Lees, A., Haddad, J. G., & Lin, S. (1984) *Biochemistry 23*, 3038–3047.
Lehrer, S. S., & Kerwar, G. (1972) *Biochemistry 11*, 1211–1216.
Markey, F., Persson, T., & Lindberg, U. (1982) *Biochim. Biophys. Acta 709*, 122–133.
Norberg, R., Thorstensson, R., Utter, G., & Fragraeus, A. (1979) *Eur. J. Biochem. 100*, 575–583.
Snabes, M. C., Boyd, A. E., & Bryan, J. (1983) *Exp. Cell Res. 146*, 63–70.
Taylor, R. S., & Weeds, A. G. (1976) *Biochem. J. 159*, 301–315.
Weeds, A. G., Harris, H. E., Gooch, J., & Pope, B. (1985) 9th European Symposium on Hormones and Regulation, Strasbourg, France.
Yin, H. L., & Stossel, T. P. (1979) *Nature (London) 281*, 583–586.
Yin, H. L., Albrecht, J. H., & Fattoum, A. (1981a) *J. Cell Biol. 91*, 901–906.
Yin, H. L., Hartwig, J. H., Maruyama, K., & Stossel, T. P. (1981b) *J. Biol. Chem. 256*, 9693–9697.
Yin, H. L., Kwiatkowski, D. J., Mole, J. E., & Cole, F. S. (1984) *J. Biol. Chem. 259*, 5271–5276.

Essential Light Chain Exchange in Scallop Myosin†

Gaku Ashiba and Andrew G. Szent-Györgyi*
Department of Biology, Brandeis University, Waltham, Massachusetts 02254
Received February 8, 1985; Revised Manuscript Received May 20, 1985

ABSTRACT: The exchange of essential light chains (SH-LCs) of scallop myosin was followed with the aid of scallop SH-LC alkylated with ^{14}C-labeled iodoacetate. More than 70% of the SH-LCs were exchanged in myosin preparations that were desensitized by removal of both regulatory light chains (R-LCs) with ethylenediaminetetraacetic acid (EDTA) treatment. Although desensitized myosin solubilized with 0.6 M NaCl or with 10 mM adenosine 5′-triphosphate (ATP) in the absence of salt equilibrated rapidly with SH-LCs even in the cold, exchange in myosin filaments required elevated temperatures. Equilibration of the SH-LCs in desensitized preparations did not depend on ATP or magnesium ions but was significantly accelerated by actin. The desensitized myosin preparations containing alkylated SH-LCs (~1 mol of thiol alkylated/mol of SH-LC) readily recombined with R-LCs. The preparations regained fully the calcium dependence of the actin-activated magnesium adenosinetriphosphatase (Mg-ATPase), contained R-LCs and SH-LCs in equimolar amounts, and had an ATPase activity similar to that of untreated myosin preparations. R-LCs interfered with the equilibration of the SH-LCs. In intact myosin preparations, the exchange of SH-LCs was slow and was frequently associated with the dissociation of the R-LCs. The blocking action of the R-LC on SH-LC exchange agrees with evidence showing that the two light chain types interact and suggests that parts of the SH-LC may lie between the R-LC and the heavy chain of myosin.

Contraction of molluscan muscles is regulated by myosin, and activation results from the binding of calcium directly to myosin (Kendrick-Jones et al., 1970). The myosin subunits responsible for regulation are the regulatory light chains (R-LCs)[1] and the essential light chains (SH-LCs). Since the R-LCs of scallop myosin can be removed reversibly without loss of contractile activity, it was possible to establish directly that R-LCs are required for regulation: their removal abolishes completely the calcium sensitivity of the actin-activated ATPase or of tension generation (Szent-Györgyi et al., 1973; Simmons & Szent-Györgyi, 1978; Chantler & Szent-Györgyi, 1980).

The suggestion that the SH-LCs are also regulatory subunits is based on findings that antibody specific to the SH-LC desensitizes scallop myosin and that in its presence the actin-activated Mg-ATPase of myosin no longer requires calcium (Wallimann & Szent-Györgyi, 1981). The close proximity of R-LCs and SH-LCs in scallop myosin is indicated by several lines of evidence. Fab fragments of antibodies specific to R-LCs bind to the same region of the myosin molecule as the ones specific to the SH-LCs (Flicker et al., 1983), antibody specific to SH-LC interferes with the binding of R-LC to myosin (Wallimann & Szent-Györgyi, 1981), and the binding site for both R-LCs and SH-LCs is on the same proteolytic fragment of M_r approximately 14000 of the myosin heavy chain (Szentkiralyi, 1984) probably corresponding to the neck region of the myosin molecule (Bagshaw, 1977; Winkelman et al., 1984). Cross-linking studies have shown that SH-LCs and R-LCs overlap at least along half of their lengths and are separated by a distance of less than 9 Å (Wallimann et al., 1982). An interaction between R-LCs and SH-LCs in myosin is suggested by the observation that the R-LCs protect the SH-LCs from papain digestion (Stafford et al., 1979) and prevent the thiol groups of the SH-LC from reacting with thiol reagents (Hardwicke et al., 1982).

†This research was supported by grants from the U.S. Public Health Service (AM15963) and the Muscular Dystrophy Association (to A. G.S.-G.) and a fellowship from the Muscular Dystrophy Association (to G.A.).

[1] Abbreviations: R-LC(s), myosin regulatory light chain(s); SH-LC(s), scallop myosin essential light chain(s); A1 and A2, vertebrate myosin alkali light chains 1 and 2; S1, myosin subfragment 1; S2, myosin subfragment 2; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; DTE, dithioerythritol; Na-DodSO$_4$, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; HMM, heavy meromyosin; ATPase, adenosinetriphosphatase; Mg-ATPase, magnesium adenosinetriphosphatase.

0006-2960/85/0424-6618$01.50/0

Regulaton is associated with movement of the light chains in scallop myosin. Cross-linking studies have established that the N-terminal portion of the R-LC is separated from the SH-LC by a distance of greater than 9 Å at rest and that the separation decreases under conditions of rigor and activity. When the N-terminal portion of the R-LC is cross-linked to the SH-LC, regulation is lost and myosin is locked in the "on" state (Hardwicke et al., 1983). Recent evidence suggests that the change in the separation between the light chains may be due to the movement of the SH-LC (Hardwicke & Szent-Györgyi, 1985).

A more precise definition of the role of the SH-LC in regulation requires the development of methods that allow the reversible dissociation or exchange of the SH-LC from scallop myosin. In the case of vertebrate skeletal myosin subfragment 1 (S1), the alkali light chains (A1 and A2, the SH-LC homologues of vertebrate fast-striated myosins) exchange readily in the presence of high concentrations of ammonium chloride (Wagner & Weeds, 1977). The alkali light chains are not required for ATPase activity, and S1 preparations freed from these light chains with the combined use of ammonium chloride and anti-alkali light chain antibodies retain considerable ATPase activity (Wagner & Giniger, 1981). In the presence of ATP at elevated temperature, the SH-LCs of rabbit S1 exchange readily and can be removed in the absence of ammonium chloride with no decrease in the enzymatic activity of the S1 (Burke & Sivaramakrishnan, 1981; Sivaramakrishnan & Burke, 1982). The same mild treatment was also reported to be effective for the equilibration of SH-LCs in whole myosin (Burke & Sivaramakrishnan, 1981). While the effects of ATP and elevated temperature on the equilibration of SH-LCs in S1 have been confirmed in several laboratories, the exchange in myosin appears to be more complex and no significant exchange was obtained in chicken (Waller & Lowey, 1983) or in rabbit myosins (Wagner & Stone, 1983) under similar conditions. However, it was observed recently that the absence of R-LCs in vertebrate S1 or their partial removal from vertebrate myosin facilitates SH-LC exchange (Wagner & Stone, 1983).

A study of the role of the SH-LCs in regulation requires the use of myosin or heavy meromyosin since S1 preparations are not regulated (Szent-Györgyi et al., 1973; Stafford et al., 1979). Because chaotropic agents inactivate scallop myosin (Wallimann and Sultan, unpublished experiments), we have developed alternative methods that result in SH-LC exchange in excess of 70% and produce myosin that remains fully regulated.

A preliminary report of the findings was presented at the 1984 meeting of the Biophysical Society (Ashiba & Szent-Györgyi, 1984).

Materials and Methods

Protein Preparations. Myosin was prepared from the striated adductor muscle of the bay scallop (*Aequipecten irradians*) by the method of Focant & Huriaux (1976) with slight modifications (Stafford et al., 1979). Myosin was desensitized by a modification of previous procedures (Wallimann & Szent-Györgyi, 1981). Regulatory light chains (R-LCs) were removed by a 5-min exposure to 10 mM EDTA in 40 mM NaCl and 5 mM phosphate, pH 7.0 at 23 °C. Myosin was pelleted by centrifugation, washed twice with 40 mM NaCl and 5 mM phosphate, pH 7.0, first at 23 °C and then at 0 °C, and taken up in the exchange medium. EDTA treatment at 23 °C removes both R-LCs from myosin. Myosin containing 1 mol of R-LC/mol of myosin was prepared similarly except that the temperature was kept at 0 °C during EDTA treatment and washes. Heavy meromyosin (HMM) was prepared from the fully regulated scallop myosin according to Craig et al. (1980).

Scallop essential light chains were isolated from desensitized myosin by treatment with 6 M guanidine hydrochloride as previously described (Kendrick-Jones et al., 1976). Further purification was carried out by urea gel electrophoresis (Perrie & Perry, 1970) on a preparative scale, and purity of more than 99% was ensured by analytical or preparative HPLC (Waters Model 262, monitored at 214 nm) using a μBondapak C_{18} column (3.9 mm × 300 mm) in 0.1% phosphate (pH 2.05) with an acetonitrile gradient (30–50%). The SH-LCs were carboxymethylated with iodo[1-^{14}C]acetic acid (Szent-Györgyi et al., 1973) except that the alkylation was performed in the absence of guanidine hydrochloride so that ca. 1 mol of thiol/mol of SH-LC was modified.

Chemicals. Iodo[1-^{14}C]acetate was purchased from New England Nuclear, Boston, MA, and the Coomassie Blue solution for Bradford's protein determination (Bradford, 1976) and Bio-Gel A-0.5m were from Bio-Rad, Rockville Center, NY. HEPES and dithioerythritol were purchased from Sigma, St. Louis, MO, and dithiothreitol was from Calbiochem-Behring, La Jolla, CA. Sephacryl S-200 superfine was purchased from Pharmacia, Piscataway, NJ, aqueous counting scintillant (ACS) was from Amersham, Arlington Heights, IL, and Fast Green was from Fisher, Pittsburgh, PA.

SH-LC Exchange. The exchange of SH-LCs was followed by measuring incorporation of the radioactively labeled SH-LCs into myosin under various conditions. Intact myosin or myosin desensitized immediately before the exchange was homogenized by a single passage in a Teflon-coated hand homogenizer in an exchange medium, and the exchange was initiated by the addition of 8–18 mol of labeled SH-LC/mol of myosin at a myosin concentration of 0.5–2 mg/mL. After incubation with gentle stirring at 4, 20, or 23 °C, the exchange was terminated by diluting the samples with 15 volumes of cold buffer solution containing 10 mM phosphate, pH 6.5, and sufficient amounts of NaCl and $MgCl_2$ to bring the NaCl concentration to 40 mM and the $MgCl_2$ concentration to 3 mM at pH 6.5 and immediately centrifuging for 10 min at 25000*g*. Unbound SH-LC was removed by dissolving the precipitated myosin in 0.6 M NaCl, 5 mM $MgCl_2$, 10 mM phosphate, pH 7.0, 0.2 mM DTT, and 0.1 mM EGTA, followed by precipitation at low ionic strength. The myosin was pelleted, redissolved, and reprecipitated. Bound SH-LC was not removed by this procedure since the SH-LC content of the myosin remained equimolar with its heavy chain as shown by NaDodSO$_4$–polyacrylamide gel electrophoresis.

Myosin was resensitized by incubating myosin solubilized in 0.6 M NaCl, 1 mM $MgCl_2$, and 5 mM phosphate, pH 7.0, with 4 mol of R-LC/mol of myosin for 20 min at 20 °C, followed by a 2-h incubation at 0 °C with gentle shaking. Myosin was precipitated by dilution with 15 volumes of 3 mM $MgCl_2$, and unbound R-LC was removed by centrifugation and reprecipitation. The resensitized myosin was dissolved in 0.6 M NaCl and dialyzed against 0.6 M NaCl, 10 mM phosphate, pH 7.0, and 1 mM $MgCl_2$.

Each step was carried out in the presence of 0.2 mM DTT and 0.2 mM EGTA at 4 °C unless stated otherwise. The repeated reprecipitations were necessary to remove excess SH-LCs that adsorbed nonspecificially to myosin filaments in significant amounts and were not removed by washing with 40 mM NaCl. Excess SH-LC could also be removed by gel filtration in a high-salt medium on a Sephacryl S-200 column or by size fractionation on a combination of HPLC columns

(Waters I-60, I-125, and I-250). However, chromatography was more time-consuming, and in most experiments unbound SH-LC was removed by reprecipitation.

The following solutions were used for exchange: ATP medium (solution A) containing 10 mM HEPES (pH 7.5), 10 mM ATP, 3 mM DTT, 0.1 mM EGTA, and ±12 mM $MgCl_2$; high-salt medium (solution H) containing 0.6 M NaCl, 10 mM HEPES (pH 7.5), 3 mM DTT, 0.1 mM EGTA, and ±3 mM $MgCl_2$; low-salt medium (solution L)containing 40 mM NaCl, 10 mM HEPES (pH 7.5), 3 mM DTT, 0.1 mM EGTA, and 3 mM $MgCl_2$. Myosin was solubilized in the ATP and in the high-salt media but formed filaments in the low-salt medium.

In each experiment a control sample was subjected to identical procedures except that it was incubated at 4 °C without added SH-LC. A zero time point was obtained by adding the labeled SH-LC to a sample that was precipitated in advance by a 15-fold dilution with cold buffer.

As a nonfilamentous control of the intact myosin, we employed HMM instead. The extra SH-LCs were removed from HMM by repeated centrifugation of a Bio-Rad A-0.5m column (Shpetner, personal communication).

Assays. Each sample was analyzed for ATPase activity, for light chain content by urea gel electrophoresis, and for SH-LC exchange by measurement of radioactivity.

ATPase activity was measured by a Radiometer pH stat, pH 7.5, at 25 °C. The 10-mL reaction medium for measuring the actomyosin–ATPase activity contained 40 mM NaCl, 50–100 μg/mL myosin, an equal weight of rabbit or chicken skeletal F-actin, 2 mM $MgCl_2$, 1 mM ATP, and 0.1 mM EGTA in the absence or in the presence of 0.2 mM $CaCl_2$. The reaction medium for measuring the high-salt Ca^{2+}-ATPase activity contained 0.6 M NaCl, 2 mM $CaCl_2$, 25 μg/mL myosin, and 1 mM ATP. The acto–HMM ATPase medium contained 20 mM NaCl, 100 μg/mL HMM, 1.4-fold weight of rabbit skeletal F-actin, 2 mM $MgCl_2$, 1 mM ATP, and 0.1 mM EGTA in the absence or in the presence of 0.2 mM $CaCl_2$.

Urea gel electrophoresis was carried out according to the procedure of Perrie & Perry (1970) using a 5 × 60 mm tube gel of 10% polyacrylamide. Protein bands were stained with Fast Green and subjected to densitometry at 660 nm. The $NaDodSO_4$–polyacrylamide gel electrophoresis system of Laemmli (1970) was used to determine the ratio between myosin heavy chain and actin for actomyosin samples and to obtain the ratio between SH-LC and heavy chain in desensitized myosin.

Incorporation of SH-LC was obtained by measuring ^{14}C content in a Beckman LS255 scintillation counter using an ACS scintillation mixture. Specific activities were adjusted to obtain >800 cpm at 60% exchange. During calculation of the exchange (in percent of the theoretical), correction was made for the unlabeled SH-LCs originally present in myosin. The molecular weights of myosin, HMM, and SH-LC were taken to be 500 000, 350 000, and 18 000, respectively.

Protein concentrations were determined by either the biuret (Gornall et al., 1949) or the Folin–Lowry (Lowry et al., 1951) methods and by Bradford's method (Bradford, 1976) when a sample contained DTT or DTE in millimolar amounts. Bovine serum albumin was used to generate a standard curve.

Results

SH-LC Exchange in Desensitized Myosin. Desensitized myosin filaments suspended in low-salt medium exchanged readily with added SH-LC. Incorporation of radioactive SH-LC depended on time and on temperature (Figure 1):

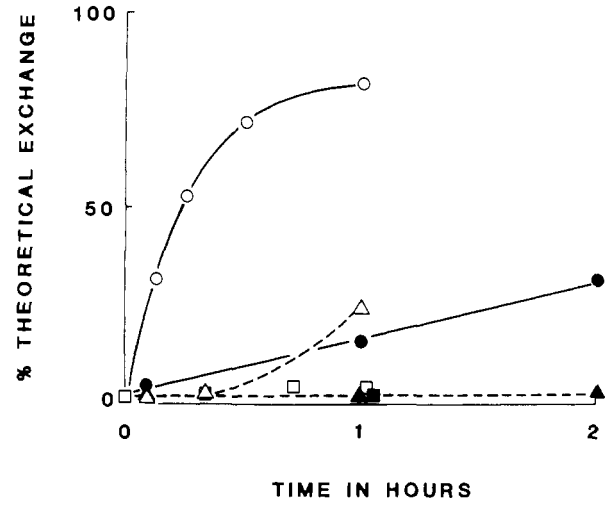

FIGURE 1: Time course of SH-LC exchange in myosin filaments. Exchange was initiated by addition of ^{14}C-labeled SH-LC (1 mol of alkylated thiol/mol of SH-LC) in 8 molar excess to myosin suspended in the low-salt medium (see Materials and Methods). The reaction was terminated by a 15-fold dilution with an ice-cold buffer solution containing 40 mM NaCl, 3 mM $MgCl_2$, and 10 mM phosphate, pH 6.5, followed by immediate centrifugation and reprecipitation (see Materials and Methods). Actual separation of unbound SH-LCs took less than 2 min. A sample from each time point was resensitized, and its radioactivity, light chain content, and ATPase activity were measured (see also Table I). (△, ▲) Intact myosin; (○, ●) myosin fully desensitized by EDTA treatment at 23 °C; (□, ■) myosin partially desensitized by EDTA treatment at 0 °C. Open symbols, exchange experiments at 23 °C; closed symbols, exchange experiments at 4 °C.

exchange was faster at higher temperature, and in 60 min an exchange of over 80% was reached at 22 °C as compared to a 15% exchange at 4 °C. Excess SH-LC was removed rapidly from myosin suspensions by centrifugation so that zero time controls were exposed to labeled SH-LC for less than 2 min. In these controls the accumulation of radioactivity in myosin, following reprecipitation, amounted to less than 3% of the value expected from full equilibration. This value represents the upper limit of SH-LC contamination that may have remained adsorbed to desensitized myosin in a nonspecific manner and indicates that reprecipitation of myosin was an effective way to remove excess SH-LC. The total SH-LC content of myosin after reprecipitation was in a mole to mole stoichiometry with the heavy chains as measured by $NaDodSO_4$–polyacrylamide gel electrophoresis (data not shown).

Alkylation of the SH-LCs did not interfere with the reuptake of R-LCs or with regulation. Rebinding of R-LCs and restoration of the calcium sensitivity of the actin-activated Mg-ATPase did not depend on alkylated SH-LC content. Myosin preparations containing 1.3 mol of alkylated SH-LCs rebound R-LCs to the same extent as the controls and had the same ATPase activities, calcium sensitivity, and R-LC content as controls not exposed to labeled SH-LCs (Table I).

Partially desensitized myosin preparations did not show SH-LC exchange. Exchange was not obtained even at 23 °C with myosins that on the average retained one of their two R-LCs. Incubation at 23 °C for 1 h or longer caused irreversible losses in ATPase activity without increasing SH-LC exchange. Although R-LCs recombined with such myosin preparations, ATPase activity was not restored.

The equilibration of SH-LCs with solubilized myosin proceeded considerably faster than in myosin filaments. Exchange was rapid even at 4 °C: over 60% of the SH-LCs exchanged in 60 min at 4 °C in desensitized myosins dissolved in 0.6 M NaCl (Table II). Because myosin dissolved in 10 mM ATP

Table I: Recovery of Ca^{2+} Sensitivity of Myosin Containing Alkylated SH-LCs[a]

sample	exchange temp (°C)	exchange time (min)	exchange (% of theor)	R-LC/SH-LC ratio	actomyosin-ATPase (nmol of P_i min^{-1} mg^{-1}) +Ca	actomyosin-ATPase (nmol of P_i min^{-1} mg^{-1}) −Ca	% Ca sensitivity
intact[b]				0.93	890	30	97
intact	4	0	2	1.01	710	50	93
intact	4	120	3.5	0.99	870	70	92
intact	23	60	29	0.98	770	45	94
desensitized[b]				0.03	290	340	−17
desensitized at 23 °C	4	0	2	1.00	780	50	94
desensitized at 23 °C	4	120	32	0.91	830	80	90
desensitized at 23 °C	23	60	82[c]	1.01	850	60	93
intact[b]				1.06	690	40	94
desensitized at 0 °C[b]				0.51	280	245	12
desensitized at 0 °C[b]	4	60	1.5	1.04	680	20	97
desensitized at 0 °C[b]	23	20	3	0.95	690	60	91
desensitized at 0 °C[b]	23	40	4	1.01	740	50	93

[a] The samples of Figure 1 were incubated with excess R-LC in high-salt medium and separated from unbound R-LC, and their radioactivity, light chain content, and ATPase activity were measured. Note that myosin containing significant amounts of alkylated SH-LC rebound R-LC and regained full calcium sensitivity. For experimental details, see Materials and Methods. Calcium sensitivity: $(1 - ATPase_{EGTA}/ATPase_{Ca}) \times 100$. [b] Samples before incubation with R-LC. [c] The sample gave a count of 6500 cpm.

Table II: SH-LC Exchange in Desensitized Myosin[a]

temp (°C)	soln	Mg^{2+}	ATP	exchange (% of theor)
4	H	+	−	65 ± 2 (5)
4	H	−	−	71
4	L	+	−	22 ± 12 (3)
4	L	+	+	10 ± 1 (2)
23	L	+	−	72 ± 9 (2)
4	A	+	+	51
4	A	−	+	56

[a] Myosin was incubated with ^{14}C-labeled SH-LC (1 mol of thiol alkylated/mol of SH-LC) for 1 h. H is high salt, L is low salt, and A is ATP medium as described under Materials and Methods. Myosin formed filaments in solution L and was solubilized in solutions H and A. In $+Mg^{2+}$ enough $MgCl_2$ was added to bring the free Mg^{2+} concentration to 2 mM. The myosin concentration in solution A was 0.5 mg/mL both in the presence and in the absence of excess Mg^{2+}; otherwise, myosin concentrations ranged between 1.5 and ~2.0 mg/mL. For details, see Materials and Methods. Figures in parentheses are number of experiments.

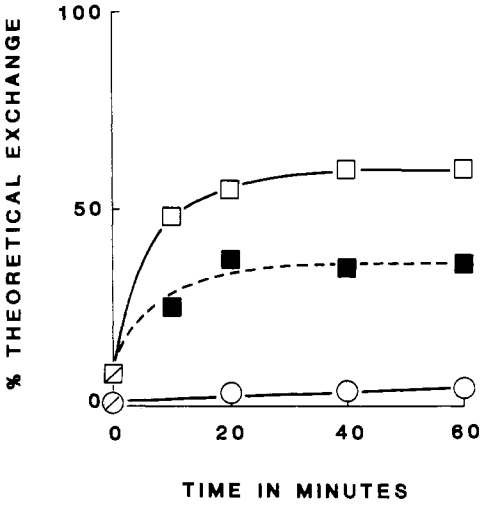

FIGURE 2: Effect of actin on SH-LC exchange. Intact (○) or desensitized (□, ■) myosin was dissolved and mixed with an equal weight of rabbit F-actin in 0.6 M NaCl, 3 mM $MgCl_2$, and 10 mM phosphate, pH 7.0. Actomyosin was precipitated by dilution, homogenized, and then incubated with labeled SH-LC in the low-salt medium either at 23 °C (open symbols) or at 4 °C (closed symbols). The reaction was terminated in the same way as described for myosin. All samples were subjected to the high-salt resensitization procedure (see Materials and Methods), followed by measurements of their radioactivity. (⊘, ⧄) Controls that were immediately centrifuged after SH-LC addition.

that contained no sodium chloride also showed a rapid exchange, the increased rate of exchange was not simply an ionic strength effect but rather reflected differences of the availability of the SH-LC in solubilized myosin as compared to that in myosin filaments.

Experiments to follow exchange in solubilized myosin, however, had several problems. The equilibration of the SH-LCs was too fast to obtain adequate time curves even at 4 °C, and the earliest point showed an approximately 30% exchange. Desensitized myosin had a low solubility in ATP, and the myosin concentration had to be kept below 0.5 mg/mL. At 23 °C desensitized myosin was less stable in 0.6 M NaCl, and after longer incubations both control and experimental samples lost their ability to rebind R-LCs fully.

Actin accelerated the exchange of SH-LCs about 2-fold at room temperature at low ionic strength (Figure 2). In most of these experiments actin was mixed with myosin in 0.6 M NaCl and the actomyosin was subsequently precipitated by dilution. Similar results, although not as reproducible, were obtained by directly adding globular or fibrous actin to preformed desensitized myosin filaments (data not shown). At 4 °C equilibration was greatly speeded up in the presence of actin and was as fast as at 23 °C although it stabilized at a lower final value (Figure 2). It is not clear whether actin changed the availability of the SH-LC binding sites on myosin or its effect was due to a disaggregation or disorganization of the myosin filaments. ATP or magnesium had no obvious effect on the exchange either at low- or high-salt concentrations (Table II).

SH-LC Exchange in Intact Myosin. The incorporation of labeled SH-LC into filaments formed from intact myosin was slow and limited, and labeled SH-LC did not exchange in significant amounts at 4 °C even after overnight exposure. The radioactivity associated with myosin was independent of incubation time and amounted to approximately 3% of the equilibrium value (Figure 1). At 23 °C equilibration began only after a 20-min lag period and was accompanied by a gradual and continuous loss of R-LCs (up to 1 mol from 1 mol of myosin in 1 h). Actin did not significantly alter the exchange rate on intact myosin filaments (Figure 2).

Exchange was more rapid and more extensive with solubilized myosin. Over 60% of the SH-LCs of myosin solubilized in 10 mM ATP exchanged in 60 min at 4 °C in the absence of magnesium. However, more than 80% of the R-LC was lost concomitantly from the preparation (Table III). Mag-

Table III: SH-LC Exchange in Intact Myosin[a]

	temp (°C)	soln	Mg^{2+}	ATP	exchange (% of theor)	R-LC/SH-LC before resensitization	R-LC/SH-LC after resensitization
myosin	4	H	+	−	29		
	4	H	−	−	38 ± (2)		
	4	L	+	+	4.6 ± 2.4 (2)		
	4	L	+	−	4.0 ± 0.1 (2)		
	4	A	+	+	40 ± 10 (3)	0.71 ± 0.03	0.97 ± 0.02
	4	A	−	+	62 ± 2 (3)	0.14 ± 0.01	0.97 ± 0.01
HMM	4	H	+	−	24 ± 1 (2)	0.96 ± 0.08	
	4	L	+	−	18 ± 2 (2)	0.90 ± 0.01	

[a] Experimental conditions for myosin as in Table II but with intact instead of desensitized myosin. In solution a with Mg^{2+}, myosin concentration was kept at 0.5 mg/mL; elsewhere, both myosin and HMM concentrations ranged between 1.5 and ~2.0 mg/mL. Figures in parentheses are number of experiments. The control HMM preparation had the actomyosin–ATPase activity ($+Ca^{2+}$) of approximately 1 μmol of P_i min^{-1} min^{-1} and the Ca^{2+} sensitivity of 85%. After the exchange procedure, HMM showed 10 to ~15% loss in the activity ($+Ca^{2+}$) and 4 to ~7% loss in the Ca^{2+} sensitivity.

nesium ions reduced both SH-LC exchange and R-LC removal. R-LC content and calcium sensitivity were both restored by incubation with R-LCs in the presence of excess magnesium ions. Myosin solubilized in 0.6 M NaCl also equilibrated with radioactive SH-LCs to a significant extent at 4 °C even in the presence of magnesium ions (Table III). Exchange was rapid and reached a plateau within 5 min, the time required for the removal of excess light chains. At this temperature there was no loss of R-LCs. Nevertheless, under the same conditions the scallop R-LCs exchanged fully with *Mercenaria* R-LCs alkylated with iodo[1-^{14}C]acetate within 20 min, while the exchange with the same *Mercenaria* R-LC was less than 10% at low-salt concentration. A 4-fold excess of scallop R-LC did not block SH-LC exchange.

The exchange of the SH-LCs in the HMM preparation that are soluble at both high- and low-salt concentrations was independent of ionic strength (Table III).

Discussion

The exchange of the SH-LCs in scallop myosin is greatly influenced by the presence of R-LCs and depends on temperature, on the filamentous state of myosin, and on the presence of actin. SH-LC exchange can be best followed on fully desensitized (i.e., no R-LCs) myosin preparations. The SH-LC exchange on intact scallop myosin is limited and is frequently associated with concomitant losses in R-LC content.

The presence of R-LCs inhibits the exchange of SH-LCs in vertebrate myosin. Chymotryptic S1 from rabbit myosin lacks R-LCs and equilibrates with alkali light chains extensively at elevated temperatures in the presence of MgATP (Burke & Sivaramakrishnan, 1981). Wagner & Stone (1983) could not demonstrate SH-LC exchange in intact rabbit myosin, although a partial exchange took place once a portion of the R-LCs was removed. They also observed that with rabbit papain S1 (containing R-LCs) the exchange was significantly slower than with chymotryptic S1 and concluded that R-LCs obstruct SH-LC exchange. Our findings with desensitized scallop myosin support this conclusion. It is noteworthy that ATP does not alter the exchange of SH-LCs in desensitized myosin and its effect on intact myosin is accompanied by losses in R-LCs.

Equilibration of SH-LCs in myosin filaments was slower than in myosin solutions. Since the exchange in solubilized myosin was faster both at high and at very low ionic strengths, the slow equilibration in myosin filaments probably results from intermolecular interactions in the filamentous assembly. The similar exchange shown by HMM in both high- and low-salt media reinforces this view. Cooperative interactions between myosin heads and S2 regions in the filament were first proposed by Harrington (1971). Intermolecular cooperativity was invoked to explain the very sharp calcium dependence of the actin-activated ATPase of myosin filaments (Chantler et al., 1981). An interaction between myosin heads and S2 regions of juxtaposed myosin molecules has been suggested from the three-dimensional reconstruction of native scallop myosin filaments (Vibert & Craig, 1983). Since both SH-LCs and R-LCs extend toward the neck region of the myosin molecule (Flicker et al., 1983), intermolecular interactions at this region could block exchange.

The facilitation of SH-LC exchange by actin may suggest a direct destabilization of the SH-LC binding site on the myosin head. It is not clear, however, whether there is any direct connection between actin and the SH-LC through the head region. The effect of actin may also be an indirect one: the binding of actin could sufficiently alter the myosin head structure so that the SH-LCs become more available for exchange. It is also possible that combination with actin interrupts possible interactions between S1 and S2 regions of different myosin molecules, thereby accelerating SH-LC exchange.

Despite the different behaviors exhibited by regulated and unregulated myosin and the different roles the light chains may play, there are similarities in their architecture and it is likely that findings on the location and some of the interactions of the light chains will be applicable to myosin molecules in general. The electron microscopic evidence locating the light chains near the S1–S2 junction in scallop myosin (Craig et al., 1980; Vibert & Craig, 1982; Flicker et al., 1983) has been extended to vertebrate-striated and smooth muscle myosins (Winkelman et al., 1983; Kendrick-Jones et al., 1982; Margossian et al., 1983). The binding sites of the R-LCs for heavy chains are conserved in different myosin species, and desensitized scallop myosin binds the R-LCs from all myosins so far tested (Kendrick-Jones, 1974; Kendrick-Jones et al., 1976; Sellers et al., 1981; Scholey et al., 1981). Although the exchange of the SH-LC homologue in vertebrate myosins requires considerably higher temperatures than the exchange in scallop, the inhibitory effect of their respective R-LCs may be taken as further evidence for similarities in the position and interactions of the light chains in the two types of myosin. The similar architecture of myosins is of considerable interest since scallop myosin is a favorable material for exchange studies due to the ease of the removal and readdition of its R-LCs.

The effect of the R-LC on SH-LC exchange provides additional evidence for the interaction between the two light chains. Such an interaction was deduced from observations that R-LCs prevent the fragmentation of the SH-LCs during papain digestion of myosin (Stafford et al., 1979), that the

thiol groups of the SH-LC become reactive only after removal of the R-LCs (Hardwicke et al., 1982), and that the R-LCs and SH-LCs are close to each other and can be cross-linked along a considerable portion of their length by short cross-linkers (Wallimann et al., 1982).

Our studies also indicate that limited alkylation of the SH-LCs does not interfere with the bindings of R-LCs. The loss of the ability of alkylated desensitized myosin to rebind R-LCs reported previously (Hardwicke et al., 1982) may have been due to a more extensive modification of the SH-LCs or/and to the alkylation of heavy chain thiol groups. It is noteworthy that while R-LCs can be removed without removal of SH-LCs, the exchange of SH-LCs requires the previous dissociation of the R-LCs. Therefore, it appears likely that a portion of the SH-LC lies between the R-LC and the heavy chain of myosin. We note, however, that the absence of the R-LC from a myosin head does not necessarily ensure SH-LC exchange as indicated by the experiments on partially desensitized myosin.

It has been shown that the distance between the N-terminal half of the R-LC and the SH-LC depends on the state of the myofibrils: in resting conditions the separation is greater than in rigor (Hardwicke et al., 1983). Recent studies suggest that movement of the SH-LC may be responsible for this subunit rearrangement (Hardwicke & Szent-Györgyi, 1985). Since the thiol groups of the SH-LC are not necessary for regulation and since the modified SH-LC can be incorporated into myosin, it is likely that markers, i.e., radioactivity, fluorescence, and cross-linkers introduced into the SH-LC, will help to test this model of light chain movement more directly.

Acknowledgments

We thank Dr. P. M. D. Hardwicke, Dr. J. Spiegel, Dr. E. M. Szentkiralyi, H. Shpetner, and M. Titus for discussions and for critically reading the manuscript.

References

Ashiba, G., & Szent-Györgyi, A. G. (1984) *Biophys. J. 45*, 229a.

Bagshaw, C. R. (1977) *Biochemistry 16*, 59–67.

Bradford, M. (1976) *Anal. Biochem. 72*, 254.

Burke, M., & Sivaramakrishnan, M. (1981) *Biochemistry 20*, 5908–5913.

Chantler, P. D., & Szent-Györgyi, A. G. (1980) *J. Mol. Biol. 138*, 473–492.

Chantler, P. D., Sellers, J. R., & Szent-Györgyi, A. G. (1981) *Biochemistry 20*, 210–216.

Craig, R., Szent-Györgyi, A. G., Beese, L., Flicker, P., Vibert, P., & Cohen, C. (1980) *J. Mol. Biol. 140*, 35–55.

Flicker, P. F., Wallimann, T., & Vibert, P. (1983) *J. Mol. Biol. 169*, 723–741.

Focant, B., & Huriaux, F. (1976) *FEBS Lett. 65*, 16–19.

Gornall, A. G., Bardawill, C. J., & David, M. M. (1949) *J. Biol. Chem. 177*, 751–766.

Hardwicke, P. M. D., & Szent-Györgyi, A. G. (1985) *J. Mol. Biol. 183*, 203–211.

Hardwicke, P. M. D., Wallimann, T., & Szent-Györgyi, A. G. (1982) *J. Mol. Biol. 156*, 141–152.

Hardwicke, P. M. D., Wallimann, T., & Szent-Györgyi, A. G. (1983) *Nature (London) 301*, 478–482.

Harrington, W. F. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 685–689.

Kendrick-Jones, J. (1974) *Nature (London) 249*, 631–634.

Kendrick-Jones, J., Lehman, W., & Szent-Györgyi, A. G. (1970) *J. Mol. Biol. 54*, 313–326.

Kendrick-Jones, J., Szentkiralyi, E. M., & Szent-Györgyi, A. G. (1976) *J. Mol. Biol. 104*, 747–775.

Kendrick-Jones, J., Jakes, R., Tooth, P., Craig, R., & Scholey, J. (1982) in *Basic Biology of Muscles: A Comparative Approach* (Twarog, B. M., Levine, R. J. C., & Dewey, M. M., Eds.) pp 255–272, Raven Press, New York.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Margossian, S. S., Bhan, A. K., & Slayter, H. S. (1983) *J. Biol. Chem. 258*, 13359–13369.

Perrie, W. T., & Perry, S. V. (1970) *Biochem. J. 119*, 31–38.

Scholey, J. M., Taylor, K. A., & Kendrick-Jones, J. (1981) *Biochimie 63*, 255–271.

Sellers, J. R., Chantler, P. D., & Szent-Györgyi, A. G. (1981) *J. Mol. Biol. 144*, 223–245.

Simmons, R. M., & Szent-Györgyi, A. G. (1978) *Nature (London) 273*, 62–64.

Sivaramakrishnan, M., & Burke, M. (1982) *J. Biol. Chem. 257*, 1102–1105.

Stafford, W. F., Szentkiralyi, E. M., & Szent-Györgyi, A. G. (1979) *Biochemistry 18*, 5273–5280.

Szent-Györgyi, A. G., Szentkiralyi, E. M., & Kendrick-Jones, J. (1973) *J. Mol. Biol. 74*, 179–203.

Szentkiralyi, E. M. (1984) *J. Muscle Res. Cell Motil. 5*, 147–164.

Vibert, P., & Craig, R. (1982) *J. Mol. Biol. 157*, 299–319.

Vibert, P., & Craig, R. (1983) *J. Mol. Biol. 165*, 303–320.

Wagner, P. D., & Weeds, A. G. (1977) *J. Mol. Biol. 109*, 455–473.

Wagner, P. D., & Giniger, E. (1981) *Nature (London) 292*, 560–562.

Wagner, P. D., & Stone, D. B. (1983) *J. Biol. Chem. 258*, 8876–8882.

Waller, G., & Lowey, S. (1983) *Biophys. J. 41*, 299a.

Wallimann, T., & Szent-Györgyi, A. G. (1981) *Biochemistry 20*, 1188–1197.

Wallimann, T., Hardwicke, P. M. D., & Szent-Györgyi, A. G. (1982) *J. Mol. Biol. 156*, 153–173.

Winkelman, D. A., Lowey, S., & Press, J. (1983) *Cell (Cambridge, Mass.) 34*, 295–306.

Winkelman, D. A., Almeda, S., Vibert, P., & Cohen, C. (1984) *Nature (London) 307*, 758–760.