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Conformation and Activity of Smooth Muscle Myosin Probed by Various Essential Light Chains

Tsuyoshi Katoh and Fumi Morita

Division of Chemistry, Graduate School of Science, Hokkaido University, Kita-ku, Sapporo 060

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Porcine aorta smooth muscle myosin contains two essential light chain (LC17) isoforms and the light chain was replaced with one of the LC17 isoforms, rabbit skeletal muscle myosin alkali light chain 2 (A2), or scallop striated muscle myosin essential light chain (SHLC). The myosin containing either an LC17 isoform or A2 showed phosphorylation-dependent properties in the monomer conformation, filament formation, ATPase activities, and superprecipitation, behaving in essentially the same way as native myosin. On the other hand, the replacement of LC17 with SHLC destabilized the 10S conformation and the myosin was predominantly filamentous under physiological conditions, irrespective of the phosphorylation state. This myosin containing dephosphorylated regulatory light chain showed higher actin-activated ATPase activity than native dephosphorylated myosin and was further activated by Ca^{2+}, resulting in a decrease of phosphorylation-dependent regulation. Superprecipitation for the myosin was observed only when the regulatory light chain was phosphorylated. Superprecipitation for myosin containing SHLC was significantly slow in the absence of Ca^{2+} in comparison with that for myosin containing LC17, and was further activated by the presence of Ca^{2+}. On the basis of the differences in amino acid sequences of these essential light chains, it appears that the N-terminal domain of LC17 may be implicated in these phosphorylation-dependent properties of smooth muscle myosin.

Key words: actomyosin ATPase, essential light chains, filament assembly, monomer conformation, smooth muscle myosin.

Smooth muscle myosin is a motor protein responsible for smooth muscle contraction and is composed of pairs of heavy chains, essential light chains (LC17), and regulatory light chains (LC20), like other myosin II. The monomer conformation and filament formation of smooth muscle myosin are regulated by the phosphorylation of LC20 *in vitro*; the phosphorylation induces the 10S-to-6S conformational transition and filament assembly under physiological conditions (for review, see Ref. *1*). The motor activities of smooth muscle myosin are also regulated by the phosphorylation. The phosphorylation switches smooth muscle myosin into the "on" state in which the myosin shows high actin-activated ATPase activity and induces the sliding movement of actin, and the dephosphorylation switches the myosin into the inactive "off" state (for review, see Ref. *2*). The requirement of LC20 for the folding into 10S myosin, the actin-activation of ATPase activity, and the sliding movement of actin has been reported (*3, 4*). The functional domains of LC20 for phosphorylation-dependent regulation of smooth muscle myosin have also been well characterized by the use of truncated and mutated LC20 (*4-10*). On the other hand, little is known about the function of LC17 in smooth muscle myosin.

Recently, we reported that porcine aorta smooth muscle myosin was depleted of its LC20 and LC17 by the use of trifluoperazine and a high concentration of NH_4Cl, and the rebinding of these light chains to the isolated heavy chain (LC-deficient myosin) restored the original properties of smooth muscle myosin (*11*). We also showed in this study that LC17 was required for folding into the 10S conformation, thus facilitating phosphorylation-dependent filament formation, and for full activation of the motor activities by the phosphorylation of LC20. Two LC17 isoforms have been found in various smooth muscle myosins, including porcine aorta myosin, and their relative content is reported to be related to the actomyosin ATPase activity (*12, 13*) and the maximal shortening velocity of smooth muscle (*14*). In contrast to smooth muscle myosin, scallop myosin is regulated by the binding of Ca^{2+} to its essential light chain (SHLC) (*15-18*), and vertebrate skeletal muscle myosin itself is not regulated. In the light of these myosin properties, we prepared porcine aorta smooth muscle myosin containing one of the LC17 isoforms, LC17nm or LC17gi (*13, 19*), rabbit skeletal muscle myosin alkali light chain 2 (A2), or scallop striated muscle myosin SHLC, and characterized the monomer conformation, filament formation, and motor activities in order to examine functional domains in LC17. The LC17 isoforms and A2 affected these properties little, whereas SHLC destabilized the 10S conformation, facilitating filament formation, and increased the ATPase activity when LC20 was dephosphorylated.

Abbreviations: A2, rabbit skeletal myosin alkali light chain 2; LC17, smooth muscle myosin 17-kDa essential light chain; LC20, smooth muscle myosin 20-kDa regulatory light chain; DP-LC20, dephosphorylated LC20; P-LC20, phosphorylated LC20; LC-deficient myosin, isolated smooth muscle myosin heavy chain free from both LC17 and LC20; SHLC, scallop (*Patinopecten yessoensis*) adductor striated muscle myosin essential light chain.

The results suggested that the N-terminal domain of LC17, whose amino acid sequence is significantly different from that of SHLC, is responsible for folding into the 10S conformation and for the low ATPase activity in the "off" state of smooth muscle myosin.

EXPERIMENTAL PROCEDURES

Protein Preparation—Aorta smooth muscle myosin, its dephosphorylated LC20 (DP-LC20), phosphorylated LC20 (P-LC20), two LC17 isoforms, and rabbit skeletal muscle actin were prepared as described (*11*). Rabbit skeletal muscle myosin A2 was prepared as described previously (*20, 21*). Scallop striated muscle myosin SHLC was prepared from *Patinopecten yessoensis* as described by Kondo and Morita (*21*).

The concentrations of A2 and SHLC were determined from the absorbance at 280 nm using the absorption coefficients of 0.24 and 0.57 $(mg/ml)^{-1} \cdot cm^{-1}$, respectively. The molecular weights used for A2 and SHLC were 16,000 and 18,000, respectively. The determination of concentrations and molecular weights for other molecules was conducted as described previously (*11*).

LC-deficient myosin was prepared and reconstituted with DP- or P-LC20 and the mixture of LC17 isoforms in an equimolar ratio, one of the LC17 isoforms, A2, or SHLC (2-3 mol of each light chain/mol head) as described previously (*11*).

Gel Electrophoresis—Urea-gel electrophoresis was carried out according to the method of Perrie and Perry (*20*). The gels were stained with Coomassie Brilliant Blue G-250.

Monomer Conformation and Filament Formation—The conformation of myosin monomers was examined at 0.15 or 0.24 M NaCl by gel filtration HPLC as described previously (*11*). The filament formation of myosin was measured by sedimentation assay. Myosin (0.1-0.2 mg/ml) was incubated at 4°C for 10 min in 0.12 M NaCl, 5 mM $MgCl_2$, 1 mM ATP, 0.1 mM EGTA or 0.1 mM $CaCl_2$, 1 mM DTT, and 20 mM imidazole (pH 7.0). The mixture was ultracentrifuged at 4°C for 10 min at 160,000 × *g* (Hitachi CP100H ultracentrifuge), then the protein concentration of the supernatant was measured by the method of Bradford (*22*) using native myosin as a standard. The concentration of soluble reconstituted myosin was estimated by subtracting the concentration of unbound light chains from the protein concentration of the supernatant. The concentration of unbound light chain was obtained by calculation, assuming that 1 mol each of essential and regulatory light chains/mol myosin heavy chain was bound. The concentration of filamentous myosin was obtained by subtracting the concentration of soluble myosin from the concentration of myosin added. The myosin filaments formed in 0.15 M NaCl were negatively stained and observed by electron microscopy as described previously (*11*).

ATPase and Superprecipitation Measurements—The ATPase assays were carried out at 25°C as described previously (*11*) except that the conditions used were 0.06-0.08 μM myosin with and without 40 μM actin, 0.1 mM EGTA or 0.1 mM $CaCl_2$, 50 mM NaCl, 6 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, and 20 mM imidazole (pH 7.0). Superprecipitation was measured at 25°C using 0.18-0.24 μM myosin and 6 μM actin and monitored in terms of the absorbance at 660 nm, as described previously (*23*).

RESULTS

Reconstitution of LC-Deficient Myosin with Isolated Light Chains—Aorta myosin was depleted of its LC20 and LC17 by incubating myosin with trifluoperazine followed by 4.5 M NH_4Cl (*11*). The reconstitution of LC-deficient myosin with isolated light chains was carried out by the addition of DP- or P-LC20 and one of the various essential light chains (2-3 mol each light chain/mol head) to LC-deficient myosin as described previously (*11*). The myosin reconstituted with P-LC20 and the mixture of LC17 isoforms in an equimolar ratio, LC17nm, LC17gi, skeletal A2, or scallop SHLC was analyzed by urea-gel electrophoresis after the removal of unbound light chains (Fig. 1). All the myosin samples contained phosphorylated LC20 and each essential light chain with the ratio of 0.8-1.1 mol LC20/mol essential light chain as determined by densitometry. When P-LC20 was replaced with DP-LC20, essentially the same results were obtained (data not shown), indicative of the stoichiometric rebinding of these light chains to the heavy chain.

Monomer Conformation of Reconstituted Myosin—The monomer conformations of the reconstituted myosins were analyzed by gel filtration HPLC at 0.24 M NaCl in the

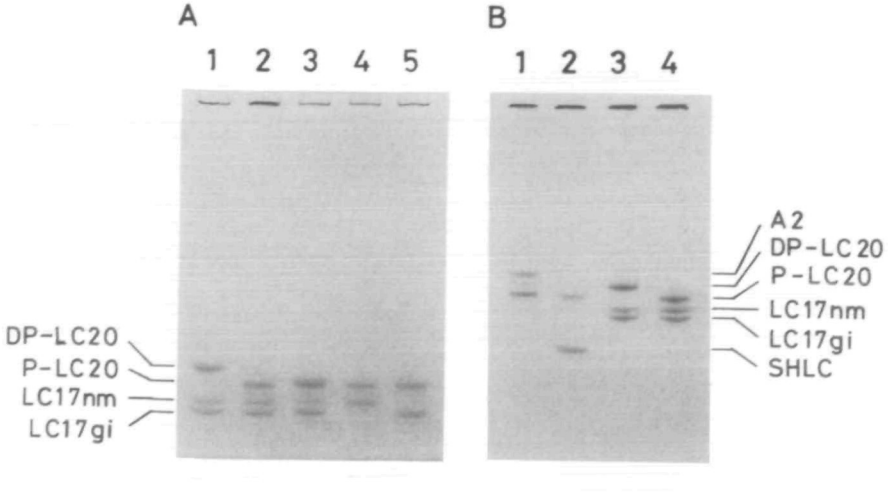

Fig. 1. **Reconstitution of LC-deficient myosin with LC20 and various essential light chains.** LC-deficient myosin was mixed with P-LC20 and the mixture of LC17 isoforms in an equimolar ratio, LC17nm, LC17gi, A2, or SHLC, and the unbound light chains were removed by a Sephacryl S-300HR spun column (*11*). The reconstituted myosin samples were subjected to urea-gel electrophoresis. Samples were native dephosphorylated myosin (A, lane 1; and B, lane 3), native phosphorylated myosin (A, lane 2; and B, lane 4), and myosin reconstituted with P-LC20 and a mixture of LC17 isoforms (A, lane 3), LC17nm (A, lane 4), LC17gi (A, lane 5), A2 (B, lane 1), or SHLC (B, lane 2).

presence of MgATP, under which conditions native dephosphorylated myosin takes the 10S conformation, but phosphorylated myosin takes the 6S conformation (*11*). The elution time for the reconstituted myosin containing LC17nm and/or LC17gi or A2 was changed from that for the 10S myosin to that for the 6S myosin by the phosphorylation of LC20, as in the case of native myosin, whereas myosin containing SHLC was eluted at the elution time for 6S myosin, irrespective of the phosphorylation state of LC20 and of the presence of Ca^{2+} (data not shown). We further examined the conformation of myosin containing SHLC at 0.15 M NaCl in the presence and the absence of Ca^{2+}. At this concentration of NaCl, native myosin is eluted at the time for 10S myosin in the absence of Ca^{2+}, irrespective of the phosphorylation state (*11*). In the absence of Ca^{2+}, almost all the myosin monomers containing SHLC were eluted at the time for 10S myosin when LC20 was dephosphorylated (Fig. 2A), while about half of the monomers were eluted as 6S myosin when LC20 was phosphorylated (Fig. 2C). The presence of Ca^{2+} increased the fraction of myosin in the 6S state, irrespective of the phosphorylation state (Fig. 2, B and D). On the other hand, such conformational transition induced by Ca^{2+} could not be observed for myosin containing LC17 (data not shown). These results indicated that the replacement of LC17 with SHLC destabilized the 10S conformation.

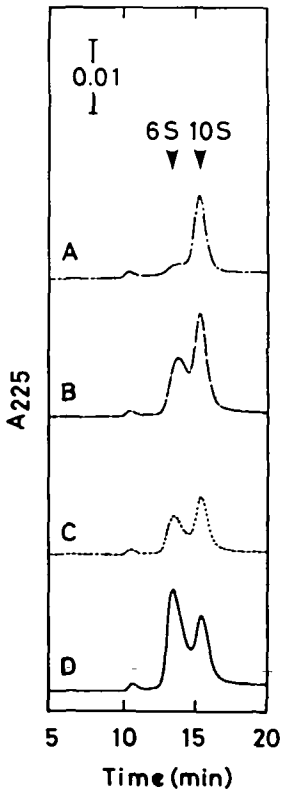

Fig. 2. **Monomer conformation of myosin reconstituted with LC20 and SHLC.** Myosin reconstituted with DP-LC20 plus SHLC (A and B) or P-LC20 plus SHLC (C and D) was analyzed by gel filtration HPLC in 0.15 M NaCl, 1 mM $MgCl_2$, 20 μM ATP, and sodium phosphate (pH 7.2) in the presence of 0.1 mM EGTA (A and C) or 0.1 mM $CaCl_2$ (B and D). The elution times for 6S and 10S myosin are indicated with arrowheads.

Filament Formation of Reconstituted Myosin—The filament formation of reconstituted myosins was examined at 0.12 M NaCl in the presence of MgATP by pelleting filamentous myosin at 160,000 × *g* (Fig. 3). The reconstituted myosin containing LC17nm and/or LC17gi or A2 was mostly soluble and only less than 19% was filamentous when LC20 was dephosphorylated, while about 50% was filamentous when LC20 was phosphorylated.

On the other hand, the reconstitution with SHLC increased the fraction of pelleted myosin in comparison with that of myosin containing LC17 or A2. Approximately 60% of the reconstituted myosin containing SHLC was pelleted even when LC20 was dephosphorylated and 80% of the myosin was pelleted when LC20 was phosphorylated. The increase may not be due to myosin oligomer formation since gel filtration HPLC at 0.4 M NaCl detected only small amounts of oligomers in these reconstituted myosin preparations, as observed in the myosins reconstituted with LC17 or A2 (data not shown). Therefore, the reconstitution with SHLC increased the fraction of filamentous myosin, consistent with the destabilization of the 10S folded conformation by SHLC described above. The filament fraction of myosin containing SHLC was essentially unaffected by Ca^{2+} under the conditions used, as in the case of myosin containing LC17. These results indicated that the reconstitution with SHLC restored neither phosphorylation-dependence nor Ca^{2+}-dependence in filament formation. The phosphorylation-dependence and/or Ca^{2+}-dependence in filament formation might be observed at lower concentra-

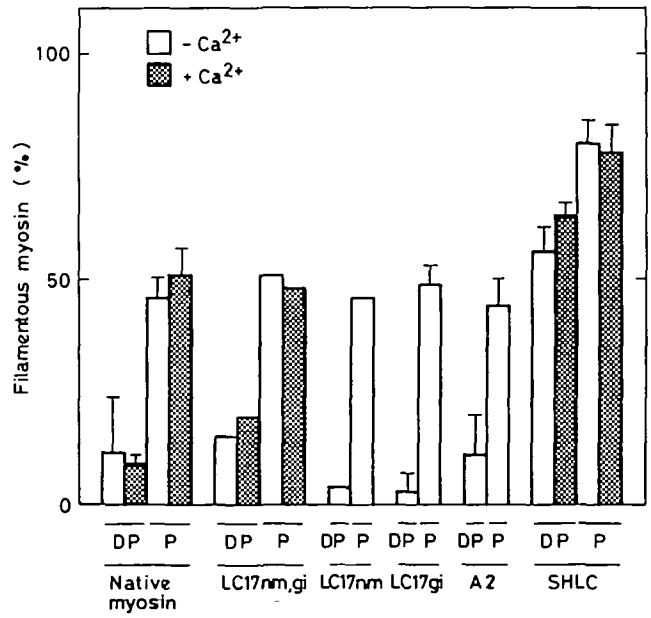

Fig. 3. **Filament formation of reconstituted myosin.** Native dephosphorylated and phosphorylated myosins and myosin reconstituted with DP- or P-LC20 and the mixture of LC17 isoforms in an equimolar ratio (indicated as LC17nm,gi), LC17nm, LC17gi, A2, or SHLC in 0.12 M NaCl, 5 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, and 20 mM imidazole (pH 7.0) in the presence of 0.1 mM EGTA (open bars) or 0.1 mM $CaCl_2$ (hatched bars) were ultracentrifuged at 160,000 × *g* for 10 min at 4°C. Values are averages of two or three determinations of two different preparations. Error bars indicate standard deviations. DP and P indicate dephosphorylated and phosphorylated states of LC20, respectively.

tions of NaCl. However, such experiments were not carried out since the difference that might be caused by phosphorylation and/or Ca^{2+} may be unclear because of the increase in the total fraction of filamentous myosin at lower NaCl concentration. The filaments of all these reconstituted myosins formed at 0.15 M NaCl were negatively stained and observed by electron microscopy (not shown). The filaments of all the reconstituted myosins showed no morphological difference from those of native myosin. Aggregates different from myosin filaments were hardly observed for these myosins either.

ATPase Activities of Reconstituted Myosin—The ATPase activities of reconstituted myosins were measured in the presence and the absence of actin and Ca^{2+} (Table I). When LC20 was phosphorylated, the actin-activated ATPase activity of reconstituted myosin containing LC17gi was slightly higher than that of reconstituted myosin containing LC17nm (Table I). The actin-activated ATPase activity of myosin containing A2 was slightly higher than that of native myosin when LC20 was phosphorylated. The degrees of phosphorylation-dependent regulation in the actin-activated ATPase activities of these myosins were more than 14-fold, comparable to the value for native myosin. On the other hand, when LC20 was dephosphorylated, the actin-activated ATPase activity of reconstituted myosin containing SHLC was considerably higher than that of myosin containing LC17 or A2, irrespective of the presence of Ca^{2+}. The activities of these myosins were, however, comparable when LC20 was phosphorylated, resulting in a decrease in the degree of phosphorylation-dependent regulation of actin-activated ATPase activity.

In addition to the phosphorylation dependence, the myosin containing SHLC showed Ca^{2+} dependence in the ATPase activities. The Ca^{2+} sensitivities of the myosin and actomyosin, $(1-ATPase_{EGTA}/ATPase_{Ca})\times 100$, were about 60% when LC20 was dephosphorylated. When LC20 was phosphorylated, the ATPase activity was also activated by the presence of Ca^{2+} but the degree was significantly lower than that in the dephosphorylated state. On the other hand, the ATPase activity of myosin containing LC17 was increased only slightly by the presence of Ca^{2+}.

Superprecipitation of Acto-Reconstituted Myosin—The motile activities of reconstituted myosins were examined by monitoring superprecipitation time courses (Fig. 4). Superprecipitation for the reconstituted myosin was completely phosphorylation-dependent and was not observed when LC20 was dephosphorylated, irrespective of the species of essential light chains. When LC20 was phosphorylated, the reconstituted myosin containing LC17nm plus LC17gi, LC17nm, or A2 showed similar superprecipitation time courses (Fig. 4, a, b, and d). Superprecipitation for these myosins reached maximal levels within 2 min, and $t_{1/2}$, the time required to reach the half-maximal level, was 55-60 s. The reconstituted myosin containing LC17gi showed slightly faster superprecipitation ($t_{1/2}$=45-50 s)

TABLE I. **ATPase activity of reconstituted myosin.** The ATPase activity was measured at 25°C using 0.06-0.08 μM myosin with or without 40 μM actin in 50 mM NaCl, 6 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, and 20 mM imidazole (pH 7.0) in the presence of 0.1 mM EGTA or 0.1 mM $CaCl_2$. Values are averages of 3-6 determinations. ELC indicates essential light chain. LC17nm,gi indicates the mixture of LC17 isoforms in an equimolar ratio. DP and P, dephosphorylated and phosphorylated, respectively.

ELC	LC20	Ca^{2+}	ATPase activity (s^{-1})		
			−Actin	+Actin	Actin-activated
Native myosin					
	DP	−	0.006±0.003	0.038±0.017	0.032
	P	−	0.032±0.005	0.611±0.065	0.579
Reconstituted myosin					
LC17nm,gi	DP	−	0.015±0.009	0.057±0.007	0.042
		+	0.012±0.010	0.064±0.007	0.052
	P	−	0.032±0.010	0.656±0.034	0.624
		+	0.045±0.005	0.762±0.037	0.717
LC17nm	DP	−	0.010±0.003	0.038±0.003	0.028
	P	−	0.026±0.005	0.601±0.023	0.575
LC17gi	DP	−	0.010±0.003	0.041±0.005	0.031
	P	−	0.027±0.004	0.664±0.042	0.637
A2	DP	−	0.013±0.001	0.030±0.001	0.017
	P	−	0.018±0.009	0.704±0.026	0.686
SHLC	DP	−	0.047±0.007	0.148±0.003	0.101
		+	0.122±0.019	0.367±0.010	0.245
	P	−	0.080±0.004	0.737±0.012	0.657
		+	0.093±0.045	0.995±0.031	0.902

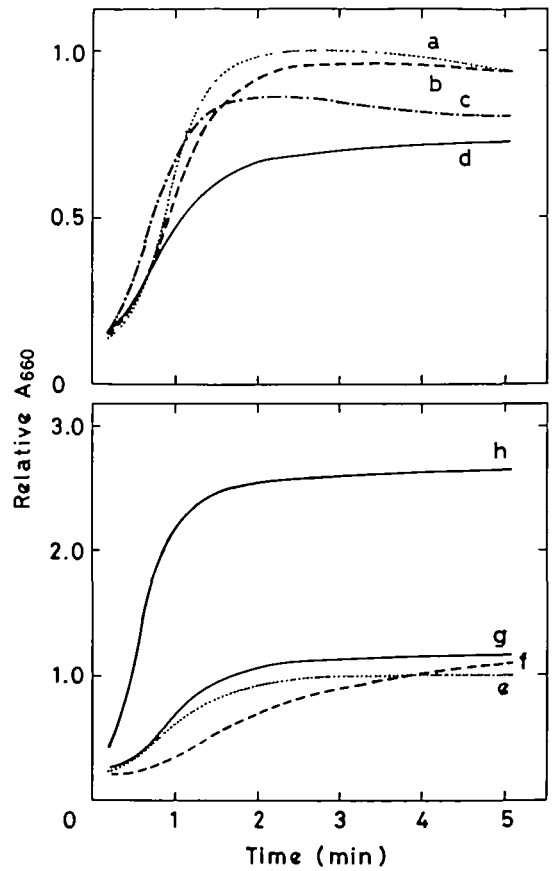

Fig. 4. **Superprecipitation of acto-reconstituted myosin.** Superprecipitation for myosin reconstituted with P-LC20 and the mixture of LC17 isoforms in an equimolar ratio (a, e, and g), LC17nm (b), LC17gi (c), A2 (d), or SHLC (f and h) was measured at 25°C with 6 μM actin in 0.1 M NaCl, 5 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, and 20 mM imidazole (pH 7.0) in the presence of 0.1 mM EGTA (a-f) or 0.1 mM $CaCl_2$ (g and h). The values of absorbance at 660 nm are relative to the maximal value for myosin reconstituted with the mixture of LC17 isoforms. When DP-LC20 was used for the reconstitution, none of the myosins showed superprecipitation up to 60 min.

than the myosin containing LC17nm (Fig. 4c), in agreement with their actin-activated ATPase activities (Table I). On the other hand, superprecipitation for the reconstituted myosin containing SHLC was significantly slow in the absence of Ca^{2+} ($t_{1/2}=160$ s; Fig. 4f), whereas it was markedly activated by the presence of Ca^{2+} ($t_{1/2}=30$ s) and the maximal level was also increased (Fig. 4h). In contrast, superprecipitation for the reconstituted myosin containing LC17 was essentially unaffected by Ca^{2+} (Fig. 4, e and g).

DISCUSSION

Effects of Various Essential Light Chains on Monomer Conformation and Filament Formation—We previously showed, using myosin lacking LC17, that LC17 is required for folding into the 10S conformation and thus for the phosphorylation-dependent conformational transition and filament formation (*11*). In the present study, this finding was confirmed by the results showing that the replacement of LC17 with SHLC destabilized the 10S folded structure (Fig. 2). On the other hand, the replacement with A2 did not affect the conformational properties. A comparison of amino acid sequences among these essential light chains showed that the similarities with LC17nm are as high as 99% for LC17gi and 88% for A2, whereas that for SHLC is only 62%. The substituted amino acid residues unique to SHLC among these light chains may be responsible for the folding of myosin into the 10S conformation, whereas the amino acid substitutions between LC17 and A2 may not. Such substitutions, including deletions and insertions, particularly with a change of charge, are threefold more frequent in the N-terminal domain than in the C-terminal. The relation of the N-terminal domain to the formation of the 10S structure also seems to be suggested by the Ca^{2+} dependence of the conformational transition of myosin containing SHLC at a lower concentration of NaCl (Fig. 2), since Ca^{2+} binds to the N-terminal domain of SHLC in scallop myosin (*18*). The three-dimensional structure of the scallop myosin regulatory domain (*18*) and chicken skeletal myosin head (*24*) showed that the helix C-loop-helix D (domain II) of essential light chain is located farther away from the heavy chain and implicated less effectively in the interactions with the heavy chain as compared with the other helix-loop-helix domains (*25*). Thus, domain II of LC17 might be implicated in the formation of the 10S structure. Liu and Uyeda (*26*) suggest that the myosin head may interact with a negatively charged region within the subfragment 2 portion. Amino acid substitutions with increase in net negative charge are found in helix D of SHLC in comparison with those of LC17 and A2 (Fig. 5). Such substitutions in SHLC might decrease the head-subfragment 2 interactions. Scallop myosin also folds into the 10S conformation in the absence of Ca^{2+} (*17, 27*), but the interactions might be somewhat different from those in smooth muscle myosin. The substitution of amino acid residues in this domain of SHLC might have inhibited the myosin containing SHLC from folding and thus resulted in the phosphorylation-independent filament formation (Fig. 3).

Effects of Various Essential Light Chains on Motor Activities—The actin-activated ATPase activity of myosin containing LC17gi in the phosphorylated state was slightly higher than that of myosin containing LC17nm. This tendency is consistent with the activities of LC17-exchanged aorta myosin (*13*) and of smooth muscle myosins containing LC17 isoforms to various extents (*12*). Similarly, the motile activity of reconstituted myosin containing LC17gi was higher than that of myosin containing LC17nm, as examined by superprecipitation (Fig. 4). However, the difference in the rates of superprecipitation did not seem to be large enough to account for the differences in the maximal shortening velocities reported for smooth muscles with different isoform distributions of LC17 (*14*). Therefore, the correlation of ATPase activity and shortening velocity with the relative content of the LC17 isoform may not result only from the differences in the two LC17 isoforms, as mentioned previously (*28*).

On the other hand, replacement of LC17 with SHLC resulted in the reduction of phosphorylation-dependent regulation of actin-activated ATPase activity, whereas that with each of the LC17 isoforms or A2 did not affect the regulation (Table I). The reduction of regulation by SHLC was mainly due to the increase in the activity in the dephosphorylated state. As mentioned above, the amino

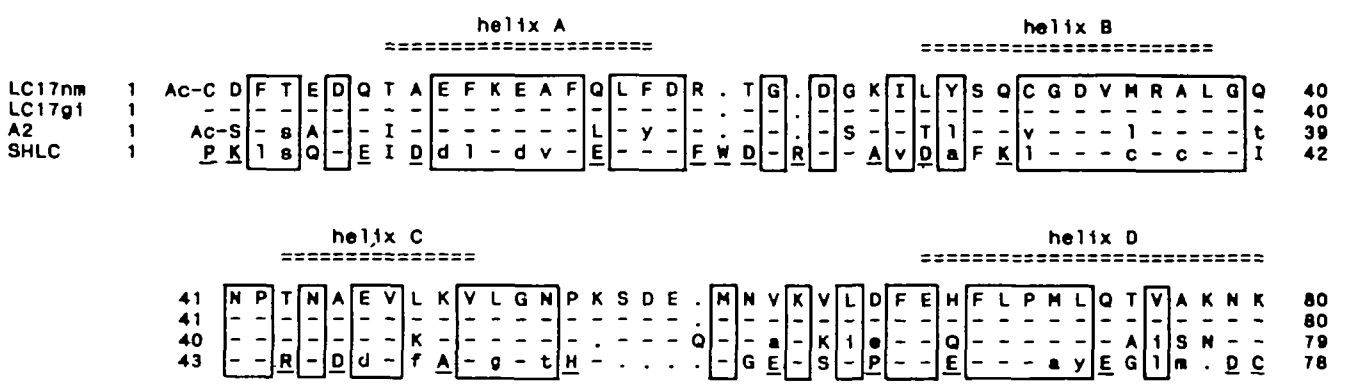

Fig. 5. Comparison of amino acid sequences of the N-terminal domains of essential light chains. The first and second rows, porcine aorta LC17nm and LC17gi (*32*), respectively; third row, rabbit skeletal A2 (*33*); fourth row, scallop (*Patinopecten yessoensis*) SHLC (*34*). Dashes represent residues identical with those of LC17nm. Periods indicate deletions. The small and capital letters in the third and fourth rows indicate conservative and non-conservative substitutions, respectively. The underlined letter in the fourth row indicates substitution with change of charge. Identical residues and those substituted for conservative ones in all these light chains are boxed. Helices A-D are also indicated above the sequences.

acid substitutions in the N-terminal domain uniquely found in SHLC among these light chains may be responsible for the effect of SHLC. In particular, such residues in domain I (helix A-loop-helix B) of essential light chain seem to be critical, since Ca^{2+} binds to this domain of SHLC in scallop myosin (*18*), and the activity of myosin reconstituted with SHLC was actually activated by Ca^{2+} (Table I). In contrast to the ATPase activity, superprecipitation for the myosin containing SHLC was completely phosphorylation-dependent, irrespective of the presence of Ca^{2+}. Such an uncoupling between the ATPase activity and motile activity has already been reported (*8, 29*). When the myosin was in the phosphorylated state, superprecipitation was slow in the absence of Ca^{2+}, but was activated by the presence of Ca^{2+} (Fig. 4). Therefore, these results suggest the importance of domain I of essential light chain in the motor activities of smooth muscle myosin.

In scallop myosin, the binding of Ca^{2+} to SHLC induces interactions among SHLC, regulatory light chain, and heavy chain, stabilizing an active structure of the regulatory domain of the myosin head (*16, 18*). Similar interactions may be formed by Ca^{2+} in reconstituted myosin containing SHLC, leading to the partial activation of ATPase activity in the dephosphorylated state, but the interactions may be insufficient for the formation of the active regulatory domain structure required for the full motor activities. The phosphorylation of LC20, in addition to the binding of Ca^{2+} to SHLC, may be required for the complete active structure for this myosin. Similar Ca^{2+}-dependent and phosphorylation-dependent regulation has also been demonstrated for desensitized scallop myosin reconstituted with gizzard LC20 (*30, 31*). Therefore, the formation of interactions among light chains and heavy chain may be required for the high activities in the "on" state of smooth muscle myosin and, conversely, the elimination of the interactions may be required for the low motor activities in the "off" state. In smooth muscle myosin, the phosphorylation of LC20 may affect the conformation of its C-terminal domain (*10*) and may induce the interactions of LC20 with domain I of LC17 and heavy chain.

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