at 140000*g* for 10 min. Resuspended pellets and supernatants were examined on SDS−PAGE (Laemmli, 1970). The intensities of actin and S1 Commassie Blue stained bands were quantified using Biomed Instruments softlaser densitometer (Fullerton, CA). The K_d values were obtained by fitting the data to

$$S/A = \{(A + S + K_d) - [(A + S + K_d)^2 - 4AS]^{1/2}\}/2A \quad (2)$$

where S/A is the molar ratio of bound S1 to actin and A and S are actin and S1 concentrations, respectively.

RESULTS

In Vitro Motility Assays with IASL-HMM. The motor function of IASL SH1-modified HMM (IASL-HMM) was tested in the *in vitro* motility assays. The motilities of several IASL-modified HMM preparations were measured using unmodified HMM of the same age as the control. With unmodified HMM, depending on its age, between 80% and 100% of actin filaments moved at mean speeds ranging between 4.0 and 5.5 μm/s (Table 1). The degree of the modification of the SH1 group in HMM varied somewhat in successive experiments (90−98%). As shown in Table 1, extensively modified IASL-HMM (95−98%) did not propel actin filaments at all. IASL-HMM at a slightly lower degree of modification (90−95%) propelled between 10% and 20% of actin filaments with a speed of ∼1 μm/s. This motility, observed at a lower degree (90−95%) of IASL-HMM modification, can be attributed to a small fraction of unmodified HMM heads present in such preparations (5−10%). Similarly, the motility of actin filaments observed with ∼50% modified IASL-HMM (Table 1) can be accounted for by the presence of unmodified HMM (∼50%) in such preparations. Since a 2-fold diluted unmodified HMM (∼0.15 mg/mL) produced similar motion of actin to that with undiluted HMM, this suggests that the IASL-HMM heads in the ∼50% modified HMM introduce some load into motility assays.

Our results suggest that IASL-HMM either does not generate motion or is a very slow motor that generates less force than the unmodified HMM. Such a motor deficiency can be in principle overcome in the *in vitro* motility assays in the presence of ADP. ADP was shown to increase the force generation in rabbit muscle fibers (Pate & Cooke, 1989) and in the *in vitro* motility assays (Miller et al., 1996) while decreasing the speed of filament sliding. Increased temperature also generally improves motility. Addition of ADP to the motility assay of IASL-HMM had marginal, if any, effect on the motion of actin filaments (Table 2). In contrast to that, when the motion of actin over unmodified HMM was impaired because of low HMM concentrations (0.02 mg/mL in the adsorption solution), ADP increased considerably the fraction of actin filaments sliding over unmodified HMM (Table 2). Similarly, while raising the temperature in the *in vitro* motility assays to 30 °C increased the sliding speed of actin over unmodified HMM, it had no effect on actin's motion over IASL-HMM. These results suggest that IASL-HMM alone either does not propel actin filaments or perhaps does so very slowly.

Table 2: Effect of ADP on the Percentage of Moving Filaments in the *in Vitro* Motility Assays[a]

	degree of modification (%)	moving filaments (%)	
		no ADP	2 mM ADP
HMM (0.3 mg/mL)	n/a	99.0	98.4
HMM (0.1 mg/mL)	n/a	85.6	99.5
HMM (0.02 mg/mL)	n/a	68.9	99.0
IASL-HMM (0.3 mg/mL)	95	19.0	24.5
IASL-HMM (0.3 mg/mL)	98	0	0

[a] The concentration of unmodified HMM used for the adsorption to the cover slip is indicated in the parentheses. Between 200 and 300 actin filaments were analyzed in each case.

In Vitro Motility Assays with Regulated Actin and SH1-Modified S1. The ability of SH1-modified myosin heads to activate regulated actin was tested in the *in vitro* motility assays. These experiments were performed on actin filaments regulated by cardiac or skeletal tropomyosin and cardiac troponin. To eliminate the possible load from the modified myosin heads, SH1-modified S1 (instead of HMM) was added to actin in solution in lieu of its adsorption to the cover slip surface. First, the motility of regulated actin filaments propelled by unmodified HMM was measured at different pCa values (Figure 1, white bars). Then, motility was measured under the same conditions except for the presence of IASL-S1 in the assay solutions (Figure 1, black bars). Addition of IASL-S1 significantly increased the sliding speed of regulated actin and the fraction of filaments moving at pCa 7 and 8, i.e., under the conditions of marginal or incomplete switching "on" of regulated actin by Ca^{2+}. Addition of unmodified S1 instead of IASL-S1 to the assays solutions had no effect on the motility of regulated actin (Figure 1, gray bars). Notably, as shown in Figure 1, IASL-S1 had no effect on the motility of actin at pCa 5. A possible reason for such a lack of additional activation of filament sliding at pCa 5 could be that the regulated actin was already fully activated under our experimental conditions. To test this explanation, we measured the *in vitro* motility of regulated actin at pCa 5 on cover slips coated with a low concentration solution of HMM. Low HMM density on the cover slip surface resulted in lower saturation of the regulated actin filaments by HMM heads and consequently, an incomplete switching "on" of the regulated actin. That, in turn, caused a decrease in the sliding speeds and in the fraction of filaments that moved (Figure 2, white bars). Under such conditions, addition of IASL-S1 to the assay solutions improved actin motility (Figure 2, gray bars) showing that IASL-S1 can activate the regulated actin also at pCa 5. Thus, IASL-S1 activates regulated actin over a wide range of calcium concentrations (pCa 5−8). The marked increase in

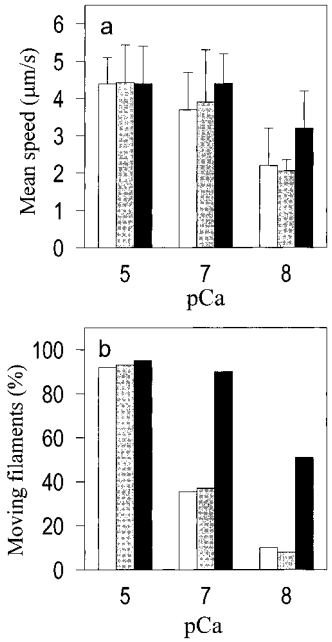

FIGURE 1: Mean speeds (panel a) and the percentage of actin filaments moving (panel b) in the *in vitro* motility assays with regulated actin. White bars, actin filaments propelled by unmodified HMM; gray bars, same as for the white bars except for the presence of 1.0 μM unmodified S1 in the assay solution; black bars, same as for the white bars except for the presence of 1.0 μM IASL-S1 in the assay solution. The standard deviations are indicated for all measurements. Between 200 and 300 actin filaments were analyzed in each experiment. The experiment was repeated four times.

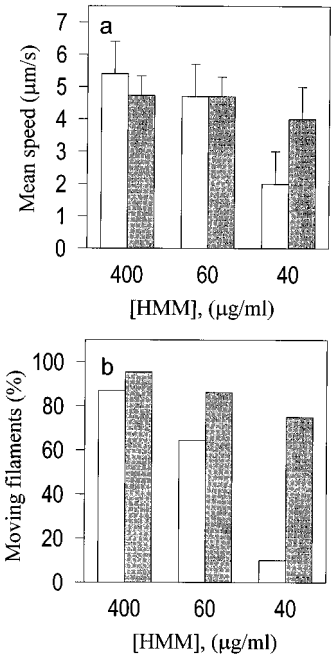

FIGURE 2: Mean speeds (panel a) and the percentage of actin filaments moving (panel b) in the *in vitro* motility assays with regulated actin at pCa 5. The concentrations of unmodified HMM solutions that were used for coating the cover slips and to produce different densities of HMM molecules on the surface are indicated under the bars. White bars, actin filaments propelled by unmodified HMM; gray bars, same as for the white bars except for the presence of 1.0 μM IASL-S1 in the assay solution. The standard deviations are indicated for all measurements. Between 200 and 300 actin filaments were monitored in each experiment. The experiment was repeated three times.

the fraction of filaments that move (panel b, Figures 1 and 2) indicates that the activation of regulated actin increases the interaction of unmodified HMM heads with actin filaments.

Table 3: Equilibrium Dissociation Constants (K_d) for Actin·IASL-S1 and Actin·S1 Complexes[a]

	K_d (μM)			
	10 mM KCl	100 mM KCl	1 mM ADP, 10 mM KCl	3 mM ATP, 10 mM KCl
S1	0.03 ± 0.01	0.07 ± 0.01	0.27 ± 0.10	39 ± 3
IASL-S1	0.03 ± 0.01	0.08 ± 0.04	0.30 ± 0.10	47 ± 12

[a] Dissociation constants for acto−S1 complexes were determined as described in Materials and Methods. At least three separate determinations were made for each K_d value.

Experiments similar to those shown in Figure 1 were also performed with S1 in which the SH1 group was modified by other thiol reagents: IAA, IAEDANS, and NEM (data not shown). Inclusion of S1 modified with any of these reagents in the motility assay had a similar effect to that of IASL-S1 (Figures 1 and 2), i.e., an increase in the speeds and the fraction of regulated actin filaments that moved over unmodified HMM. Thus, any SH1-modified S1 tested in this work activates regulated actin in the *in vitro* motility assays.

Solution Interactions of IASL-S1 and Actin. The impairment of the myosin motor function and the activation of the regulated actin filaments reveal important changes in acto-myosin interactions after SH1 modification. To shed light on these changes, the strong and weak binding of IASL-S1 to actin and the activation of its ATPase activity by actin were compared to those with unmodified S1. The strong binding affinities of S1 to actin were determined by titrations of pyrenyl-actin with S1. As shown in Table 3, IASL-S1 and unmodified S1 have similar affinities to actin at low (10 mM KCl) and high (100 mM KCl) ionic strength conditions and in the presence of 1 mM MgADP. The weak binding affinities of S1 to actin were determined by cosedimentation assays. As shown in Table 3, SH1 modification does not change substantially the weak binding of S1 to actin. Representative plots of the ATPase activities of unmodified S1 and IASL-S1 versus actin concentration are shown in Figure 3. The fitting of these curves to the Michaelis−Menten equation yielded K_m and V_{max} values of 20.0 ± 2.0 μM and 7.8 ± 0.2 s^{-1} for unmodified S1 and 2.0 ± 0.2 μM and 0.93 ± 0.02 s^{-1} for IASL-S1. This agrees well with the previously reported results (Mulhern & Eisenberg, 1978). Thus, modification of the SH1 group with IASL decreases strongly the activation of S1 Mg-ATPase by actin.

DISCUSSION

According to current views, myosin plays a dual role in the contractile cycle in muscle. First, myosin heads act as motors and transducers of energy, generating force and propelling actin filaments. Second, in conjunction with the Ca^{2+} switch of troponin−tropomyosin, myosin activates the regulated actin filaments. In the McKillop and Geeves (1993) model, this myosin-induced activation of the regulated actin corresponds to a shift between the "closed" and "open" states of regulated actin. In this work we presented new evidence demonstrating that SH1 modification on myosin alters both the motor and the actin activation properties of myosin heads in the *in vitro* motility assays.

It was shown previously that the modification of the SH1 group with phenylmaleimide (Root & Reisler, 1992) and with

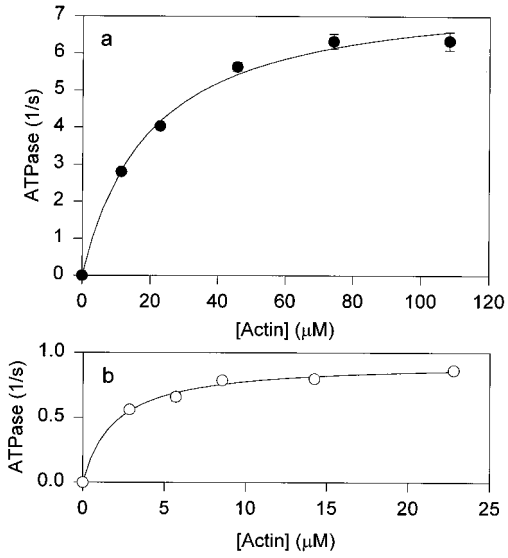

FIGURE 3: Representative plots of the S1 Mg^{2+}-ATPase activity vs actin concentration. Panel a, unmodified S1; panel b, IASL-S1. The activities in the absence of actin (0.05 s^{-1} for S1 and 0.4 s^{-1} for IASL-S1) were subtracted from the activities determined in the presence of actin. The ATPases were measured at 20 °C in 10 mM PIPES at pH 7.0, 10 mM KCl, 3.0 mM $MgCl_2$, and 3.0 mM ATP. Fitting of the data to the Michaelis−Menten equation yielded in the K_m and V_{max} values of 20.0 ± 2.0 μM and 7.8 ± 0.2 s^{-1} for unmodified S1 and 2.0 ± 0.2 μM and 0.93 ± 0.02 s^{-1} for IASL-S1. The experiment was repeated three times.

1-(bromomethyl)-2-nitro-4,5-dimethoxybenzene (Marriott & Heidecker, 1996) abolished the *in vitro* motility of myosin and HMM, respectively. This time, we have chosen the spin probe IASL to study the effects of SH1 modification on the motor functions of HMM. This probe has high specificity for SH1 and has been widely used in solution and muscle fiber experiments, and the effects of its attachment to SH1 on force production in fibers are well documented (Crowder & Cooke, 1984; Bell et al., 1995). All precautions were taken to avoid nonspecific modification of HMM; the reactions were limited to about 90−98% labeling of HMM, and the Ca^{2+}-ATPase and K^+-EDTA-ATPase activities were routinely monitored. The IASL-modified HMM had a limited ability to propel actin filaments in the *in vitro* motility assays at a 90−95% modification and lost that ability at a slightly higher degree of modification (95−98%). Our results support previous observations that SH1 modifications abolish the motor function of myosin in the *in vitro* motility assays.

The most important result of this study is that SH1-modified myosin heads can activate the *in vitro* sliding of regulated actin. Such activation was most prominent at the low calcium concentrations (pCa 7 and 8; Figures 1 and 3) and at low HMM density (Figure 2), i.e., under conditions at which the amounts of unmodified HMM and Ca^{2+} were insufficient to completely switch "on" the regulated actin filaments. SH1-modified S1, when added into motility assay solutions, activated actin filaments, increasing their sliding speeds and the fraction of filaments that moved (Figures 1 and 2). It is important to note, that under the same conditions unmodified S1 did not affect actin motility (Figure 1, gray bars). Thus, SH1-modified S1 is a more effective activator of regulated actin than unmodified S1. The fact that all SH1-modified S1 had similar activating effects in the *in vitro* motility assays suggests that the mechanism of regulated actin activation is not affected significantly by the nature of the SH reagents.

The activation of regulated actin by the SH1-modified S1, but not by unmodified S1, raised questions about modification-related changes in actin-binding properties of S1. Our results showed that IASL-S1 and unmodified S1 had similar affinities to actin in the absence of nucleotides and in the presence of ADP and ATP. However, the actin activation of S1 Mg-ATPase was decreased drastically by the modification. It is unclear at the moment how SH1 modification converts the modified S1 into a more efficient activator of the regulated actin than the unmodified S1. We ruled out simple explanations such as changes in actin strong binding and the desensitization of this binding to ATP (Table 3). A detailed kinetic study of the acto-IASL-S1 will be required to clarify the mechanism of activation of regulated actin.

The two main conclusions of our study are that SH1 modification disrupts the motor function of myosin heads and that it converts myosin heads into more effective activators of regulated actin. The first conclusion appears to be in conflict with the results of muscle fiber experiments. For example, one study showed that the fibers with up to 95% IASL-modified myosin heads produced about 25% of active force (Bell et al., 1995). It was assumed on the basis of this and similar observations in fibers that, in contrast to the implications of solution experiments, SH1-modified myosin can generate force and propel actin filaments, albeit less effectively than the unmodified cross-bridge. At a qualitative level, our results suggest an alternative explanation for these observations. If the SH1-modified myosin heads do not generate force in the muscle fibers but instead activate the actin filaments, it is possible that the remaining unmodified heads generate more force than predicted by their percentage in the total population of myosin heads in the fiber. Such a possibility is demonstrated for the *in vitro* motility case in the experiment with diluted HMM at pCa 5. Figure 2 shows that the motor function of unmodified HMM at 0.04 mg/mL is facilitated, or appears improved, through the activation of regulated actin filaments by IASL-modified S1.

Active shortening of intact muscle fibers during a twitch reduces the force production and the duration of the twitch (Edman, 1975). Similarly, Caputo et al. (1994) showed that muscle shortening during relaxation from a tetanus produces a transient rise in cytosolic [Ca^{2+}] which is thought to be released from the troponin. These results suggest that the presence of strongly bound force-bearing cross-bridges promotes thin filament activation. Reductions in force exerted by attached cross-bridges or the number of cross-bridges *per se* may deactivate the thin filament. These physiological results are consistent with the actin-activating effects of modified myosin heads reported above. Activation of regulated actin motion with unmodified S1 was not achieved in our assays despite the above physiological indications. This lack of activation may be due to the competition for actin binding between the solution S1 and the necessarily small amount of HMM on the cover slip. Either a kinetic or mechanistic advantage of SH1-modified over unmodified S1 leads then to the activation of regulated actin with the former and not the latter S1.

It was shown before that S1 modified at more than one thiol group by NEM accelerated fiber shortening velocity and activated force production at submaximal calcium

concentrations (Swartz & Moss, 1992). We observed in the *in vitro* motility assays that such modified S1 and SH1-modified S1 activate regulated actin filaments in a qualitatively similar manner (unpublished data). Thus, it can be assumed that SH1-modified S1, by analogy to NEM-S1, should be able to activate actin in the muscle fibers. In addition to an activation of regulated actin in muscle fibers, the modified head on the myosin molecule could act as a latch to actin, facilitating the interaction of the unmodified head with actin and potentiating the force output from the modified fibers. This suggestion is based on the fact that in the extensively modified myosins very few if any myosin molecules are left with two unlabeled heads.

Overall, our study supports the concern of other researchers (Mulhern & Eisenberg, 1978; Titus et al., 1989; Root & Reilser, 1992; Marriott & Heidecker, 1996) about the suitability of the SH1 group as an attachment site for probes of myosin cross-bridges in the force-generating states. The modification of this group alters major functions of the myosin head.

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