

An Immunological Approach to Myosin Light-Chain Function in Thick Filament Linked Regulation. 2. Effects of Anti-Scallop Myosin Light-Chain Antibodies. Possible Regulatory Role for the Essential Light Chain[†]

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ABSTRACT: Specific antibodies directed against the regulatory light chains (R-LC) or essential light chains (SH-LC) of scallop myosin abolished calcium regulation in myofibrils, myosin, and heavy meromyosin by elevating the actin-activated Mg^{2+} -ATPase activity in the absence of calcium. Calcium dependence was completely eliminated at molar ratios of 2.5-3 antibodies bound per myosin. Monovalent anti-R-LC Fab and anti-SH-LC Fab fragments also desensitized myofibrils fully. High Ca^{2+} -ATPase activity remained unaffected by the antibodies. Anti-SH-LC IgG reduced to about one-half the actin-activated Mg^{2+} -ATPase in the presence of calcium and the potassium-activated ethylenediaminetetraacetic acid (EDTA)-ATPase activities. Anti-SH-LC Fab, however, desensitized without inhibiting the actin-activated Mg^{2+} -ATPase. The desensitizing effect of both antibodies was abolished by prior absorption with the homologous myosin light chain. Calcium binding and R-LC content remained unaffected by anti-R-LC and anti-SH-LC IgG's and by anti-SH-LC Fab. The anti-R-LC Fab fragment induced a significant (70%) dissociation of R-LC from myofibrils and myosins with concomitant losses in calcium binding. In contrast, anti-R-LC IgG prevented the dissociation of R-LC from myosin by

EDTA. Binding of anti-R-LC IgG to myofibrils was proportional to their R-LC content. Increased amounts of anti-SH-LC IgG were bound by myofibrils devoid of R-LC. Bound anti-SH-LC antibody significantly inhibited the reuptake of R-LC by EDTA-treated myofibrils as well as the full binding of anti-R-LC antibody. Certain rabbits produced a population of anti-SH-LC antibodies which were specific for this light chain and bound extensively to myosin but failed to desensitize it (nondesensitizing anti-SH-LC antibody). The desensitizing and nondesensitizing anti-SH-LC IgG populations bound to different regions of the SH-LC on the myosin, and the binding of the two types of antibody to the SH-LC was nearly additive. The nondesensitizing SH-antibody inhibited the reuptake of R-LC less, and its binding to myofibrils was not influenced by the absence of R-LC. These studies indicate a direct or indirect involvement of the SH-LC's in myosin-linked regulation, raise the possibility of an interaction between the R-LC and SH-LC, and confirm the regulatory function of the scallop R-LC. A model for a relative location of the two types of light chains and the involvement of the subfragment-2 region of myosin in myosin linked regulation is discussed.

The myosins of molluscan adductor muscles and of the muscles of many invertebrates are regulatory myosins (Kendrick-Jones et al., 1970; Lehman & Szent-Györgyi, 1975). For myosin-linked regulation the presence of a class of light chains, the regulatory light chains (R-LC),¹ is necessary. The role of the regulatory light chains has been directly shown in scallop myosin which is the only myosin from which these light chains can be reversibly dissociated by removal of divalent cations with EDTA. EDTA treatment at low temperature (<10 °C) removes ~1 mol of this light chain per myosin from myofibrils (Szent-Györgyi et al., 1973; Kendrick-Jones et al., 1976). At slightly elevated temperatures both light chains may be reversibly dissociated (Chantler & Szent-Györgyi, 1980). In the absence of regulatory light chains calcium sensitivity is abolished, i.e., the actin-activated Mg^{2+} -ATPase activity in the absence of calcium is elevated, and the specificity and high affinity of the calcium binding sites of scallop myosin are also lost (Chantler & Szent-Györgyi, 1980). The role of regulatory light chains in vertebrate skeletal myosins is poorly understood. Several studies indicate that the DTNB light chains are not part of the catalytic or actin-binding sites (Gazith et al., 1970; Weeds & Lowey, 1971; Holt & Lowey, 1975; Wagner &

Weeds, 1977; Higuchi et al., 1978). These light chains are phosphorylatable (Perrie et al., 1973; Frearson & Perry, 1975), and phosphorylation occurs during contractile activity (Barany et al., 1979).

The function of the essential light chains (alkali or essential light chains) is unknown. Dissociation of this class of light chains without denaturation of myosin has not been possible so far. Experiments with chaotropic agents have led to the suggestion that essential light chains directly participate in the catalytic site (Stracher, 1969; Gershman & Dreizen, 1970; Dow & Stracher, 1971). This interpretation, however, has not been substantiated, and the effects were explained on the basis of incomplete dissociation of the light chains, partial

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¹ Abbreviations used: RIA, radioimmunoassay; HVE, high-voltage electrophoresis; R-LC, myosin regulatory light chain; SH-LC, myosin "essential" light chain; HMM, heavy meromyosin; GarFc, goat anti-rabbit Fc antibody; S1, subfragment-1; CNBr, cyanogen bromide; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); Gdn-HCl, guanidine hydrochloride; FITC, fluorescein isothiocyanate; OD, optical density; PMSF, phenylmethanesulfonyl fluoride; EGTA, [ethylene(oxyethylenetriolo)]-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; DEAE, diethylaminoethyl; DTT, dithiothreitol; NaDodSO₄, sodium dodecyl sulfate; BSB, borate/saline buffer (0.15 M NaCl and 14 mM boric acid, titrated to pH 8.0 with NaOH); PBS, phosphate-buffered saline (0.15 M NaCl and 7.5 mM phosphate, pH 7.2); EBT, EDTA/boric acid/Tris buffer (0.75 mM EDTA, 25 mM boric acid, and 45 mM Tris, pH 8.6, at 4 °C); wash, 40 mM NaCl, 3 mM NaN₃, and 5 mM phosphate, pH 7.0; Mg^{2+} wash, wash containing 1 mM MgCl₂ and 0.1 mM EGTA.

denaturation of myosin, and poor recovery of ATPase activities (Kim & Mommaerts, 1971; Wagner & Weeds, 1977). Recently, myosin S1 and HMM isozymes containing different alkali light chains have been isolated (Weeds & Taylor 1975; Wagner, 1977). Exchange studies of rabbit S1 with different essential light chains of fast myosin (A1 and A2) and of slow myosins led to changes in K_m and V_{max} of the actin-activated ATPase (Wagner & Weeds, 1977; Winstanley et al., 1979), provided the ionic strength remained low (Wagner et al., 1979). However, the K-EDTA and high-calcium ATPase activities were not altered by the different essential light chains (Wagner & Weeds, 1977; Okamoto & Yagi, 1977; Higuchi et al., 1979), indicating that the catalytic site is not involved in the effects mediated by the essential light chain. Binding of specific antibodies directed against the essential light chains had no effect on ATPase activities or on actin binding (Holt & Lowey, 1975).

In a manner similar to the immunological approach applied earlier by Holt & Lowey (1975) to vertebrate myosin, we have used antibodies as probes of light-chain function in scallop myosin. In particular, we explored the role of essential light chains in the regulation of scallop myosin. A preliminary report of part of this work has been presented (Szent-Györgyi et al., 1978; Wallimann & Szent-Györgyi, 1979).

Experimental Procedures

Preparation of Myofibrils. Glycerinated myofibrils were prepared from striated adductor muscles of the scallop, *Aequipecten irradians*. The muscles were stretched while still attached to the shell by cutting the smooth adductor as described previously (Lehman & Szent-Györgyi, 1975). The washed myofibrils were filtered through two layers of fine cheese cloth, centrifuged, and resuspended with a Teflon-glass homogenizer in 40 mM NaCl, 1 mM MgCl₂, 0.1 mM EGTA, 3 mM NaN₃, and 5 mM phosphate, pH 7.0 (referred to as Mg²⁺ wash) to give a protein concentration of ~5 mg/mL.

Incubation of Myofibrils and Myosin with Antibodies. To 1 mL of myofibrillar suspension were added increasing amounts of preimmune IgG, anti-R-LC IgG, or anti-SH-LC IgG, previously dialyzed against Mg²⁺ wash and centrifuged prior to use. The volume was kept constant (6–8 mL) by the addition of Mg²⁺ wash. The suspension was gently rotated on a rotary shaker for 6–12 h at 4 °C. Unbound antibody was removed by centrifugation, the first supernatant was saved for polyacrylamide gel electrophoretic analysis, and the myofibrils were washed 3 times and finally resuspended in 40 mM NaCl, 3 mM NaN₃, and 5 mM phosphate, pH 7.0 (referred to as wash). Aliquots were taken for ATPase measurements, polyacrylamide gel electrophoretic analysis, calcium binding, and protein determination. If necessary, after completion of the ATPase measurements, the precipitated actomyosin was recovered from the pH-stat vessel by centrifugation and then processed for polyacrylamide gel electrophoretic analysis. Myosin, prepared as described in the preceding paper, was incubated with antibodies either as a suspension in low-salt Mg²⁺ wash or solubilized in Mg²⁺ wash containing 0.3 M NaCl.

Quantitation of Antibody Uptake. Antibody content of myofibrils was obtained by polyacrylamide gel electrophoresis in NaDodSO₄ in phosphate buffer (Weber & Osborn, 1969). The molar ratios of IgG to myosin were determined by densitometry of Acid Fast Green stained gels assuming similar stain uptake of both proteins and M_r of 50 000 and 200 000 for the heavy chains of IgG and myosin, respectively. Different amounts of protein were loaded on gels to make certain that the measurements were within the linear range of stain uptake.

Rough estimates of antibody uptake, usually exceeding the ones obtained by densitometry by 10–20%, could also be obtained by measuring the increase in protein while assuming the myosin content to be 65% of the total myofibrillar protein and by taking M_r of 470 000 for myosin and 150 000 for IgG. Release of myosin light chains was determined by measuring the molar ratio of myosin light chains to heavy chains after electrophoresis in 5% and 10% polyacrylamide gels with actin as an internal standard as described previously (Szent-Györgyi et al., 1973). The molar ratio of R-LC to SH-LC and the ratio of tropomyosin to SH-LC were determined by densitometry of 10% polyacrylamide gels run in the presence of 8 M urea using a Tris/glycine buffer system at pH 8.6 as described by Perrie & Perry (1970) and Kendrick-Jones et al. (1976).

ATPase Assays and Calcium Sensitivity Measurements. ATPase activity was measured at pH 7.5 and 25 °C with a Radiometer pH stat as described previously (Szent-Györgyi et al., 1973). Ca^{2+} -ATPase was measured in 0.5 M NaCl, 10 mM CaCl₂, and 2.5 mM ATP, K^+ /EDTA-ATPase in 0.6 M KCl, 2 mM EDTA, and 2.5 mM ATP, and actin-activated Mg²⁺-ATPase in 20–40 mM NaCl, 1 mM MgCl₂, 0.1 mM EGTA, and 1–2 mM ATP, before and after adding 0.15 mM CaCl₂. Typically, an aliquot containing 0.5–1 mg of myofibrils or myosin (in 40 mM NaCl, 1 mM MgCl₂, 0.1 mM EGTA, 3 mM NaN₃, and 5 mM phosphate pH 7.0) was made 0.6 M in NaCl and 1–5 mM in ATP. Subsequently, a 10 molar excess of rabbit F-actin over myosin was added, and the mixture was allowed to stand for 5–10 min on ice. Then it was transferred into a pH-stat vessel containing the above assay solution titrated to pH 7.5 to give a final volume of 10 mL. Percent calcium sensitivity is defined as $(1 - [ATPase\ in\ EGTA]/[ATPase\ in\ Ca^{2+}]) \times 100$.

Desensitization of Myofibrils. Myofibrils were desensitized at low temperature (0–10 °C) by incubation with 10 mM EDTA at pH 7.0 (final concentration of myofibrils was 1–2 mg/mL) whereby 1 mol of R-LC is usually released (Kendrick-Jones et al., 1976) and at 35 °C whereby 2 mol of R-LC is released (Chantler & Szent-Györgyi, 1980). By variation of the temperature of the EDTA treatment between 0–35 °C, myofibrils could be obtained with molar ratios of R-LC to SH-LC or myosin head ranging between 0 and 0.7 (Figure 12).

Reuptake of R-LC following Incubation with Anti-SH-LC Antibody. Desensitized myofibrils previously incubated with an excess of anti-SH-LC antibody and washed with Mg²⁺ washed were rotated gently with a 2–10-fold molar excess of R-LC at 4 °C for 12 h. After removal of unbound R-LC by centrifugation and washings, aliquots were taken for ATPase assays and polyacrylamide gel electrophoretic analysis to determine antibody uptake and rebinding of R-LC.

Calcium Binding. Calcium binding of myofibrils was determined by double-labeling techniques using ⁴⁵Ca²⁺ and [³H]glucose added to a EGTA/Ca²⁺ buffer system as described earlier (Kendrick-Jones et al., 1970). Free calcium and magnesium were 2.1×10^{-6} M and 1–2 mM, respectively, at pH 7.0 and 0 °C. After centrifugation the myofibrillar pellet was dissolved in 6 mL of Aquasol (New England Nuclear) and counted in a Beckman LS 255 liquid scintillation counter.

Absorption of Anti-SH-LC IgG with Myosin Subfragment-1. Anti-SH-LC IgG (desensitizing antibody from rabbit no. 5) was absorbed at the precipitin equivalence point with myosin subfragment-1, (EDTA)S1, containing a fragment of M_r 8000 of the SH-LC only (Stafford et al., 1979). An excess of rabbit F-actin was added and, upon binding to actin, the

(EDTA)S1 was spun down by centrifugation at 60 000 rpm for 3 h. The supernatant was analyzed for cross-reactivity with intact SH-LC and (EDTA)S1 by double immunodiffusion and added to myofibrils to determine its effect on calcium sensitivity.

Preparation of Scallop Heavy Meromyosin. HMM was prepared by a 5-min digestion of myosin (10–15 mg/mL) in 0.6 M NaCl and 20 mM phosphate at pH 7.0 and 25 °C by using a 1:400 w/w ratio of trypsin to myosin. The reaction was stopped with a 3-fold (w/w) excess of soybean trypsin inhibitor over trypsin. After dialysis against low-salt buffer followed by centrifugation, the HMM was precipitated by ammonium sulfate between 40 and 65% saturations and dialyzed against wash.

Double-Reciprocal Plots of HMM. Two milligrams of HMM and 4 mg of either preimmune IgG, anti-R-LC IgG, or anti-SH-LC IgG were mixed and dialyzed overnight against Mg^{2+} -ATPase assay buffer. Aliquots were assayed for actin-activated Mg^{2+} -ATPase first in the absence and then in the presence of calcium by adding increments of 0.3 mg of pure rabbit F-actin to the antibody-treated HMM. Concentration changes of HMM due to dialysis were estimated by comparing the high Ca^{2+} -ATPase activity before and after dialysis.

Preparation of Monovalent Antibody Fab Fragments. Fab was prepared by digestion for 12 h at 30 °C of crude IgG or affinity-purified IgG with papain (Porter, 1959) at a ratio of 1 mg of papain/100 mg of IgG in 0.1 M phosphate buffer, pH 7.0, containing 5 mM cysteine and 2 mM EDTA. Papain was preactivated for 30 min at 20 °C in 0.1 M NaCl, 2 mM EDTA, 5 mM DTT, and 10 mM phosphate, pH 7.0. The reaction was stopped by the addition of iodoacetic acid at pH 7.0 to give a final concentration of 20 mM. After being stirred for 30 min at room temperature, the digest was dialyzed extensively against ice-cold BSB. Crystallized Fc fragment was removed by centrifugation. The supernatant Fab was concentrated by vacuum dialysis, dialyzed against Mg^{2+} wash, and stored at -20 °C. The extent of digestion was followed by using goat anti-rabbit IgG and Fc antibodies in double-immunodiffusion tests as markers for the unsplit and completely cleaved product and by NaDodSO₄-polyacrylamide gel electrophoresis.

Incubation of Myofibrils with Fab. Myofibrils were incubated with preimmune Fab, anti-R-LC Fab, or anti-SH-LC Fab as described for IgG. Special precautions were taken to prevent any activation of traces of papain which were introduced by the Fab preparations and bound with high affinity to the myosin-Fab complexes by adding samples of Fab-treated myofibrils directly to boiling NaDodSO₄ loading buffer prior to polyacrylamide gel electrophoresis in NaDodSO₄. For polyacrylamide gel electrophoresis in 8 M urea, samples were preincubated for 10 min at 30 °C in urea loading buffer containing 5 mM iodoacetic acid instead of DTT and then made 25 mM with DTT and incubated for another 60 min at 30 °C.

Other Procedures. Materials and experimental procedures not referred to here are described in the preceding paper.

Results

(1) Effects of Anti-Scallop Myosin Light-Chain Antibodies on ATPase Activity. (a) *Anti-Regulatory Light-Chain Antibodies.* All batches of anti-R-LC IgG interfered with the calcium dependence of the actin-activated Mg^{2+} -ATPase activity of myofibrils by elevating the ATPase activity in the absence of calcium. The antibody bound by myofibrils could be readily quantitated by gel electrophoresis in NaDodSO₄ since the bands associated with the antibody moved separately

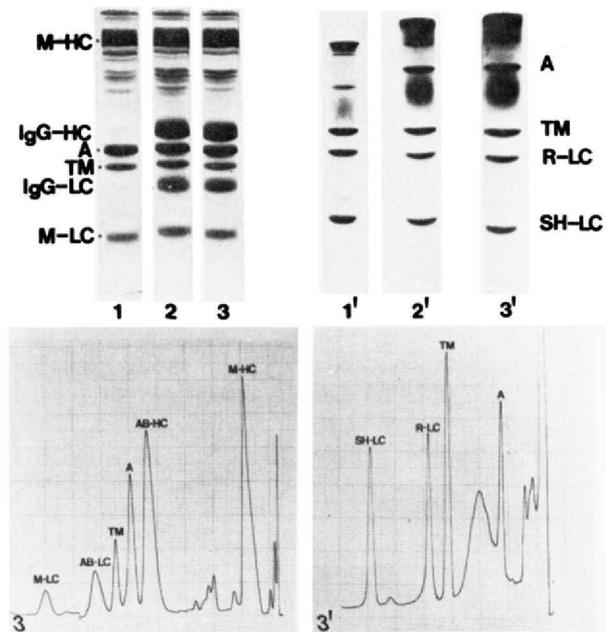


FIGURE 1: Measurement of antibody uptake by scallop myofibrils and determination of the molar ratio of R-LC to SH-LC. 7.5% polyacrylamide gel electrophoresis in NaDodSO₄ (upper left, 1–3) and 10% polyacrylamide gel electrophoresis in 8 M urea (upper right, 1'–3') of scallop myofibrils incubated with control IgG (1, 1'), anti-R-LC IgG (2, 2'), or anti-SH-LC IgG (3, 3'). M-HC, myosin heavy chain; M-LC, myosin light chains; IgG-HC, immunoglobulin heavy chains; IgG-LC immunoglobulin light chains; R-LC, regulatory light chain; SH-LC, essential light chain; A, actin; TM, tropomyosin. (Lower panels) Densitometry tracings of gels 3 and 3'. The diffusive band between actin and tropomyosin on urea gels is a component of IgG, probably IgG-LC.

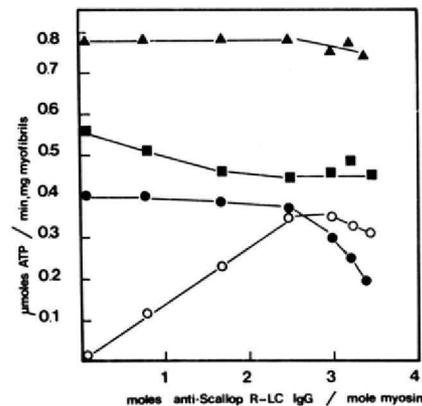


FIGURE 2: Effect of anti-scallop R-LC IgG on ATPase activities of scallop myofibrils. Ca^{2+} -ATPase activity measured in 0.5 M NaCl, 10 mM $CaCl_2$, and 2.5 mM ATP at pH 7.5 (\blacktriangle); K^+ /EDTA-ATPase activity measured in 0.5 M KCl, 2 mM EDTA, and 2.5 mM ATP at pH 7.5 (\blacksquare); actin-activated Mg^{2+} -ATPase activity measured in 40 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EGTA, and 1 mM ATP at pH 7.5 (\circ) and subsequently in 0.2 mM $CaCl_2$ (\bullet). The initial points are control values obtained after incubation with an excess of pre-immune IgG equaling the largest amount of anti-R-LC IgG used.

from those of the myofibrillar proteins (Figure 1, 1–3). The presence of antibody did not interfere with the separation of R-LC and SH-LC on urea gels (Figure 1, 1'–3'). Calcium sensitivity was fully abolished when antibody uptake exceeded 2 mol of IgG/mol of myosin (Figure 2). The actin-activated Mg^{2+} -ATPase in the absence of calcium was fully elevated at these antibody to myosin ratios. At higher antibody concentrations the actin-activated ATPase declined both in the presence and absence of calcium. High calcium- and K^+ /EDTA-activated ATPase activities were not changed significantly by this antibody (Figure 2). Desensitization was not

Table I: Effect of Anti-Scallop Myosin Light-Chain Antibodies on Binding of Calcium to Scallop Myofibrils

	mol of Ca ²⁺ bound ^a / mol of myosin
untreated scallop myofibrils	1.75
myofibrils treated with 10 mM EDTA at 10 °C	1.1
myofibrils treated with 10 mM EDTA at 35 °C	0.12
myofibrils treated with anti-R-LC IgG, followed by EDTA treatment at 35 °C	1.67 ^b
myofibrils treated with R ₅ -anti-SH-LC IgG, ^d followed by EDTA treatment at 35 °C	0.16
myofibrils treated with control IgG	1.85
myofibrils treated with anti-R-LC IgG	1.91
myofibrils treated with R ₅ -anti-SH-LC IgG ^d	2.10
myofibrils treated with control Fab	1.82
myofibrils treated with anti-R-LC Fab	0.65 ^c
myofibrils treated with R ₅ -anti-SH-LC Fab	1.91

^a Moles of calcium bound per mole of myosin measured at 2.1×10^{-6} M free [Ca²⁺] at pH 7.0 and 0 °C. ^b Anti-R-LC IgG prevents dissociation of R-LC by EDTA, hence high value of calcium binding is retained. ^c Anti-R-LC Fab causes partial dissociation of R-LC, hence calcium binding is lowered. ^d Desensitizing R₅-anti-SH-LC antibody.

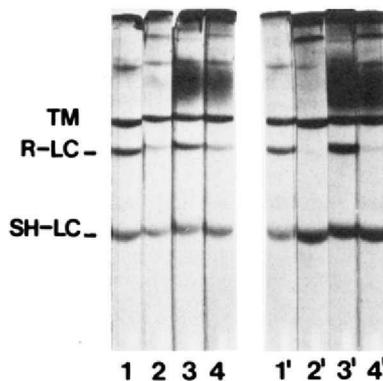


FIGURE 3: Prevention of dissociation of R-LC by anti-R-LC antibody. Scallop myofibrils were incubated with anti-scallop myosin light-chain antibodies prior to treatment with 10 mM EDTA at 10 or 35 °C. Untreated scallop myofibrils (1, 1'); myofibrils incubated with preimmune IgG (2, 2'), anti-R-LC IgG (3, 3'), and anti-SH-LC IgG (4, 4'), followed by a treatment with 10 mM EDTA at 10 °C (2-4) or 35 °C (2'-4'), shown after electrophoresis on 10% polyacrylamide urea gels. TM, tropomyosin; R-LC, regulatory light chain; SH-LC, essential light chain.

due to dissociation of the R-LC's by the antibodies since calcium binding was not altered by antibody binding and isolated R-LC's do not bind calcium (Table I). Moreover, incubation of antibody-treated myofibrils with excess R-LC's did not restore calcium sensitivity and did not increase the R-LC content, although calcium sensitivity was regained in control myofibrils that were desensitized by EDTA instead of by antibodies (not shown). Anti-R-LC IgG prevented the removal of R-LC's by EDTA, at both 10 and 35 °C, and the loss in calcium binding (Figure 3, Table I). Anti-SH-LC IgG did not protect against the same treatment.

Anti-R-LC Fab like anti-R-LC IgG, desensitized the actin-activated Mg²⁺-ATPase of myofibrils by specifically elevating this activity in the absence of calcium. The activities of the EDTA and high calcium activated ATPase remained unaffected (Figure 4). However, treatment with anti-R-LC Fab reduced the light-chain content and lowered the R-LC to SH-LC ratio (Figure 4 and Figure 5, gels 2 and 2').

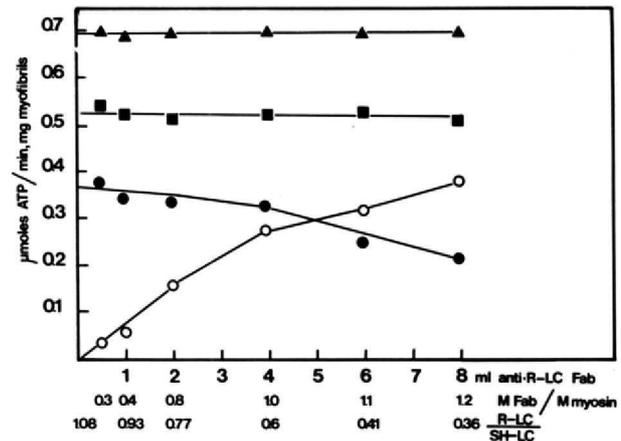


FIGURE 4: Effect of anti-R-LC Fab on ATPase activities of scallop myofibrils. In addition to the amount of antibody added (milliliters of anti-R-LC Fab), the uptake of antibody in moles per mole of myosin and the amount of R-LC which was dissociated off the myosin by this monovalent antibody (expressed as the molar ratio of R-LC to SH-LC) are also shown. Ca²⁺-ATPase activity (▲); K⁺/EDTA-ATPase activity (■); actin-activated Mg²⁺-ATPase activity in the absence (○) and in the presence (●) of calcium. Assay conditions and controls are as described in Figure 2.

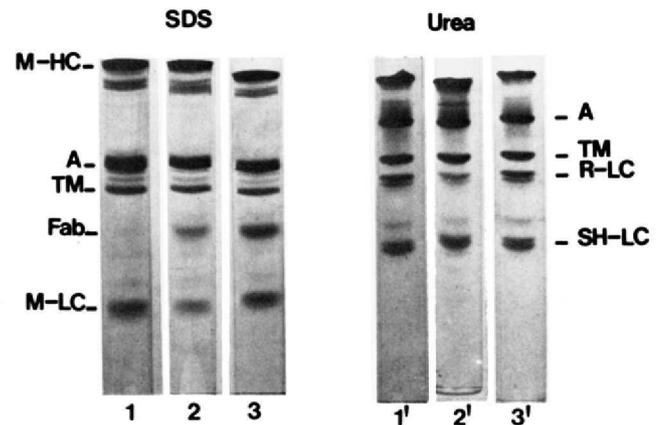


FIGURE 5: Binding of anti-LC Fab's by myofibrils. 10% polyacrylamide gel electrophoresis in NaDodSO₄ (left panel, 1-3) and 10% polyacrylamide gel electrophoresis in 8 M urea (right panel, 1'-3') of scallop myofibrils incubated with preimmune Fab (1, 1'), anti-R-LC Fab (2, 2'), or desensitizing R₅-anti-SH-LC Fab (3, 3'). M-HC, myosin heavy chain; M-LC, myosin light chains; Fab, monovalent fragment of IgG; R-LC, regulatory light chain; SH-LC, essential light chain; A, actin; TM, tropomyosin. Note that the light-chain bands on urea gels are split. This is caused by partial alkylation during preincubation of the urea gel samples with iodoacetic acid to prevent reactivation of traces of papain.

Dissociation of R-LC's from myosin and their release into the supernatant by anti-R-LC Fab was directly demonstrated with immunoreplicas (Figure 6). This effect caused by anti-R-LC Fab was a specific one, R-LC or SH-LC content was not altered by preimmune, control Fab or by anti-SH-LC Fab (Figure 5, gels 1, 3, 1', and 3'). Correspondingly, anti-R-LC Fab bound to a lesser extent to myofibrils and to myosin than anti-R-LC IgG or anti-SH-LC Fab (Figure 4; Figure 5, gel 2; Table II). Calcium binding by myofibrils was reduced to about one-third after incubation with anti-R-LC Fab (0.65 mol of calcium/mol of myosin, in contrast to 1.82 mol of calcium/mol of myosin in control Fab-treated myofibrils) (Table I). The lowered calcium binding corresponds to an ~70% loss of R-LC which is in agreement with the 0.36 molar ratio of R-LC to SH-LC obtained from analysis of urea gels (Figure 4; Figure 5, gel 2'). Calcium binding of myofibrils was not altered by incubation with control IgG, R₅-anti-SH-

Table II: Effect of Anti-Scallop Myosin Light-Chain Antibodies on Scallop Myosin^a

scallop myosin incubated with	actin-activated Mg ²⁺ -ATPase ^b		% Ca ²⁺ sensitivity ^d	ATPase ^b		mol of AB bound ^c /mol of myosin
	EGTA	Ca ²⁺		K ⁺ /EDTA	Ca ²⁺	
control IgG	0.02	0.59	97	0.84	1.12	0
R ₁ -anti-R-LC IgG	0.29	0.26	<0	0.85	1.13	4.3
R ₅ -desensitizing anti-SH-LC IgG	0.26	0.26	0	0.52	1.12	3.7
R ₄ -nondesensitizing anti-SH-LC IgG	0.06	0.34	84	0.83	1.1	4.0
control Fab	0.06	0.56	90	0.79	1.11	0
R ₁ -anti-R-LC Fab	0.47	0.37	<0	0.77	1.07	1.5
R ₅ -desensitizing anti-SH-LC Fab	0.49	0.49	0	0.49	1.08	4.4

^a Scallop myosin, purified by (NH₄)₂SO₄ precipitation and Sepharose-4B chromatography, incubated with an excess of specific, affinity-purified rabbit anti-scallop myosin light-chain antibodies: preimmune, control IgG and Fab, anti-R-LC IgG and Fab, and both types (R₄ and R₅) of anti-SH-LC antibodies. R₁, R₄, and R₅ refer to rabbits no. 1, 4, and 5, respectively. ^b Actin-activated Mg²⁺-ATPase, K⁺/EDTA-ATPase, and Ca²⁺-ATPase activities are given in micromoles of ATP per minute per milligram of myosin. ^c Maximal uptake of antibodies (AB) by myosin, determined by densitometry of 7.5% polyacrylamide-NaDodSO₄ gels stained with Fast Green, is expressed as moles of IgG bound per mole of myosin. ^d Percent calcium sensitivity is defined under Experimental Procedures.

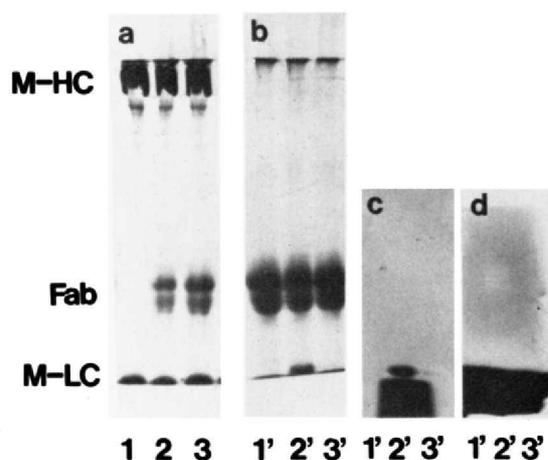


FIGURE 6: Microslab polyacrylamide gel electrophoresis in NaDodSO₄ (Matsudaira & Burgess, 1978) of (a) scallop myosin incubated with preimmune Fab (1), affinity-purified anti-R-LC Fab (2), and R₅-anti-SH-LC Fab (desensitizing antibody) (3), after extensive washing, (b) first supernatants of antibody-treated myosin (1'-3'), (c) anti-R-LC immunoreplica of the gel of the supernatants (1''-3''), and (d) anti-SH-LC immunoreplica of the gel of the supernatants (1'''-3'''). M-HC, myosin heavy chain; M-LC, myosin light chains; Fab, monovalent antibody fragment. The lower part of the immunoreplica appears dark due to precipitation and subsequent staining of some antibody within the overlap gel caused by the low pH below the front of the microslab gel.

LC IgG or Fab, or anti-R-LC IgG, except that calcium binding after anti-SH-LC IgG was consistently slightly higher (Table I). The desensitizing effect by anti-R-LC Fab therefore was a combination of loss of R-LC and antibody binding. In the presence of calcium the actin-activated Mg²⁺-ATPase activity dropped below that obtained in the absence of calcium. Such negative sensitivity is characteristic for myofibrils from which the R-LC's have been fully removed (Chantler & Szent-Györgyi, 1980). Incubation with an excess of R-LC partially resensitized myofibrils which were previously treated with anti-R-LC Fab (not shown). Purified myosin preparations bound affinity-purified anti-R-LC antibodies specifically (Figure 7) and behaved very similarly to myofibrils when treated with anti-R-LC antibodies. Calcium sensitivity of the actin-activated ATPase of myosin was selectively abolished by anti-R-LC IgG or Fab. In addition, anti-R-LC Fab dissociated a significant portion of the R-LC's (Table II, Figure 6). Antibody binding by myosin (4-4.5 mol of antibody/mol of myosin) somewhat exceeded binding by myofibrils (3-3.5 mol/mol of myosin) probably due to better accessibility of

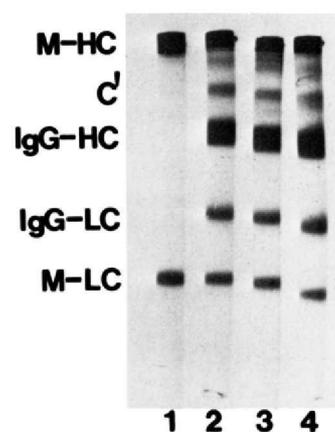


FIGURE 7: 10% polyacrylamide-NaDodSO₄ gels of Sepharose-4B-purified scallop myosin incubated with monospecific, affinity-purified anti-scallop myosin light-chain antibodies: control, preimmune IgG (1); anti-R-LC IgG (2); R₄-anti-SH-LC IgG, nondesensitizing antibody (3); R₅-anti-SH-LC IgG, desensitizing antibody (4). M-HC, myosin heavy chain; M-LC, myosin light chains; C, complement proteins; IgG-HC, immunoglobulin heavy chains; IgG-LC, immunoglobulin light chains.

myosin for the antibodies in the absence of actin (Figure 7, Table II).

Calcium sensitivity of scallop HMM preparations was also abolished by anti-R-LC IgG, indicating that activation of ATPase activity by antibodies in the absence of calcium did not depend on the filamentous form of myosin (Figure 8). Calcium had no effect on the actin-activated Mg²⁺-ATPase activity of HMM which bound 4 mol of anti-R-LC IgG/mol, although, similar to myofibrils at this high antibody concentration, the actin-activated Mg²⁺-ATPase activity in the presence of calcium was less than in control preparations. Antibody elevated the maximum turnover rate (V_{max}) in the absence of calcium. The measurements were not sufficiently accurate to determine to what degree, if at all, the K_m had changed (Figure 8).

(b) *Anti-Essential Light-Chain Antibodies.* Two populations of anti-SH-LC antibodies were produced in different rabbits (Wallimann & Szent-Györgyi, 1981). R₅-anti-SH-LC was a desensitizing antibody that interfered with regulation by elevating the actin-activated Mg²⁺-ATPase in the absence of calcium (Figure 9). A full effect was obtained at ratios of 3 mol of antibody/mol of myosin. At the higher antibody concentrations, both the K⁺/EDTA-ATPase and the actin-activated Mg²⁺-ATPase activities were reduced by about

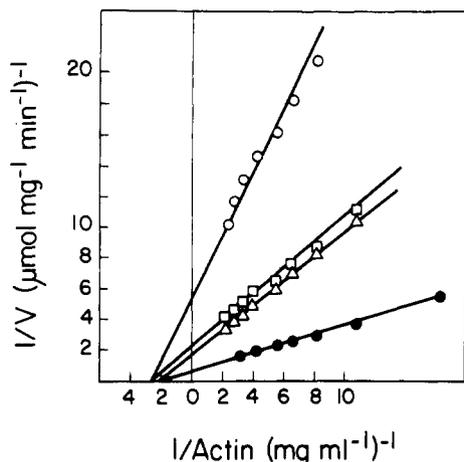


FIGURE 8: Effect of anti-scallop myosin light-chain antibodies on actin-activated Mg^{2+} -ATPase of heavy meromyosin (HMM). Lineweaver-Burk plot of the actin-activated Mg^{2+} -ATPase activity for scallop HMM after incubation with an excess of control IgG (○, ●), anti-R-LC IgG (△), and R_5 -anti-SH-LC IgG (□). Approximately 4 mol of anti-myosin light-chain antibodies were bound/mol of HMM. Open and filled symbols represent activities measured in the absence and presence of calcium, respectively. In the case of HMM treated with anti-myosin light-chain antibodies, the values obtained in the presence of calcium have been omitted for reasons of clarity since they overlapped with the ones obtained in the absence of calcium indicating that HMM was completely desensitized by both antibodies.

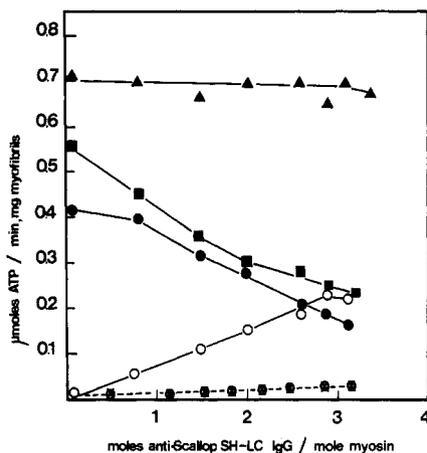


FIGURE 9: Effect of desensitizing R_5 -anti-SH-LC IgG on the ATPase activities of scallop myofibrils. Ca^{2+} -ATPase activity (▲); K^+ /EDTA-ATPase activity (■); actin-activated Mg^{2+} -ATPase activity in the absence (○) and in the presence (●) of calcium. Effect of nondesensitizing R_4 -anti-SH-LC IgG on the actin-activated Mg^{2+} -ATPase activity in the absence of calcium (⊙). This antibody has no effect on the Ca^{2+} -ATPase or on the K^+ /EDTA-ATPase activities. The actin-activated Mg^{2+} -ATPase in the presence of calcium began to decline only when antibody was bound in excess of 2 mol/mol of myosin. Assay conditions and controls with preimmune IgG are as described in Figure 2.

50–60%. The high Ca^{2+} -ATPase was not influenced by R_5 -anti-SH-LC antibody (Figure 9). In contrast, the nondesensitizing R_4 -anti-SH-LC antibody, although bound by myofibrils, had only marginal effects, at best, on the actin-activated Mg^{2+} -ATPase measured in the absence of calcium (Figure 9). The nondesensitizing R_4 -anti-SH-LC antibody was without effect on the K^+ /EDTA- and high Ca^{2+} activated ATPase activities and started to depress the calcium-activated Mg^{2+} -ATPase only when more than 2 mol of antibody were bound.

Desensitizing R_5 -anti-SH-LC Fab elevated the actin-activated Mg^{2+} -ATPase activity in the absence of calcium. Desensitization was full at a ratio of ~2.75 mol of Fab

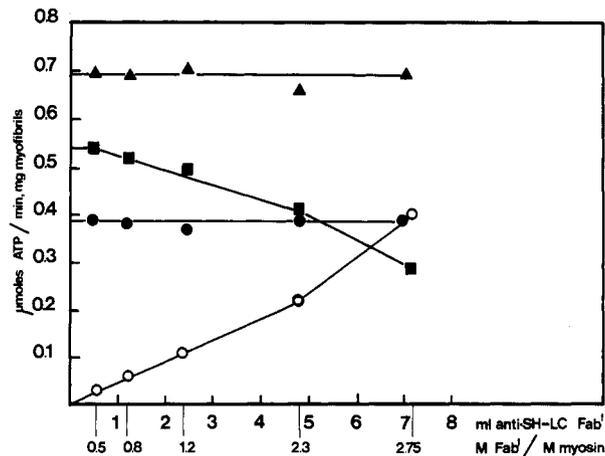


FIGURE 10: Effect of desensitizing R_5 -anti-SH-LC Fab on ATPase activities of scallop myofibrils. In addition to the amount of antibody added (milliliters of anti-SH-LC Fab), the uptake of antibody in moles per mole of myosin is also shown. Ca^{2+} -ATPase activity (▲); K^+ /EDTA-ATPase activity (■); actin-activated Mg^{2+} -ATPase activity in the absence (○) and in the presence (●) of calcium. Assay conditions and controls with preimmune Fab are as described in Figure 2.

bound/myosin (Figure 10). The high Ca^{2+} -ATPase was not affected and the K^+ /EDTA-ATPase was reduced by ~50%, similar to the effects of the corresponding IgG. In contrast to anti-SH-LC IgG, however, the monovalent antibody did not inhibit the actin-activated Mg^{2+} -ATPase in the presence of calcium (Figure 10), and binding of up to 3 mol of Fab did not interfere with actin activation. Since Fab fragments are monovalent, the desensitization could not be ascribed to cross-linking of myosin heads. Anti-SH-LC Fab did not dissociate R-LC's or SH-LC's (Figure 6), and calcium binding by myofibrils was not altered by anti-SH-LC Fab (Table I). Addition of excess R-LC's failed to resensitize myofibrils. Therefore desensitization is likely the consequence of specific binding of the monovalent anti-SH-LC antibody to a particular region of the SH-LC within the myosin molecule.

When R_5 -desensitizing anti-SH-LC IgG was absorbed at equivalence with (EDTA)S1, it lost its ability to desensitize myofibrils, although it still cross-reacted to some extent with intact SH-LC. R_5 -anti-SH-LC which was not absorbed with (EDTA)S1 fully desensitized the same myofibrils (not shown). Since (EDTA)S1 contains a 8000–10000 M_r fragment of the SH-LC and no R-LC's (Stafford et al., 1979), it appears that binding of R_5 -anti-SH-LC antibody to this remaining SH-LC fragment is responsible for the desensitization.

Affinity-purified anti-SH-LC antibodies bound to purified myosin and had effects on myosin similar to those they had on myofibrils (Table II). Significantly, R_5 -anti-SH-LC Fab fully desensitized scallop myosin by elevating the actin-activated Mg^{2+} -ATPase activity measured in the absence of calcium, and thus abolished the calcium dependence of the ATPase. Remarkably, binding of more than four anti-SH-LC Fab's by myosin did not significantly lower its actin-activated Mg^{2+} -ATPase activity in the presence of calcium (Table II). R_5 -anti-SH-LC IgG also desensitized HMM by increasing its V_{max} in the absence of calcium (Figure 8).

The desensitization by anti-R-LC and anti-SH-LC antibodies was the result of a specific interaction of these antibodies with the homologous light chains. Desensitization was prevented by adding to the antibody a slight excess of the homologous light chain before incubation with the myofibrils but not by the addition of heterologous light chain (Table III). Anti-R-LC and anti-SH-LC antibodies obtained by affinity

Table III: Prevention of Desensitization by Homologous Myosin Light Chain^a

scallop myofibrils incubated with	actin-activated Mg ²⁺ -ATPase ^b		% Ca sensitivity ^c
	EGTA	Ca ²⁺	
control IgG	0.01	0.35	97
anti-R-LC IgG	0.27	0.265	<0
anti-R-LC IgG + R-LC	0.02	0.28	93
anti-R-LC IgG + SH-LC	0.28	0.27	<0
R ₅ -anti-SH-LC IgG ^d	0.21	0.20	<0
R ₅ -anti-SH-LC IgG ^d + SH-LC	0.02	0.29	93
R ₅ -anti-SH-LC IgG ^d + R-LC	0.21	0.215	2.3

^a Scallop myofibrils incubated with a 3 M excess of anti-scallop myosin light-chain antibodies over myosin or with antibody plus a slight excess of either of the two light chains (R-LC, SH-LC).

^b Actin-activated Mg²⁺-ATPase activity, measured in the absence (EGTA) and presence of calcium (Ca²⁺), is expressed in micro-moles of ATP per minute per milligram of myofibrils. ^c Calcium sensitivity as defined under Experimental Procedures. ^d Desensitizing type of anti-SH-LC antibody elicited in rabbit no. 5.

chromatography of two different batches of anti-total-scallop myosin antisera also desensitized myosin indicating the structural similarity of isolated and bound myosin light chains.

(2) *Binding Studies.* (a) *Anti-Myosin Light-Chain Antibody Binding by Myofibrils.* Maximal antibody binding somewhat depended on the type of antibody. Binding was highest with anti-R-LC IgG ($3.7 \pm 5\%$ IgG/myosin), intermediate with R₅-desensitizing anti-SH-LC IgG ($3.4 \pm 5\%$ IgG/myosin), and lowest with R₄-nondesensitizing anti-SH-LC IgG ($3.2 \pm 5\%$ IgG/myosin) (not shown). The binding of anti-SH-LC and anti-R-LC antibodies was not completely additive when these antibodies were added simultaneously or successively to myofibrils. The degree of inhibition of antibody uptake depended on the antibody type. R₅-desensitizing anti-SH-LC IgG interfered more with anti-R-LC IgG uptake (20% inhibition) than R₄-nondesensitizing-anti-SH-LC IgG uptake (6% inhibition) (not shown). On the other hand, most of the R₄- and R₅-anti-SH-LC antibodies did not seem to compete for the same antigenic sites of the SH-LC, although full additivity was not obtained (23% inhibition) when these antibodies were added simultaneously to myofibrils (5.1 mol of IgG bound/mol of myosin vs. 6.6 mol which would represent full additivity).

(b) *Antibody Binding and R-LC Content.* Anti-R-LC IgG binding was proportional to the R-LC content of scallop myofibrils (Figure 11). The binding of antibody decreased as the R-LC content was reduced by EDTA treatment at elevated temperature (0–35 °C). Myofibrils from which R-LC's have been fully removed failed to bind anti-R-LC IgG in measureable quantity (Figure 11).

Binding of R₅-desensitizing anti-SH-LC IgG also depended on R-LC content. Removal of R-LC increased the uptake of this antibody. Binding increased by 50–60% in the absence of R-LC. The blocking of R₅-anti-SH-LC IgG binding sites by R-LC's was specific to the desensitizing anti-SH-LC IgG; the uptake of R₄-nondesensitizing anti-SH-LC IgG was essentially independent of R-LC content (Figure 11).

(c) *Inhibition of R-LC Uptake by Anti-SH-LC Antibody.* Scallop myofibrils from which R-LC's had been completely removed by EDTA treatment bound R₅-desensitizing anti-SH-LC IgG in excess (Figure 11). This antibody in turn significantly inhibited (72%) the reuptake of R-LC (Table IV). The monovalent R₅-anti-SH-LC Fab fragment, although only

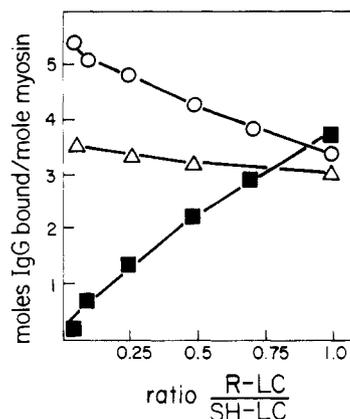


FIGURE 11: Binding of anti-scallop myosin light-chain antibodies to scallop myofibrils as a function of regulatory light-chain (R-LC) content. Anti-R-LC IgG (■); R₄-nondesensitizing anti-SH-LC IgG (Δ); R₅-desensitizing anti-SH-LC IgG (○). Antibody binding was determined by densitometry of 7.5% polyacrylamide gels in Na-DodSO₄, and the molar ratio of R-LC to SH-LC was obtained by densitometry of 10% polyacrylamide-urea gels stained with Fast Green.

Table IV: Inhibitory Effect of Anti-Scallop SH-LC Antibodies on Reuptake of R-LC^a

	ratio of R-LC ^b /SH-LC	% inhibition of reuptake of R-LC
normal scallop myofibrils	1.02	
myofibrils treated with 10 mM EDTA at 35 °C (desensitized myofibrils)	0.02	
desensitized myofibrils incubated with control IgG, followed by R-LC	0.98	2
R ₅ -anti-SH-LC IgG, followed by R-LC	0.28	72
R ₅ -anti-SH-LC Fab, followed by R-LC	0.48	52
R ₄ -anti-SH-LC IgG, followed by R-LC	0.65	35

^a Scallop myofibrils treated with 10 mM EDTA at 35 °C (desensitized myofibrils) and subsequently incubated with antibodies against the "essential" light chain (SH-LC), followed by incubation with an excess of regulatory light chain (R-LC) of scallop myosin. R₄ and R₅ refer to the two types of anti-SH-LC antibodies: R₄-anti-SH-LC IgG (nondesensitizing type, elicited in rabbit no. 4) and R₅-anti-SH-LC IgG (desensitizing type, elicited in rabbit no. 5). ^b Molar ratio of R-LC to SH-LC determined by densitometry of 10% polyacrylamide urea gels stained with Fast Green (see Figure 1).

about one-third of the size of IgG, still had a 52% inhibitory effect. In comparison, the inhibition with nondesensitizing R₄-anti-SH-LC IgG was less than half (35%), while preimmune, control IgG was not inhibitory. These effects were confirmed with desensitized myosin (not shown).

Discussion

Effects of Antibodies Specific for Scallop R-LC. Considerable evidence has been accumulated over the past years for the direct involvement of the R-LC in thick filament linked calcium control (Kendrick-Jones et al., 1970, 1976; Szent-Györgyi et al., 1973; Kendrick-Jones, 1974; Simmons & Szent-Györgyi, 1978; Stafford et al., 1979; Chantler & Szent-Györgyi, 1980). The desensitizing effect of the anti-R-LC antibodies is therefore not surprising and in line with the previous studies on R-LC function. The specificity of the effect of these antibodies, however, is somewhat unexpected. Up to 3 mol of anti-R-LC IgG may be bound by myosin without inhibiting the ATPase activities. Desensitization by these antibodies is the consequence of the specific elevation of the actin-activated Mg²⁺-ATPase in the absence of calcium to a point where the ATPase is no longer dependent upon the presence of calcium. These antibodies act by combining with

the R-LC's thereby abolishing their inhibitory effect on actin-activation in the absence of calcium. Desensitization by anti-R-LC IgG is not due to the dissociation or loss of R-LC, and calcium binding was not reduced by this antibody.

Anti-R-LC Fab also desensitized myofibrils. The Fab fragment, however, in addition, significantly dissociated R-LC's and decreased calcium binding. The desensitization by the anti-R-LC Fab fragment therefore is a complex combination of two effects: loss of R-LC and binding of antibody.

Partial dissociation of DTNB-LC from chicken myosin by certain batches of anti-DTNB-LC antisera has been reported earlier (Lowey & Steiner, 1972; Holt & Lowey, 1975). However, in these cases even the divalent anti-DTNB-LC IgG dissociated the LC's. With scallop, a dissociation of R-LC could only be achieved with the monovalent Fab fragment. Anti-R-LC IgG was not only ineffective in releasing R-LC's but also prevented R-LC dissociation by EDTA. It is unclear at present whether the dissociation of R-LC is due to a conformational change, actively induced by the specific antibody, which then results in the release of R-LC as a soluble R-LC-Fab complex, whether dissociation is simply a question of a higher affinity of the antibody for the R-LC, or whether the R-LC has a higher "off" rate for myosin. The way anti-R-LC IgG prevents dissociation of the R-LC is not clear. Cross-linking of the two R-LC's on the myosin molecule by the divalent IgG remains a possibility. These results are analogous in many respects to those obtained with anti-creatine phosphokinase (CPK) antibodies. Antibody-IgG specific for the M-line protein, CPK, binds specifically to the M line of chicken skeletal myofibrils and prevents the extraction of the electron-dense material within the M line which is usually achieved by low ionic strength buffers (Wallimann et al., 1977). Monovalent anti-CPK Fab fragments destabilize this structure by inducing a dissociation of significant amounts of CPK from the M line with a concomitant loss of the M-bridge material within the M line (Wallimann et al., 1978). The results obtained with anti-scallop R-LC antibodies are in accord with the greater ease by which the regulatory light chains may be removed from myosin compared to the SH-LC. While R-LC's are released readily from scallop myosin by EDTA or monovalent anti-R-LC antibodies, so far, no comparable treatment has been found for the reversible removal of SH-LC's. Similarly, no procedure has yet been found for the removal of alkali light chains from vertebrate myosin without its denaturation.

Effects of Antibodies Specific for Scallop SH-LC. The observation that R₅-anti-SH-LC antibodies interfered with regulation is the most unexpected result of this study. Since this finding at present is the only one that suggests a role for this class of light chains, it is important to evaluate how compelling the evidence is for assigning a role to the SH-LC in myosin-linked regulation.

Desensitization by the R₅-anti-SH-LC antibodies is the result of the elevation of the actin-activated Mg²⁺-ATPase in the absence of calcium. This activation effect is particularly specific with the R₅-anti-SH-LC Fab which desensitizes fully without inhibiting the actin-activated Mg²⁺-ATPase in the presence of calcium when on the average 1.5 mol of Fab is bound/myosin head (Figure 10). The desensitization by the monovalent antibody cannot be due to cross-linking of the myosin heads.

R₅-anti-SH-LC antibodies desensitized by combining with the SH-LC of myosin and their effect can not be explained by an artifact of cross-reactivity with R-LC's. It has been conclusively shown that the anti-scallop myosin LC antibodies used for this study are specific for the homologous light chain

(Wallimann & Szent-Györgyi, 1981). In addition, desensitization by R₅-anti-SH-LC antibody was prevented by preincubation with SH-LC but not by preincubation with R-LC. Anti-SH-LC antibodies did not release R-LC's, prevent their dissociation by EDTA, or alter calcium binding. Finally, the results obtained with antibodies, purified on affinity columns, and pure myosin agreed with the effects of crude antibody preparation on myofibrils. Therefore, the desensitization of scallop myosin reflects a specific interaction of the desensitizing anti-SH-LC antibody (divalent or monovalent) with the SH-LC.

Experiments with the R₄-nondesensitizing anti-SH-LC antibody population demonstrate that desensitization is not simply due to steric hindrance caused by a mere attachment of antibodies onto myosin. Although these antibodies were specific for the SH-LC (Wallimann & Szent-Györgyi, 1981) and bound extensively to myosin, they did not desensitize myofibrils and did not lower the K⁺/EDTA-ATPase. The fact that the binding of R₄- and R₅-anti-SH-LC IgG's was nearly additive and that only 23% of the two antibody populations competed for the same sites shows that these two antibody populations bind to different regions of the SH-LC. The two antibodies differed also in their reactivity with CNBr peptides (Wallimann & Szent-Györgyi, 1981). The experiments indicate that calcium sensitivity is influenced by a particular region of the SH-LC and binding of antibody (monovalent or bivalent) to that region interferes with the regulatory function of myosin.

R-LC and SH-LC of scallop myosin do not appear to participate directly in ATPase and actin-binding sites. This is in agreement with previous results of Holt & Lowey (1975) obtained with anti-chicken DTNB-LC and alkali-LC antibodies. Actin did not significantly interfere with antibody uptake by the light chains since pure myosin bound antibodies only in slight excess over myofibrils. The anti-LC antibodies had no effect on the high Ca²⁺-ATPase, and, although the K⁺/EDTA-ATPase and the actin-activated Mg²⁺-ATPase in the presence of calcium were reduced to half of their initial value by R₅-anti-SH-LC IgG, the inhibition disappeared or was greatly lessened when Fab fragments were used. The important effect of all the antibodies with the exception of the R₄-nondesensitizing anti-SH IgG consisted of the increase of the turnover rate of the actin-activated Mg²⁺-ATPase in the absence of calcium. This effect has also been demonstrated with HMM at varied actin concentrations. The results with HMM indicate, furthermore, that the antibody effects do not depend on the constraints imposed by the myofibrillar structure.

Evidence for Interaction between R-LC and SH-LC. The desensitizing effect of the R₅-anti-SH-LC antibodies may mean that the SH-LC is a component of the regulatory system of myosin. A direct role of the SH-LC in regulation would imply an interaction between the two kinds of light chains and their close proximity. It is possible, however, that the role of the SH-LC is a passive one and it simply occupies a region of the myosin molecule that is particularly important for regulation.

It has been shown recently that R-LC protected SH-LC from digestion by papain (Stafford et al., 1979) which suggests an interaction between the two kinds of light chains of scallop myosin. Our results, although based on indirect evidence, also indicate an interdependence of the two light chains. The extent of the binding of R₅-desensitizing anti-SH-LC antibody to myofibrils depended on the presence of R-LC. Removal of R-LC exposed additional binding sites on the SH-LC previously covered by the R-LC, e.g., EDTA-treated myofibrils

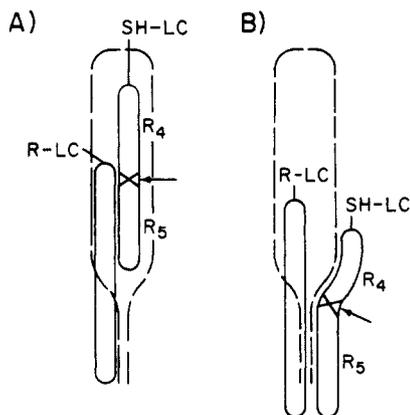


FIGURE 12: Schematic models for relative position of R-LC and SH-LC in scallop myosin: staggered (A) and forked (B) arrangement of LC's. The models are complemented by a tentative outline of the myosin head portion. The two domains to which R_5 -desensitizing and R_4 -nondesensitizing anti-SH-LC antibodies are preferentially binding are designated R_5 and R_4 , respectively. The position where the SH-LC may be clipped by papain in the absence of divalent cations is marked by X.

lacking R-LC bound R_5 -anti-SH-LC antibody in excess of nearly 2 mol/mol of myosin over intact myofibrils. Anti-R-LC and R_5 -anti-SH-LC antibody uptake showed a reciprocal inhibition when added simultaneously or consecutively. The 20% inhibition appears to be significant since these antibodies were highly specific for the homologous light chains and therefore did not compete for the same antigenic sites. Finally, R_5 -anti-SH-LC IgG and Fab significantly inhibited (50–70%) the reuptake of R-LC by desensitized myofibrils and myosin. Antibody bound to the SH-LC must therefore be within the range of the attachment sites of R-LC.

The evidence obtained with R_5 -anti-SH-LC antibodies thus indicates a putative interaction between R-LC and SH-LC and suggests that the antigenic sites against which the R_5 -anti-SH-LC antibody population are directed may be located on a particular region of the SH-LC close to the R-LC. The binding of R_4 -anti-SH-LC antibodies was far less affected by R-LC or by anti-R-LC antibodies. Antigenic sites against which these R_4 antibodies are directed may be found on particular regions of the SH-LC farther away from the R-LC. The simplest explanation of these and previous results on desensitizing and nondesensitizing anti-SH-LC antibodies is that these antibodies bind to different domains of the SH-LC. This is supported by the observed differences in their cross-reactivity with the CNBr peptides of the SH-LC (Wallimann & Szent-Györgyi, 1981). The domain reacting with the desensitizing-anti-SH-LC antibody (R_5) appears to be closer to the R-LC and may be involved in myosin-linked regulation.

These results suggest interactions between the different light chains. This interpretation is, however, limited by the large size of the antibodies used as probes and remains tentative until more direct evidence is obtained.

Model for LC Location on Scallop Myosin. R-LC's are elongated molecules of nearly 100-Å length (Stafford & Szent-Györgyi, 1978; Alexis & Gratzer, 1978; Hartt & Mendelson, 1979). A similarly elongated structure has been inferred for the SH-LC from immunological studies (Wallimann & Szent-Györgyi, 1981). Indirect evidence suggests that the DTNB-LC's in rabbit (Weeds & Taylor, 1975; Weeds & Pope, 1977; Bagshaw, 1977) extend to the S1–S2 hinge region of the myosin molecule, since they protect this region of the heavy chain from digestion. An involvement of the S2 region in calcium regulation was indicated by studies showing that

the myosin fragments from which S2 has been removed (S1) lack calcium sensitivity while myosin fragments containing S2 (HMM and single-headed myosin) are calcium sensitive (Stafford et al., 1979). In addition, antibodies specific to myosin rod fragment also desensitized myofibrils (T. Wallimann & J. M. Stallmeyer, unpublished observation). It is reasonable to assume therefore that the regulatory light chain extends to the S2–S1 hinge region. Recent electron microscopic studies on myosin and myosin fragments performed in collaboration with Dr. Carolyn Cohen's group demonstrate directly the presence of a portion of the R-LC at the neck toward the hinge region of myosin.

We propose that the SH-LC contains two domains, one of which (R_5) is in rather close proximity to the R-LC (Figure 12). This is the domain that reacts with R_5 -desensitizing antibody and may somehow be involved in regulatory function. This is also the domain that is present on the SH-LC fragment of the (EDTA)S1 representing about half of the SH-LC (M_r 8000–10000) since absorption of R_5 -desensitizing anti-SH-LC antibody with (EDTA)S1 removes the population of antibodies responsible for the desensitizing effect. This fragment (R_5) then would be the one close to the R-LC.

In the model it is suggested that the SH-LC domain (R_5) containing antigenic sites for R_5 antibodies is close to and interacts with the R-LC. The domain binding R_4 antibodies (R_4), on the other hand, does not interact with and is at a distance from the R-LC. In two variants of the model the two light chains are either staggered or forked (Figure 12). The place where papain cleaves the SH-LC is marked by an X. The model also shows a schematized outline of the myosin head. Naturally, more direct evidence establishing the proximity of R-LC and SH-LC would be highly desirable.

We propose that there is an interplay between R-LC, SH-LC, and S2–S1 hinge that is altered as a result of calcium action. Structural studies are needed to test this model (Vibert et al., 1978; Craig et al., 1980).

Acknowledgments

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Mechanism of Inactivation of γ -Aminobutyric Acid- α -Ketoglutaric Acid Aminotransferase by 4-Amino-5-halopentanoic Acids[†]

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ABSTRACT: (S)-4-Amino-5-halopentanoic acids were previously shown to irreversibly inhibit pig brain γ -aminobutyric acid- α -ketoglutaric acid aminotransferase, and a mechanism for the inactivation was proposed (Silverman, R. B., & Levy, M. A. (1980) *Biochem. Biophys. Res. Commun.* 95, 250). Evidence is presented to show that these compounds are mechanism-based inactivators, and experiments are described to elucidate their mechanism of action. The enzyme must be in

the pyridoxal phosphate form for inactivation to occur, the γ proton of the inactivators is removed in a rate-determining step, and one fluoride ion is released from 4-amino-5-fluoropentanoic acid per active site labeled. The change in the optical spectrum of the enzyme during inactivation suggests that the coenzyme is converted into the pyridoxamine phosphate form. Every turnover of inactivator leads to an inactivation event, i.e., the partition ratio is zero.

It is well established that γ -aminobutyric acid (GABA)¹ is a major CNS inhibitory neurotransmitter and that the brain level of GABA is important in CNS dysfunctions such as seizures (Krnjević & Schwartz, 1967; Obata & Takeda, 1969; Baxter, 1970; Mandel & DeFeudis, 1979). The brain concentration of GABA is principally controlled by two PLP-dependent enzymes, glutamate decarboxylase, which catalyzes the biosynthesis of GABA, and GABA-T, which is responsible for its catabolism. Since GABA is not transported across the blood-brain barrier, many compounds which inhibit GABA-T have been synthesized in order to increase the brain concentration of GABA for use as potential anticonvulsant agents

(Mandel & DeFeudis, 1979; Roberts et al., 1976). Numerous compounds have been shown to be irreversible inhibitors of GABA-T both in vitro and in vivo, e.g., 4-amino-5-ynoic acid (Jung & Metcalf, 1975), 4-amino-5-enoic acid (Lippert et al., 1977), gabaculine (Rando & Bangerter, 1977), isogabaculine (Metcalf & Jung, 1979), and ethanolamine O-sulfate (Fletcher & Fowler, 1980). Concurrent with the

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¹ Abbreviations used: GABA, γ -aminobutyric acid; GABA-T, γ -aminobutyric acid- α -ketoglutaric acid aminotransferase (EC 2.6.1.19); PLP, pyridoxal 5'-phosphate; PMP, pyridoxamine 5'-phosphate; AFPA, 4-amino-5-fluoropentanoic acid; ACPA, 4-amino-5-chloropentanoic acid; ABPA, 4-amino-5-bromopentanoic acid; α -KG, α -ketoglutarate; SSADH, succinic semialdehyde-NADP oxidoreductase (EC 1.2.1.16); NADP, nicotinamide adenine dinucleotide phosphate; Tris, tris(hydroxymethyl)aminomethane; Gabase, a commercial preparation from *Pseudomonas fluorescens* of GABA-T and SSADH.