Uncoupling of Actin-Activated Myosin ATPase Activity from Actin Binding by a Monoclonal Antibody Directed against the N-Terminus of Myosin Light Chain 1†

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ABSTRACT: The role of the N-terminal region of myosin light chain 1 (LC1) in actomyosin interaction was investigated using an IgG monoclonal antibody (2H2) directed against the N-terminal region of LC1. We defined the binding site of 2H2 by examining its cross-reactivity with myosin light chains from a variety of species and with synthetic oligopeptides. Our findings suggest that 2H2 is directed against the N-terminal region of LC1 which includes the trimethylated alanine residue at the N-terminus. In the presence of 2H2, the rate of actomyosin superprecipitation was reduced, although the extent was not. 2H2 caused a reduction in the V_{max} of both myosin and chymotryptic S1(A1) actin-activated ATPase activity, while the K_m appeared to be unaltered. The Mg^{2+}-ATPase activity of myosin alone was also unaffected. Binding studies revealed that 2H2 did not prevent the formation of acto–S1 complex, either in the presence or in the absence of ATP, nor did it affect the ability of ATP to dissociate S1 from F-actin. Our findings suggest that the N-terminal region of LC1 is not essential for actin binding but is involved in modulating actin-activated ATPase activity of myosin.

Actin and myosin are essential for movement and the generation of force in skeletal muscle. Despite an increasing understanding of the nature and function of these proteins, the basic underlying molecular mechanism of motility is still to be determined. Using an in vitro assay, Toyoshima et al. (1987) demonstrated that, in the presence of Mg^{2+}-ATP, the papain-generated subfragment 1 (S1)[1] portion of myosin alone is capable of moving actin filaments. This showed that only one of the two "heads" is necessary for movement, thus narrowing the molecular mechanism down to a single head.

Each myosin head is associated with two noncovalently attached myosin light chains (LCs). One is referred to as the alkali light chain (because it could be dissociated under alkaline conditions) and the other, the regulatory light chain. The alkali light chains were originally thought to be essential for myosin function. It has since been shown that S1 heavy chain devoid of the alkali light chains was still capable of binding actin and hydrolyzing ATP, though actin-activated ATPase activity in the absence of the alkali light chains was reduced (Wagner & Giniger, 1981). The role of the regulatory light chains in skeletal muscle is not clear, though they are believed to be involved in Ca^{2+}-mediated modulation of myosin activity in scallop muscle (Kendrick-Jones et al., 1976).

There are two types of alkali light chains in rabbit fast twitch skeletal myosin, LC1 (A1) and LC3 (A2). They occur at molar ratios of 1.3 and 0.7, respectively, per mole of myosin. The primary structures of LC1 and LC3 are identical except that LC1 possesses an additional 41 residues at the N-terminus, which is rich in proline, lysine, and alanine. S1 prepared by digesting myosin with chymotrypsin consists of the head region of the myosin heavy chain with only an alkali light chain bound. The regulatory light chain (LC2) is removed in the preparation. Chymotryptic S1 can be separated by their alkali light chain content, S1(A1) containing LC1 and S1(A2) containing LC3 (Weeds & Taylor, 1975). The isoenzymes have different actin-activated ATPase activities which appear to be due to the particular alkali light chain present (Wagner & Weeds, 1977). It has also been observed that the affinity of S1(A1) for actin is greater than S1(A2) at low ionic strength (Wagner et al., 1979) and in the presence of Ca^{2+} at raised ionic strengths (Trayer & Trayer 1985). Chaussepied and Kasprzak (1989) also made the observation that unlike S1(A1), S1(A2) does not induce actin polymerization. The N-terminal region of LC1 is believed to be highly mobile (Prince et al., 1981). The N-terminal residue is a trimethylated alanine (Me_3A), and ^{1}H-NMR studies have suggested that this residue interacts with actin (Henry et al., 1985). It was of interest, therefore, to study the effects of blocking this interaction on (i) the ability of the myosin head to bind to F-actin, (ii) the myosin ATPase activity and its activation by actin, and (iii) superprecipitation of actomyosin, the in vitro functional analogue of contraction.

In the present study, specific blocking of the N-terminus of LC1 (and LC2) was achieved by the use of an IgG monoclonal antibody (2H2) which we show to bind to the N-terminal residue. Our data suggest that blocking of the N-

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[1] Abbreviations: BSA, bovine serum albumin; CM, carboxymethyl; DEAE, diethylaminoethyl; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; F-actin, filamentous actin, ^{1}H-NMR, proton nuclear magnetic resonance; LC1 (A1), alkali light chain 1; LC3 (A2), alkali light chain 2; LC2, regulatory light chain; Me_3A, trimethylalanine; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; S1, chymotryptic subfragment 1 of myosin; S1(A1), S1 containing LC1; S1(A2), S1 containing LC3; SDS, sodium dodecyl sulfate.

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terminus of LC1 can affect the rate of actin-activated ATPase and superprecipitation of actomyosin without interfering with the binding of actin to myosin either in the absence or in the presence of ATP at low salt.

Materials and Methods

Protein Preparations. Myosin was prepared according to Tonomura et al. (1966) from rabbit skeletal muscle. Myosin light chains were extracted from myosin by dissociation in 6 M guanidine hydrochloride followed by ethanol precipitation, and further purified by ion-exchange chromatography according to Holt and Lowey (1975). Actin was prepared from acetone-dried powder of rabbit skeletal muscle according to Spudich and Watt (1971). S1 was prepared by chymotryptic digestion of myosin purified by DEAE ion-exchange chromatography (Weeds & Taylor, 1975). Separation of S1 into its isoenzymes S1(A1) and S1(A2) was achieved by passage of purified S1 through a CM-Sepharose (Pharmacia) column according to Prince et al. (1981). Crude muscle extracts were obtained by denaturation of whole muscle tissue in 6 M guanidinium isothiocyanate (Somerville & Wang, 1987). The preparation of the LC2 mutant 442 has been described elsewhere (Reinach et al., 1986). Trimethylated alanine was prepared by refluxing L-alanine in methanol with methyl iodide for 8 h under alkaline conditions (Henry et al., 1982). An alkaline pH was maintained by the addition of saturated NaOH. Excess reagent was removed by rotary evaporation in vacuo. The synthetic peptide PKK was synthesized according to the Merrifield method (1963) using an LKB Biolynx manual peptide synthesizer. Protein concentrations were determined using extinction coefficients of $E^{0.1\%}_{290nm} = 0.63$ cm^{-1} for G-actin (Lehrer & Kerwar, 1972) and $E^{0.1\%}_{280nm} = 0.56$ cm^{-1} for myosin (Chock & Eisenberg, 1979), 0.76 cm^{-1} for S1 (Weeds & Pope, 1977), 0.22 cm^{-1} for LC1 and LC3 (Wagner, 1982), 0.6 cm^{-1} for LC2 (Holt & Lowey, 1975), and 1.4 cm^{-1} for monoclonal IgG (Goding, 1983).

Monoclonal Antibody 2H2. The preparation and partial characterization of the antibody "2H2" have been described elsewhere (Noakes et al., 1986). The antibody was purified from ascites fluid using an Affi-Gel protein A MAPS kit (Bio-Rad) and digested with papain (1–2 mg/mL) under reducing conditions to produce Fab fragments. Undigested antibody and Fc fragments were removed by another passage over the protein A support. The specificity of 2H2 was further evaluated by Western blot and enzyme-linked immunosorbent assay (ELISA). SDS-solubilized human, rabbit, and bovine ventricular muscle extract, purified myosin light chains, and LC2 mutant 442 were separated on 15% SDS–PAGE according to Laemmli et al. (1970). The gels were then electroblotted onto nitrocellulose according to Towbin et al. (1979). A portion of the nitrocellulose was then stained with amido black while the remainder was treated overnight with 2% BSA in 20 mM PBS, pH 7.5. The nitrocellulose was then incubated with a 1:500 dilution of ascites antibodies. After the membranes were washed in 0.4 M NaCl, 20 mM Tris, pH 7.5, and 0.1% Tween 20, antibody binding was visualized by incubating the nitrocellulose with a 1:150 dilution of a peroxidase-conjugated goat anti-mouse antibody (Sigma) and then by adding the substrate 4-chloro-1-naphthol. Cross-reactivities of 2H2 with Me_3A, mutant 442, and the synthetic peptide PKK were evaluated by ELISA according to the method described by Axiak et al. (1987). Microtiter wells were coated with antigen by incubation at 37 °C for 1 h with 50 μL of Me_3A, 442, PKK, LC2, unmodified L-alanine, or BSA. Unbound antigen was removed, and unblocked sites were saturated with 1% BSA. 2H2 at 1:1000 dilution of ascites was then added and incubated for another 1 h at 37 °C, and binding was assessed by the addition of alkaline phosphatase-conjugated sheep anti-mouse antibody (Tago). Color was developed by the addition of substrate (*p*-nitrophenyl phosphate, Sigma) at 2 mg/mL, and absorbance was determined at 405 nm using a Flow Titertek Multiscan.

Superprecipitation. Solvent conditions for superprecipitation measurements were 25 mM KCl, 20 mM Pipes, pH 7.0, and 1 mM $MgCl_2$. Actomyosin was reconstituted by combining F-actin and myosin at a molar ratio of 4:1. The final protein concentrations were 2 μM (0.1 mg/mL) F-actin and 0.5 μM (0.25 mg/mL) myosin. The effect of 2H2 on this reaction was studied either (1) by the preincubation of 2H2 with myosin for 30 min at different molar ratios of 2H2 to myosin or (2) by the addition of 2H2 to preformed actomyosin. The proteins were mixed thoroughly in a Teflon–glass homogenizer, and 2-mL aliquots were transferred to a 1-cm-path-length cuvette. The superprecipitation reaction was initiated by the addition of ATP to 0.1 mM which was rapidly mixed in the cuvette, and the time course of the turbidity change was monitored at 650 nm using a Philips PU8800 spectrophotometer.

ATPase Activity. ATPase activities of myosin and S1 were determined using a pH stat apparatus (Radiometer), as described by Eisenberg and Moos (1970). The pH was set at 7.0 and maintained by titration with 2 mM KOH. Solvent conditions were 3 mM $MgCl_2$, 20 mM KCl, 1 mM imidazole, pH 7.0, and 3 mM ATP. The actin-activated ATPase activity was obtained by the addition of F-actin to myosin or S1 in the solvent conditions described above. Starting protein concentrations were 1.5 μM (0.75 mg/mL) myosin or 3 μM (0.36 mg/mL) S1, and F-actin was titrated in 1.6-nmol steps until activation rates plateaued. The starting volume of each sample was 1.5 mL, and the titrations were stopped when the volumes exceeded 2.0 mL. To investigate the effects of 2H2 on ATPase rates, 2H2 was either (1) incubated with the myosin or S1 for 30 min prior to assay using 2H2 molar concentrations of 0.16–0.6 μM (0.25–1.0 nmol in 1.5 mL) or (2) titrated in 0.25-nmol steps into a 1.5-mL solution containing 1.5 μM myosin and 2 μM F-actin until complete inhibition was achieved.

Airfuge Binding Assays. The binding of 2H2 and actin to chymotryptic S1 was measured in both the absence (rigor) and presence of ATP; 2 μM (0.2 mg/mL) S1 and 4 μM (0.16 mg/mL) F-actin were incubated with 0–3 μM (0–0.5 mg/mL) 2H2 for 20 min at room temperature, followed by centrifugation at 30 psi for 15 min using the Beckman airfuge to pellet the F-actin and separate free and bound 2H2 or S1. The solvent was 10 mM KCl, 10 mM imidazole, pH 7.0, 3 mM $MgCl_2$, and 1 mM EGTA. The total volume of each sample was 150 μL. Pellets were resolubilized in SDS gel buffer, and the protein contents of supernatants and pellets were analyzed by SDS gel electrophoresis using 15% polyacrylamide gels. Binding of purified S1(A1) and S1(A2) to F-actin and 2H2 in rigor was also compared using the same conditions described.

Acto–S1 binding in the presence of 2H2 and ATP was measured using protein concentrations of 2 or 20 μM S1 and 4 μM F-actin in the same solvent conditions as above, except that 5 mM ATP was added immediately prior to centrifugation at 4 °C at 30 psi for 15 min using a Beckman airfuge.

Results

Characterization of the 2H2 Binding Site. Cross-reactivity of 2H2 with various muscle extracts and myosin light chains was analyzed by Western blot analysis and the results are shown in Figure 1. These demonstrate that 2H2 specifically

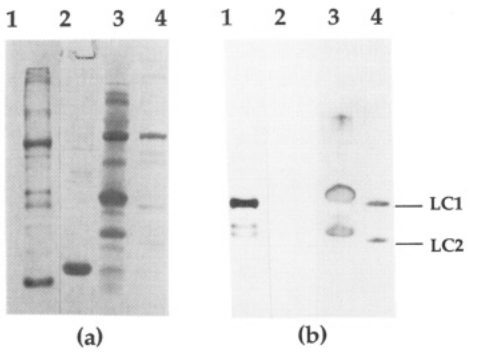

FIGURE 1: Western blot of 2H2 with various muscle extracts. Panel a shows the amido black stained membrane and panel b the corresponding immunoblotted membranes. Lanes: (1) human skeletal muscle extract; (2) LC2 mutant 442; (3) bovine ventricular muscle extract; and (4) rabbit skeletal muscle extract.

Table I: Summary of 2H2 Binding Specificities

positive binding	negative binding
rabbit fast skeletal LC1, LC2	rabbit LC3
chicken skeletal LC1, LC2[a]	chicken LC3[a]
human skeletal LC1, LC2	chicken LC2 mutant 442
human ventricular LC1, LC2[b]	L-alanine
bovine ventricular LC1, LC2	synthetic PKK
synthetic trimethylalanine	

[a] Noakes et al. (1986). [b] Trahair (1990).

binds only to myosin LC1 and LC2 of human skeletal, rabbit skeletal, and bovine ventricular muscle. 2H2 does not bind LC3. The binding specificities of 2H2 are summarized in Table I. The C-terminal 141 residues of LC1 and LC3 are identical, and LC1 has an additional sequence of 41 residues at its N-terminus. Between this additional sequence and the sequence common to both proteins are eight amino acid residues which contain only three residues common to both LC1 and LC3 (Frank & Weeds, 1974). Therefore, it is probable that the antigenic determinant is located at the N-terminus of LC1, within the first 49 residues. We searched for homologous sequences of at least 3 residues in length within the N-terminal 49 residues of LC1, and LC2. The search included published amino acid sequences of chicken and rabbit skeletal (Perisamy et al., 1984; Kurma et al., 1986), human skeletal (Seidel et al., 1987), and human ventricular light chains (Hoffman et al., 1988). We found only one sequence common to both LC1 and LC2 which is not found in LC3. This sequence is the N-terminal tetrapeptide of both LC1 and LC2, Me_3APKK. Thus, we have by a process of elimination identified the N-terminal four residues of LC1 and LC2 as the putative epitope.

To test our hypothesis, we cross-reacted 2H2 with various synthetic peptide fragments of the putative epitope by ELISA. Figure 2 is a histogram illustrating the binding specificities of 2H2 to synthetic Me_3A, PKK, unmodified L-alanine, LC2, and BSA. The results show that 2H2 binds synthetic Me_3A and LC2 but not PKK, L-alanine, or BSA.

Cross-reactivity of 2H2 with the LC2 mutant 442 was also examined by ELISA and Western blot. 442 is a bacterially expressed mutant chicken skeletal LC2 which has a 16-residue deletion from 5 to 20 inclusive and does not contain the posttranslational trimethylation of the N-terminal alanine. Thus, it still contains the N-terminal tetrapeptide APKK, but with an unmodified alanine at the N-terminus. The ELISA (Figure 2) and Western blot (Figure 1, lane 3) show that 2H2 does not bind mutant 442. Taken together, we have defined the binding site of 2H2 to the N-terminal region of LC1 and LC2, and it appears that the N-terminal residue Me_3A is

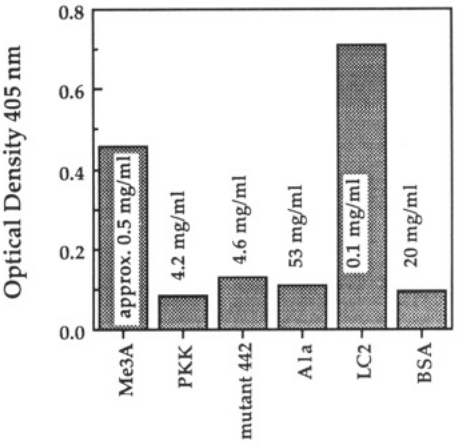

FIGURE 2: Relative binding affinities of 2H2 to synthetic Me_3A, PKK, mutant 442, unmodified L-alanine, LC2, and BSA as revealed by ELISA. The stock concentration of each antigen used is displayed with each column.

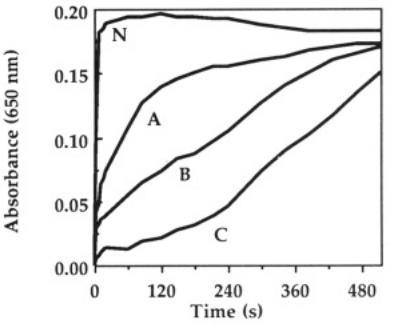

FIGURE 3: Change in turbidity of actomyosin at 650 nm with respect to time. The superprecipitation was initiated with the addition of ATP at time 0. Protein concentrations were 2 μM F-actin and 0.5 μM myosin in either the absence (N) or the presence of 1:1 (A), 2:1 (B), or 4:1 (C) molar ratios of 2H2 to myosin. Solvent conditions were 25 mM KCl, 20 mM Pipes, pH 7.0, and 1 mM $MgCl_2$.

essential for the antigenicity of 2H2.

Inhibition of Actomyosin Superprecipitation by 2H2. Figure 3 shows the change in turbidity of actomyosin with time following the addition of ATP. Trace N shows the superprecipitation of actomyosin in the absence of 2H2. We observed the rate of superprecipitation in the presence of a 1–4 molar excess of 2H2/myosin, where 2H2 was added to myosin before mixing with F-actin. The time taken for superprecipitation to reach half-maximum ($t_{1/2}$) increased from 2.5 ± 0.5 (SEM) s in the absence of 2H2 to 102 ± 15 s at a ratio of 1:1, 225 ± 45 s at 2:1, and 340 ± 76 s at a 4:1 2H2:myosin ratio. Thus, in the presence of increasing amounts of 2H2, there is a progressive slowing of the rate of superprecipitation with little or no effect on the final extent of the reaction. The same result was also obtained when 2H2 was added to a preformed complex of actin and myosin (data not shown). To eliminate possible "steric artefacts" due to cross-linking by the divalent antibody, the monovalent Fab fragments of 2H2 were also tested. The results showed that the $t_{1/2}$ time was increased from 2.5 s to over 70 s in the presence of a 4:1 molar ratio of Fab to myosin. Thus, there was still significant inhibition of the superprecipitation rate by the Fab fragments, although its effect was not as dramatic. Control experiments using an IgG monoclonal antibody directed against κ light chains (Axiak et al., 1987) had no effect on these two superprecipitation parameters.

Inhibition of Actin-Activated ATPase Activity by 2H2. The presence of 2H2 antibody did not affect the Mg^{2+}-ATPase activity of myosin alone, although it inhibited the actin-ac-

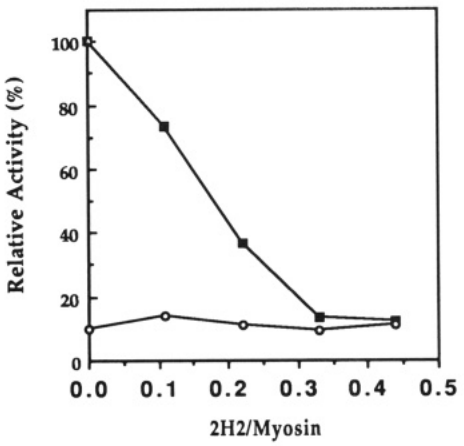

FIGURE 4: Mg^{2+}-ATPase activity (O) and the actin-activated ATPase activity (■) of myosin in the presence of increasing amounts of 2H2. The actin-activated ATPase rate in the absence of 2H2 was 0.43 μmol·mg^{-1}·min^{-1}, expressed as 100% activity. All other rates are expressed relative to it.

tivated ATPase activity both of intact myosin and of its chymotryptic S1 fragment. Figure 4 illustrates the extent of inhibition of actin-activated myosin ATPase activity. The results show an increasing inhibition with increasing concentrations of 2H2 present. It is of interest to note, however, that at a 0.2 molar ratio of 2H2 to myosin, we see up to 60% inhibition of ATPase activity. This discrepancy in stoichiometry may be due to cooperativity between the two heads of myosin. Further measurements using single-headed S1 were also made; the results are described later in the text. Inhibition of actin-activated ATPase activity by 2H2 was equally effective when 2H2 was preincubated with myosin before addition of F-actin, or when 2H2 was added to preformed actomyosin. The uninhibited actin-activated ATPase rate is normalized to 100% (0.43 μmol of P_i·mg^{-1}·min^{-1}), and all other values are expressed in relation to it. All reported ATPase activities are the average of at least three sets of measurements. The V_{max} and K_m of the ATPase activity of myosin in the absence and presence of 0.16 and 0.33 μM 2H2 were also compared. They were determined from the intercepts of a Lineweaver–Burk plot (not shown) with regression coefficients for all three plots being $\geq$0.965. In the presence of 2H2, the V_{max} was reduced (1.1, 0.53, and 0.26 μmol·mg^{-1}·min^{-1} in the absence and in the presence of 0.16 and 0.33 μM 2H2, respectively) while the K_m remained unchanged (1.7 ± 0.02 μM), suggesting that 2H2 is a noncompetitive inhibitor of the actin activation of myosin. The binding affinity of actin and myosin remains unaffected while the maximal rate of hydrolysis was reduced.

The above experiments do not distinguish between the effects of blocking LC1 or LC2, since both contain the putative epitope. Therefore, the effect of 2H2 was also determined using chymotryptic S1(A1) and S1(A2) which lacks LC2, but containing LC1 and LC3, respectively. In the presence of a 1:2 molar ratio of 2H2 to S1(A1), complete inhibition of actin-activated S1(A1) ATPase activity was achieved. However, at molar ratios up to 1:1 2H2 to S1(A2), the actin-activated activity of S1(A2) remained unaffected. This demonstrates that the inhibitory effect of 2H2 is derived from the blocking of the N-terminus of LC1. Fab fragments of 2H2 were also used in some of the measurements and they were observed to similarly inhibit.

Binding of 2H2 to Acto–S1. In the absence of ATP, the binding of 2H2–S1 complex to F-actin was observed by sedimenting the mixture and analyzing the protein contents of

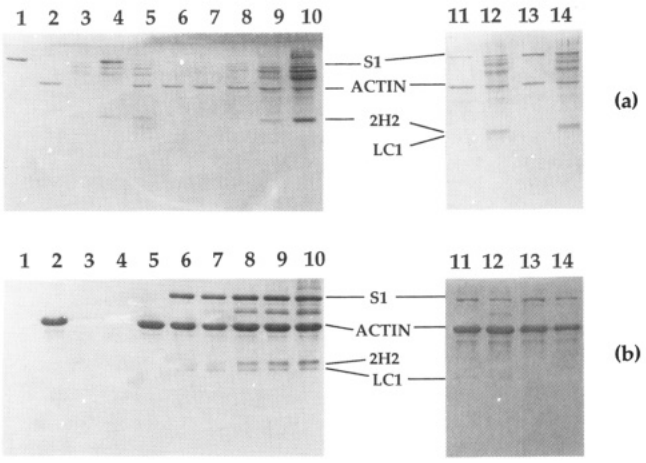

FIGURE 5: Supernatant (a) and pellet (b) contents of a binding assay in rigor separated by SDS–PAGE after centrifugation at 30 psi for 15 min (Beckman airfuge). Protein contents in each lane prior to centrifugation were the following: (1) 30 μg of S1; (2) 25 μg of F-actin; (3) 20 μg of 2H2; (4) S1 and 2H2; (5) F-actin and 2H2; (6) S1 and F-actin; (7–10) S1 and F-actin with 8, 20, 40, and 80 μg of 2H2, respectively, in 150 μL of solvent; (11 and 12) S1(A1) and F-actin in the absence and presence of 40 μg of 2H2, respectively; (13 and 14) S1(A2) and F-actin in the absence and presence of 40 μg of 2H2, respectively.

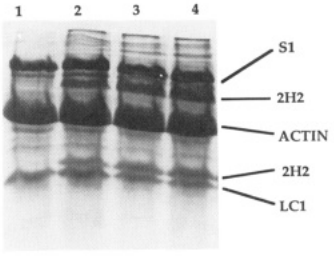

FIGURE 6: Pellet contents of binding assay in the presence of ATP separated by SDS–PAGE after centrifugation at 30 psi for 15 min (Beckman airfuge). Protein contents in each lane prior to centrifugation were as follows: (1) 300 μg of S1 and 23 μg of F-actin; (2–4) S1 and F-actin with 100, 150, and 200 μg of 2H2, respectively, in 150 μL of solvent. 5 mM ATP was added immediately prior to centrifugation at 4 °C.

the supernatants and pellets by PAGE (Figure 5a,b). Under our experimental conditions, 2H2 was not sedimented in the presence of either S1 or F-actin alone. However, 2H2 was sedimented in the presence of both S1 and F-actin, indicating the concomitant binding of both 2H2 and F-actin to S1 in rigor. The amount of S1 sedimented with actin does not appear to be affected by the presence of 2H2. This demonstrates that 2H2 does not bind to actin, but cosediments with S1 and F-actin as a ternary complex, and that there is no noticeable effect on the ability of S1 to bind to F-actin in the absence of ATP. Using purified S1(A1) or S1(A2), we found that 2H2 is sedimented only in the presence of S1(A1) but not S1(A2), indicating the specificity of 2H2 for LC1.

Formation of acto–S1 complex was significantly reduced in the presence of ATP, with or without 2H2. Using protein concentrations of 2 μM S1 and 4 μM F-actin, the presence of the S1 heavy-chain band in the pellet when analyzed by SDS–PAGE was very weak, such that quantitation was difficult. Therefore, the assay was performed at an increased S1 concentration of 20 μM. The results of this assay are shown in Figure 6. The amount of S1 sedimented with actin does not appear to be affected by the presence of 2H2, which was cosedimented with the acto–S1 complex. The extent of acto–S1 binding was analyzed using a Molecular Dynamics densitometer. The amount of S1–2H2 bound with F-actin was

estimated from the density of the S1 heavy chain, 2H2, and F-actin bands. The ratios of sedimented S1 to F-actin remained consistent with increasing amount of 2H2 (result not shown). This result shows that 2H2 does not dissociate the acto–S1 complex in the presence of ATP.

Discussion

In this report, we have characterized a monoclonal antibody, 2H2, and determined its probable epitope. The major antigenic determinant of 2H2 appears to be the N-terminal residue of LC1 and LC2, which is a charged trimethylated alanine. This epitope bears remarkable similarities to that described by Tokunaga et al. (1987), despite the fact that the antibodies were raised with antigens from different species. These authors located the epitope by noting that their antibody failed to bind LC3 but was specific for LC1 and LC2. In a series of cross-reactivity experiments with proteolytic fragments of the light chains as well as a histone protein (which also contains a Me_3APKK N-terminal sequence), they concluded that their epitope was the N-terminal tetrapeptide.

On the basis of ^{1}H-NMR, Henry et al. (1985) have proposed that this N-terminal segment is capable of "exceptional" mobility. Others (Tainer et al., 1985) have suggested that charged or highly mobile segments of peptide chains are potentially highly antigenic. The fact that we have generated a monoclonal antibody which is apparently identical to that described by Tokunaga et al., is consistent with this conclusion.

In the present study, 2H2 was used to specifically block the N-terminal region of the light chains to determine the importance of this region in the interaction of LC1 with actin. We found the following when the N-terminus of LC1 was blocked: (a) the rate of superprecipitation of acto–myosin was significantly reduced; (b) the actin-activated ATPase activity was inhibited such that the V_{max} was reduced while the K_m remained unaffected; (c) the ATPase activities of myosin and S1 alone were unaffected; (d) binding of acto–S1 in the presence or absence of ATP remained unchanged in the presence or absence of 2H2; and (e) ATP-mediated dissociation of acto–S1 was not inhibited.

Thus, the presence of 2H2 bound to the N-terminus of LC1 had no effect on the ability of the myosin heavy chain to bind or dissociate from actin in an ATP-dependent manner, nor on its ability to hydrolyze ATP. However, it had a profound effect on the ability of F-actin to accelerate ATPase activity of the myosin head. On the basis of kinetic studies, the activation of myosin or S1 ATPase activity by F-actin is due to an acceleration in the release of ATPase products which requires the reattachment of the myosin head to actin [see Cooke (1986) for a review]. There are several lines of evidence supporting the interaction of the N-terminus of LC1 with actin. Sutoh (1983) suggested that the N-terminal peptide of LC1 interacts directly with the C-terminal residues of actin, while Henry et al., (1985) reported a direct interaction of Me_3Ala with actin. ^{1}H-NMR of S1 shows that the mobility of this segment is quenched by actin binding (Highsmith & Jardetzsky, 1980).

One interpretation of our observations would suggest that the release of hydrolysis products has been hindered by blocking the interaction of LC1 with actin. However, we have also demonstrated that the binding of actin both in the presence and in the absence of ATP has not been affected. This indicates that the N-terminus of LC1 is not essential for actin binding in both the strong and weak binding states, even though this region appears to play a crucial role in the activation of ATPase by actin. Suzuki et al. (1987, 1990) reported that a synthetic heptapeptide analogue of an actin binding site on the myosin head (which includes the SH1 region) completely inhibited actin-activated S1 ATPase activity without dissociating the acto–S1 complex during the ATPase steady state. This observation is not unlike ours. However, in contrast to our findings, they also showed that their heptapeptide inhibits acto–S1 complex formation in rigor. Katoh et al. (1984) and Katoh and Morita (1984) proposed that there are at least two actin binding sites on the myosin head, one close to the reactive SH1 and SH2 (S-site) and the other near the junction between the 50- and 20-kDa domains (J-site). Suzuki et al. suggested that the S-site (high-affinity site) binds transiently with actin to accelerate the release of products while the J-site (weak binding site) keeps acto–S1 linked during steady-state ATPase activity. The authors also suggested that there may be several other contact regions which have stationary or transient interaction with F-actin and that the tertiary structure around the S-site may be constructed with both the peptide region around SH1 and the N-terminal region of LC1. In order to reconcile our results into this model, both the N-terminal region of LC1 together with the S-site would have to bind with actin transiently to accelerate hydrolysis product release. Hence, the inhibition of this transient interaction by 2H2 resulted in a decrease of ATPase activity.

Another possible interpretation of our results is that the N-terminal region of LC1 is part of the "communication pathway" between the actin binding site and the ATPase site in S1. This communication is lost when 2H2 is bound to LC1, so that even though S1–ADP–P_i heads are still capable of binding to actin, the release of ADP and P_i cannot be effected. However, communication between the binding sites for ATP and actin does not appear to be lost in the presence of 2H2 as demonstrated by our binding study, which showed significantly less acto–S1–2H2 complex formation in the presence of ATP than in rigor. Thus, it would appear that the N-terminus of LC1 plays a role in modulating the release of hydrolysis products, but not in the release of actin by ATP binding. This conclusion was also made by Chaussepied et al. (1986), who observed that the ATP-mediated dissociation of F-actin was independent of the type of alkali light chain present in a 30-kDa heavy-chain–light-chain complex prepared by thrombic digestion of S1.

The mechanism of interaction between the actin and ATPase sites is still poorly understood. Our findings examine the functional role of this N-terminal peptide of LC1. Complete absence of this region of the light chain [e.g., S1(A2)] has little effect on its function. On the balance of these observations, the latter interpretation is the more plausible one, given that 2H2 does not appear to affect any aspect of acto–S1 binding.

It is possible that movement (or attachment to actin) of this highly mobile N-terminal peptide of LC1 causes a conformational change within the myosin head. This conformational change is required to facilitate the transmission of information from the actin binding site to the ATPase site, inducing product release. The role of this extra peptide on the myosin head is therefore to inhibit communication from the actin binding site to the ATPase site, and thus product release. This inhibition is reversed when the N-terminal region is removed or located at a different site, coupled perhaps with a transient attachment to actin. In the presence of LC3 instead of LC1, this conformational change is not required, as the pathway of communication has not been inhibited, but is always "open". Thus, the presence of 2H2 bound to LC1 could have the effect of locking this communication pathway in the "closed" position, either by quenching its mobility or by sterically inhibiting the

required conformational change. This model is consistent with previous observations that S1(A2) has a larger K_m than S1(A1) and the V_{max} of S1(A2) is about twice that of S1(A1) at low ionic strength (Wagner & Weeds, 1977).

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