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Electron Microscopy of Scallop Myosin
Location of Regulatory Light Chains

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The heads of the Ca^{2+}-sensitive myosin molecules from scallop muscle, contrasted for electron microscopy by rotary shadowing, display two appearances depending on the presence or absence of the regulatory light chains. The heads of intact myosin appear "pear-shaped" as described for vertebrate myosin (Elliott & Offer, 1978): they are widest at the end remote from the tail and taper to a narrower neck near their junction with the tail. In contrast, myosin heads that lack the regulatory light chains appear more globular. The neck region is no longer visible: the rounded heads appear directly attached to the tail or there is an apparent gap between the head and the tail. Two preparations of myosin subfragment-1 that differ in light chain content show a similar difference in appearance.

Fab fragments of antibodies specific for the light chains bind to the myosin heads and can also be visualized in the electron microscope using rotary shadowing. Both Fab fragments specific for the regulatory light chains and Fab fragments specific for the essential light chains bind preferentially to intact scallop myosin in the narrow region of the myosin head near its junction with the tail.

1. Introduction

Muscle contraction is controlled by calcium-sensitive myofibrillar proteins that regulate the force-producing interactions of myosin and actin. In many muscle types, the myosin molecule itself contains the regulatory sites; they are located in the globular head regions and involve the small polypeptides known as "light chains" (Kendrick-Jones *et al.*, 1970,1976; Lehman & Szent-Györgyi, 1975). In vertebrate smooth muscle and in non-muscle cells, Ca ions activate a calmodulin–kinase complex that phosphorylates the regulatory light chains (Dabrowska *et al.*, 1978; Adelstein & Klee, 1981). A related but simpler form of myosin regulation is found in molluscan muscles, where Ca^{2+} binds directly to the myosin head at a site that requires interaction of the regulatory light chain and the rest of the head (Chantler & Szent-Györgyi, 1980). Phosphorylation or Ca^{2+}

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binding relieves the inhibitory effect of the light chains, resulting in elevation of the actin-activated ATPase activity of myosin.

The striated adductor muscle of the scallop has proved particularly useful for study of myosin-linked regulation, since the regulatory light chains can be reversibly detached from myosin in the absence of divalent cations. Removal and re-addition of regulatory light chains have established that Ca^{2+}-sensitive control depends on the presence of these chains (Szent-Györgyi *et al.*, 1973; Chantler & Szent-Györgyi, 1980; Simmons & Szent-Györgyi, 1978,1980). Recent evidence has also implicated the second ("essential") class of light chains in regulation (Wallimann & Szent-Györgyi, 1981*a*,*b*), and shown that the regulatory and essential light chains move relative to one another on activation (Hardwicke *et al.*, 1983).

The precise location of the two types of light chain on the myosin head is unknown. Both classes of light chain are elongated (Stafford & Szent-Györgyi, 1978), and thus have the potential for interaction with a considerable length of the myosin head. Studies using cross-linking reagents suggest the close proximity and substantial overlap of the two kinds of light chain (Wallimann *et al.*, 1982). Previous structural studies have indicated that the regulatory light chains affect the shape or conformation of the myosin head. Scallop thin filaments decorated with proteolytic fragments of scallop myosin, S-1† or heavy meromyosin display two different arrowhead shapes depending on the presence or absence of the regulatory light chain (Craig *et al.*, 1980). Three-dimensional reconstructions of the arrowheads revealed that the regulatory light chains contribute to the narrow neck region of the head (Vibert & Craig, 1982). Studies of muscle fibers by X-ray diffraction suggested that both the shape and distribution of myosin crossbridges attached to actin in rigor might be influenced by the light chains (Vibert *et al.*, 1978). To determine more directly the location of the light chains and their effect on the conformation of myosin heads, we have examined individual scallop myosin molecules contrasted for electron microscopy by rotary shadowing. In addition, we have used antibody labeling to localize both types of light chain on the myosin head.

2. Experimental Procedures

(a) *Preparation of scallop myosin and desensitized myosins*

Myofibrils from the bay scallop, *Aequipecten irradians*, were prepared as described by Wallimann & Szent-Györgyi (1981*b*). One or 2 regulatory light chains per myosin were dissociated, as required, from scallop myofibrils by treatment with EDTA at 10°C or 35°C (Kendrick-Jones *et al.*, 1976; Chantler & Szent-Györgyi, 1980). Myosin was prepared by ammonium sulfate fractionation according to the method of Focant & Huriaux (1976) as modified by Stafford *et al.* (1979) and Wallimann & Szent-Györgyi (1981*a*). Intact scallop myosin was also directly desensitized by treatment with EDTA at 25°C. A total of 2 mol of R-LC is dissociated by this procedure. Scallop myosin previously desensitized by treatment with EDTA at 25°C was resensitized by incubation with a 2 to 3-fold molar

† Abbreviations used: S-1, subfragment 1; R-LC, scallop regulatory light chain; EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N*-*N*′-tetraacetic acid; S-2, subfragment 2; SH-LC, scallop essential (or sulfhydryl-containing) light chain.

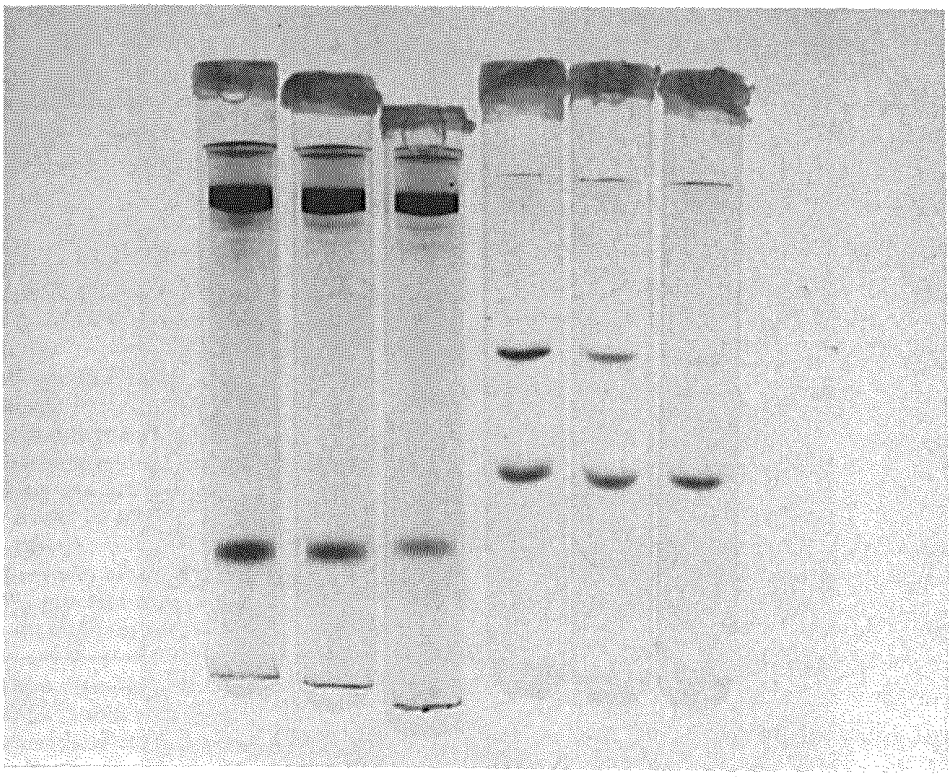

FIG. 1. Heavy chain and light chain content of myosin preparations. Lane 1, myosin prepared from intact scallop myofibrils; lane 2, from myofibrils exposed to 10 mM-EDTA at 10°C for 5 min, a treatment which removes roughly 1 mol R-LC per mol myosin; lane 3, from myofibrils subjected to a treatment with 10 mM-EDTA at 35°C for 5 min by which 2 mol R-LC are dissociated. (a) After electrophoresis on 7·5% (w/v) polyacrylamide gels in the presence of sodium dodecyl sulfate (lanes 1 to 3); and (b) on 10% (w/v) polyacrylamide gels in the presence of 8 M-urea (lanes 1′ to 3′). The preparations are shown before purification on Sepharose 4B by which most of the minor contaminants are removed.

excess of purified scallop R-LC in 40 mM-NaCl, 1 mM-$MgCl_2$, 0·1 mM-EGTA, 3 mM-NaN_3, 5 mM-phosphate (pH 7·0) (referred to as Mg^{2+} wash).

Scallop myosin was stored conveniently for more than 6 weeks in 40% (w/v) $(NH_4)_2SO_4$ containing 1 to 5 mM-Mg^{2+}ATP, 0·1 to 0·2 mM-EGTA (pH 7·0) at 0 to 4°C without a significant loss of either specific activity of the actin-activated Mg^{2+}ATPase or calcium sensitivity (Wallimann & Szent-Györgyi (1981*a*); Table 1). Furthermore, exposure of dilute solutions of scallop myosin to a dispersion buffer similar to that used for electron microscopy (0·7 M-ammonium acetate, 70% (v/v) glycerol, pH 7·0) did not significantly affect its calcium sensitivity, although the specific activity of the actin-activated Mg^{2+} ATPase was somewhat lowered (Table 1). This finding demonstrates that under these conditions the regulatory light chains are not dissociated from the myosin and that the myosin is not irreversibly denatured.

(b) *Other procedures*

Gel electrophoresis in the presence of 0·1% (w/v) sodium dodecyl sulfate or 8 M-urea was performed as described by Kendrick-Jones *et al.* (1976). Gels were stained with acid fast

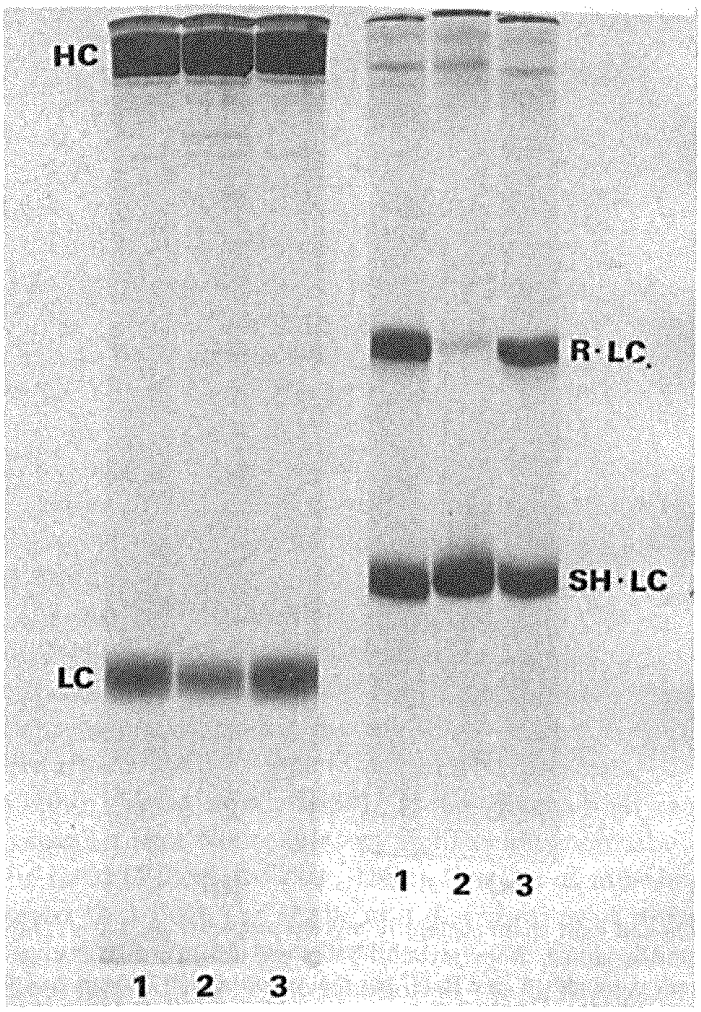

FIG. 2. Electrophoretic characterization of resensitized myosin. Scallop myosin after electrophoresis on 7·5% polyacrylamide gels in the presence of sodium dodecyl sulfate (a), and on 10% polyacrylamide gels in the presence of 8 M-urea (b). Lane 1, intact myosin; lane 2, directly desensitized myosin from which both R-LC have been removed by a treatment with 10 mM-EDTA for 5 min at 25°C; lane 3, resensitized myosin after incubation for 12 h at 4°C in Mg^{2+} wash with a 2-fold molar excess of purified scallop regulatory light chains followed by 3 washes with Mg^{2+} wash.

green prior to densitometry and planimetry. Protein determinations, calcium binding, ATPase assays, double immunodiffusion and immunoreplication were performed as described previously (Wallimann & Szent-Györgyi, 1981*a*,*b*).

(c) *Characterization of subfragment-1 preparations*

Subfragment-1 was prepared directly from purified scallop myosin according to the procedure used for the preparation of S-1 from scallop myofibrils described by Stafford *et al.* (1979). CaMg S-1 and EDTA S-1 refer to the myosin subfragment-1 preparations obtained by papain digestion in the presence and absence of divalent cations, respectively. CaMg S-1 contains 1 mol intact SH-LC and one slightly clipped R-LC (M_r = 16,000) (Stafford *et al.*, 1979; Craig *et al.*, 1980; Fig. 3 (a) 4, (c) 4 and (e) 1). EDTA S-1 contains no R-LC (Stafford *et al.*, 1979; Fig. 3 (a) 3, (c) 3 and (e) 6), but retains an electrophoretically

TABLE 1

Characterization of myosin preparations

	Actin-activated Mg^{2+} ATPase (μmol/min per mg) $-Ca^{2+}$	$+Ca^{2+}$	Calcium¶ sensitivity %	Mol Ca^{2+} bound / mol myosin	Ratio R-LC / SH-LC
Normal scallop myosin	0·01	0·65	98	1·82	1·03
Myosin from 10°C desensitized myofibrils	0·52	0·56	7	1·1	0·54
Myosin from 35°C desensitized myofibrils	0·36	0·34	0	0·12	0·09
Desensitized myosin†	0·28	0·25	0	0·13	0·08
Resensitized myosin‡	0·03	0·53	94	1·65	0·95
Normal myosin after storage for 6 weeks in 40% $(NH_4)_2SO_4$§	0·013	0·46	97	—	0·98
Normal myosin after 12 h exposure to 70% glycerol containing 0·7 M-ammonium acetate‖	0·03	0·36	92	—	0·96

† Scallop myosin (1 to 2 mg/ml) directly treated with 10 mM-EDTA, 40 mM-NaCl, 5 mM-phosphate (pH 7·0) for 5 min at 25°C.

‡ Desensitized scallop myosin essentially free of regulatory light chains (Fig. 2(a), lane 2 and (b), lane 2) after incubation overnight at 4°C in 40 mM-NaCl, 1 mM-$MgCl_2$, 5 mM-phosphate (pH 7·0) with a 2 M excess of scallop regulatory light chains (Fig. 2(a), lane 3 and (b), lane 3).

§ In 40% $(NH_4)_2SO_4$, 0·5 M-NaCl, 1 mM-MgATP, 0·1 mM-EGTA, 5 mM-phosphate (pH 7·0).

‖ In 70% glycerol, 0·7 M-ammonium acetate, 1 mM-$MgCl_2$, 0·1 mM-EGTA (pH 7·0).

¶ Percentage calcium sensitivity is defined as: $[1-(\text{ATPase in EGTA})/(\text{ATPase in } Ca^{2+})]\times 100$.

fast migrating peptide (M_r = 8000 to 10,000) which was identified by the immunoreplica technique as being a fragment of the SH-LC (Fig. 3 (a) 3, (b) 3 and (d) 6).

The two S-1 preparations not only differed in their light chain content but also showed some minor differences in the apparent molecular weights of their heavy chains. EDTA S-1 isolated from purified myosin consistently showed a major heavy chain band (89,000 to 90,000 M_r) followed by a minor fraction of a polypeptide with somewhat higher molecular weight (95,000 to 96,000 M_r). CaMg S-1 contained only one heavy chain band at 92,000 to 93,000 M_r (Fig. 4). When co-electrophoresed, the 89,000 to 90,000 M_r component of the EDTA S-1 and the 92,000 to 93,000 M_r chain of the CaMg S-1 are resolved as a doublet (Fig. 4(b), lane 3).

The apparent V_{max} of the actin-activated Mg^{2+} ATPase activity of the S-1s were $8{\cdot}5\pm2$ μmol min^{-1} mg^{-1} for EDTA S-1 and $7{\cdot}5\pm2$ μmol min^{-1} mg^{-1} for CaMg S-1. The inclusion of 1 mM-ATP in the imidazole buffer and the DEAE column buffer (Stafford *et al.*, 1979) seems to prevent aggregation of S-1 preparations and improves the apparent V_{max} value of the actin-activated ATPase of both S-1s.

(d) *Incubation of myosin with antibodies*

Fab fragments of 3 classes of antibodies specific for light chains were used to label the light chains on the myosin head. One class is specific for the regulatory light chain (anti-R-LC-Fab). Two classes of Fab fragment specific for the essential light chain (anti-SH-LC-Fab) were used; one class desensitizes myosin while the other class does not (Wallimann & Szent-Györgyi, 1981*a*,*b*). The affinity-purified rabbit anti-scallop myosin light chain antibodies used were monospecific for the homologous light chain and showed

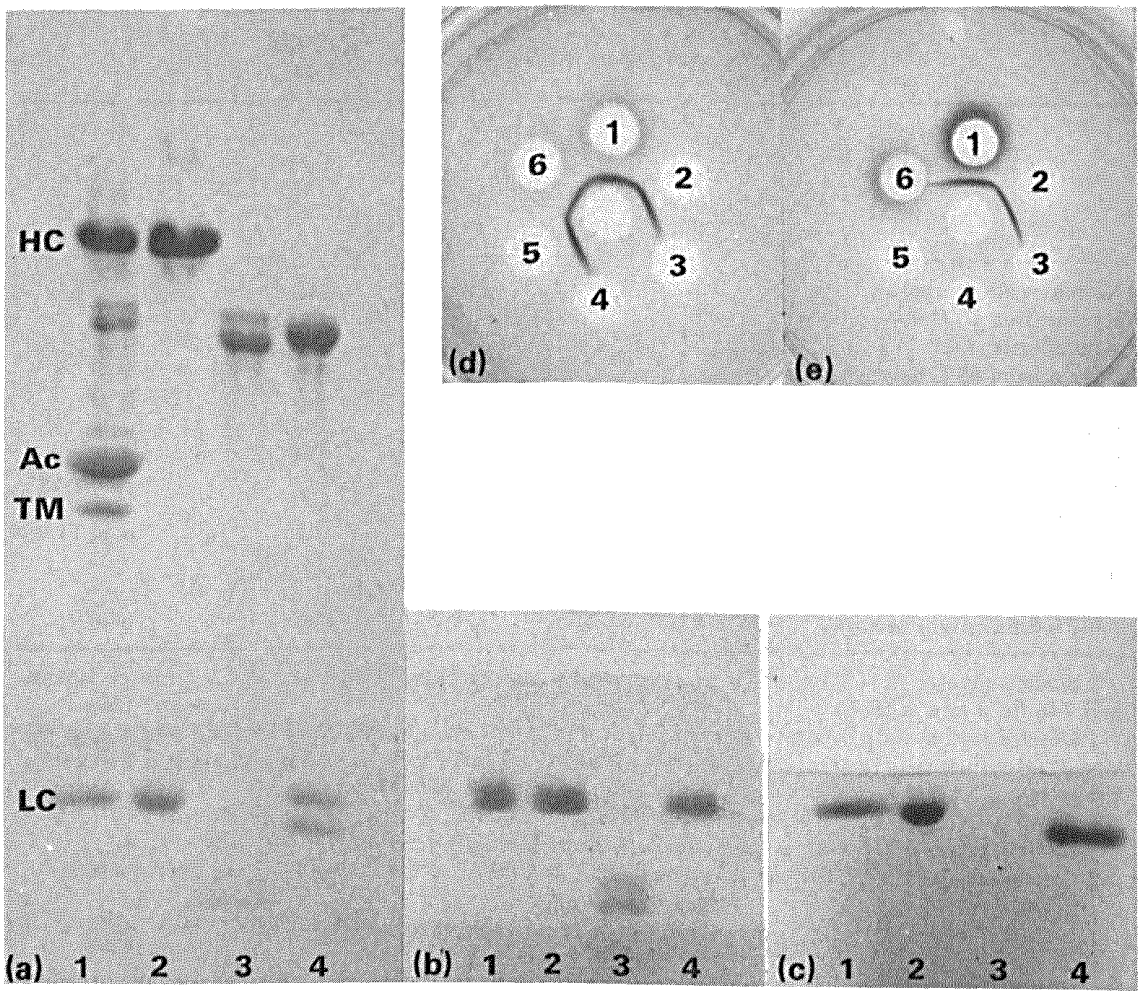

FIG. 3. Characterization of myosin subfragment-1 preparations by immunoreplication and double immunodiffusion. Lane 1, scallop myofibrils; lane 2, myosin; lane 3, EDTA S-1; lane 4, CaMg S-1 after electrophoresis on a 13% polyacrylamide microslab gel (Matsudaira & Burgess, 1978) in the presence of sodium dodecyl sulfate (a). Myosin heavy chain (HC); actin (Ac); tropomyosin (TM); myosin light chains (LC). Rabbit anti-essential light chain immunoreplica (b) of the lower portion of the gel in (a) and anti-regulatory light chain immunoreplica (c) of the same. Double immunodiffusion (d): rabbit anti-scallop SH-LC IgG, in the center well; 1 mg CaMg S-1/ml (1); 0·1 mg SH-LC/ml (2); 0·1 mg R-LC/ml (3); 1 mg R-LC/ml (4); 0·1 mg SH-LC/ml (5); 1 mg EDTA S-1/ml (6) of scallop myosin. Double immunodiffusion (e): rabbit anti-scallop R-LC IgG, in the center well; 1 mg CaMg S-1/ml (1); 0·1 mg R-LC/ml (2); 0·1 mg SH-LC/ml (3); 0·1 mg SH-LC/ml (4); 1 mg SH-LC/ml (5); 1 mg EDTA S-1/ml (6) of scallop myosin.

no cross-reactivity with the heterologous ones, as judged by solid-phase radioimmunoassays (Wallimann & Szent-Györgyi, 1981*a*).

Typically, 0·5 mg portions of normal or desensitized myosin suspended in 0·5 ml of low ionic strength buffer (20 mM-NaCl, 1 mM-$MgCl_2$, 1 mM-$CaCl_2$, 3 mM-NaN_3, 5 mM-phosphate (pH 6·7)) were used and increasing amounts (1 to 4 molar excess of Fab over myosin) of either anti-R-LC-Fab or anti-SH-LC-Fab were added. The mixture was incubated overnight, either without stirring or while gently shaking in plastic centrifuge tubes at 4°C; 0·1 to 1·0 mM-iodo-acetic acid (pH 6·8) was added if traces of residual papain activity in the Fab preparation (stemming from the digestion of immunoglobulin by papain) led to

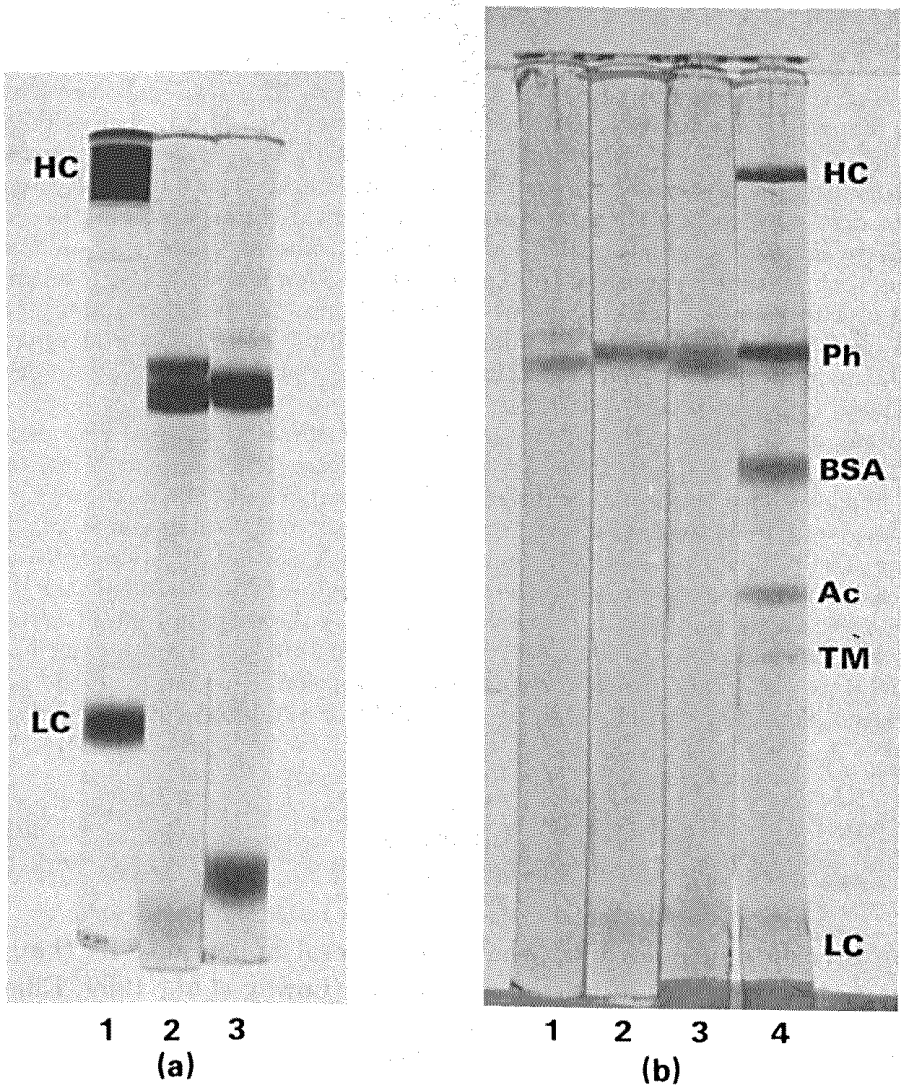

FIG. 4. Electrophoretic characterization of subfragment-1 heavy chains from scallop. (a) Scallop myosin, starting material subjected to papain digestion, after electrophoresis on 7·5% polyacrylamide/sodium dodecyl sulfate gels (lane 1); and subfragment-1 preparations after electrophoresis on 5% polyacrylamide/sodium dodecyl sulfate gels: 50 μg of purified EDTA S-1 (lane 2) and 40 μg of CaMg S-1 (lane 3). (b) Lane 1, 5 μg of EDTA S-1; lane 2, 5 μg of CaMg S-1; lane 3, mixed S-1s after electrophoresis on 5% polyacrylamide/sodium dodecyl sulfate gels. Lane 4, standards: myosin heavy chain (HC, M_r = 200,000); myosin light chains (LC, M_r = 17,500); phosphorylase b (Ph, M_r = 94,000); bovine serum albumin (BSA, M_r = 45,000); actin (Ac, M_r = 45,000); tropomyosin (TM, M_r = 37,000).

partial degradation of myosin molecules to subfragment-1 and myosin rod (as seen by electron microscopy). Concentrations of up to 10 mM-iodo-acetic acid did not inhibit the actin-activated Mg^{2+} ATPase activity nor affect the calcium sensitivity or R-LC content of scallop myosin (unpublished observations). Myosin was then freed of unbound Fab by 3 washes with 1 ml each of a buffer containing 20 mM-NaCl, 1 mM-$MgCl_2$, 5 mM-phosphate (pH 6·7). After a final centrifugation for 10 min at 5000 revs/min (Sorvall HS-4 swinging bucket rotor) the pellets were dissolved in 0·5 ml of an ice-cold 0·7 M-ammonium acetate buffer (pH 7·0) containing 1 mM-$MgCl_2$. Any precipitate was removed by centrifugation and the preparation was kept on ice.

(e) *Rotary shadowing*

Myosin or S-1s were prepared for rotary shadowing following the method described by Shotton *et al.* (1979) (cf. also Tyler & Branton, 1980). The proteins were diluted into solutions prepared from 3 parts 1 M-ammonium acetate, 1 mM-$MgCl_2$, 1 mM-ATP, 0·1 mM-EGTA, and 7 parts glycerol. The protein solution (3 to 10 μg/ml) was sprayed at room temperature using a laboratory aerosol sprayer onto freshly cleaved mica. Samples were allowed to dry *in vacuo* at room temperature in an Edwards evaporator by evacuating for 0·5 to 1 h to a vacuum of at least 10^{-5} Torr. A 6° shadow angle was used for rotary shadowing with platinum vaporized from a heated tungsten filament. The resulting replicas were coated with a supporting film of carbon, floated onto distilled water and picked up on bare 400-mesh grids. They were examined in a Philips EM301 electron microscope at 80 kV. The magnification was calibrated using negatively stained tropomyosin paracrystals (395 Å repeat: Cohen & Longley, 1966).

Corrections of the dimensions of the molecules for metal thickness were estimated as follows: the apparent widths of clearly resolved myosin tails were first measured from enlarged images. Since the width of 2-stranded α-helical coiled coils is known to be about 20 Å, this gives an estimate for the broadening and lengthening effect of the rotary shadowing. The tail widths observed were typically 40 Å, so that 20 Å was subtracted from other measured dimensions to give the corrected values. Measurements of dimensions where one end was located within the molecule, such as the length of intact myosin heads, were corrected for only one thickness of metal (typically 10 Å). Separate determinations of metal thickness were made for each shadowed preparation. The length of the myosin tail was measured on molecules whose tails were straight or exhibited only one or two bends.

3. Results

(a) *Myosin molecules*

Scallop myosin molecules have the same general appearance as that of other muscle myosins examined by rotary shadowing (Lowey *et al.*, 1969; Elliott *et al.*, 1976; Elliott & Offer, 1978). Two globular heads and a long narrow tail can be clearly distinguished (Fig. 5). The shape of the heads depends on the presence or absence of the regulatory light chain. The heads of intact myosin appear widest at the end remote from the tail and taper to a narrower neck near their junction with the tail (Figs 5 and 6); their shape thus resembles an elongated pear. Both straight and curved heads can be seen. The average length of the head, measured from the junction of the head with the tail, is 195 ± 30 Å. The width of the head at its widest point is 80 ± 15 Å (Fig. 7 and Table 2).

The heads of desensitized myosin, which contain no regulatory light chains, appear shorter and more rounded than the heads of intact myosin (Fig. 6). The narrow neck region is no longer visible; either the rounded heads appear to be attached directly to the tail, or there is an apparent gap between the head and the tail. The long dimension of the rounded heads is 135 ± 25 Å, and their width is 85 ± 15 Å (Fig. 7 and Table 2).

Although a range of head shapes can be seen in any field of molecules, the type of myosin (intact or desensitized) in a given field is easily recognized from the predominant appearance of the molecules. About 20 to 25% of the molecules cannot be identified as belonging to a particular class because the shape of their heads is unclear. In about 75% (750 counted) of the remaining intact myosin

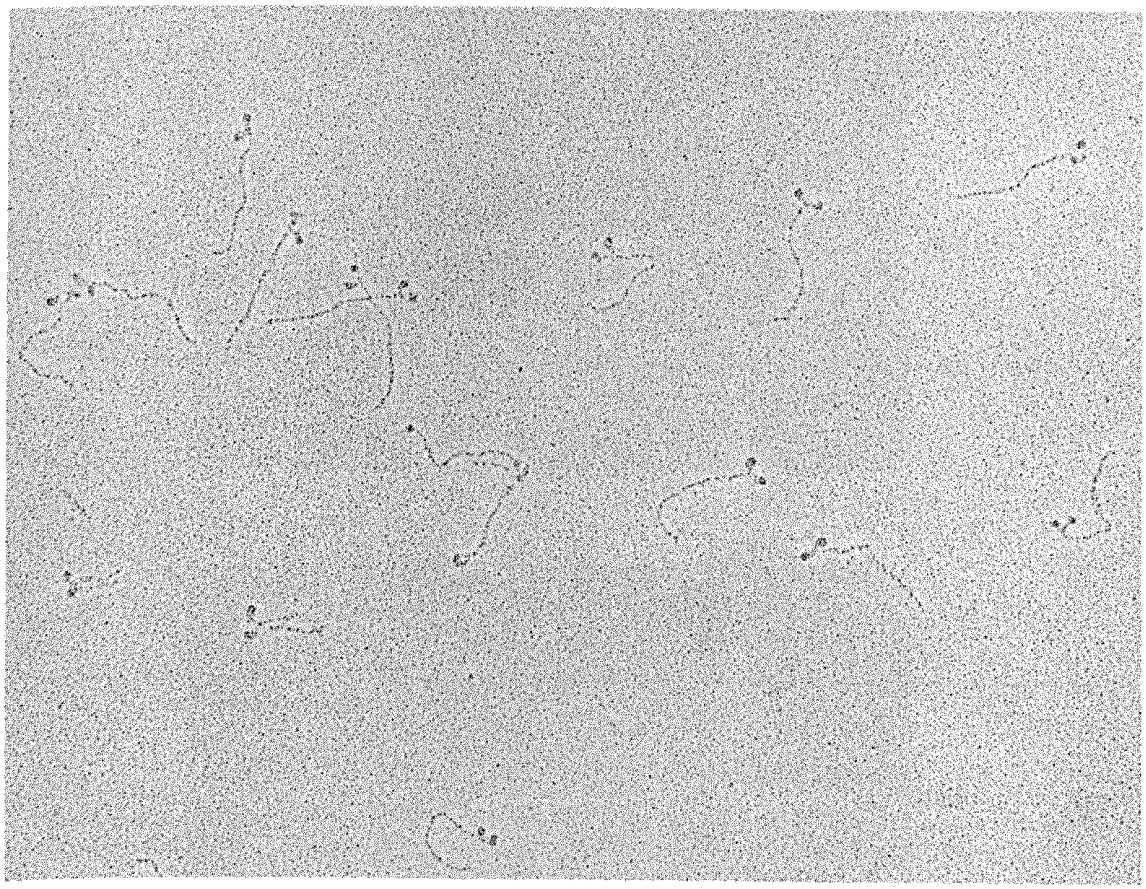

FIG. 5. A field of intact myosin molecules. Magnification, 100,000×.

molecules the heads appeared sensitive according to the description outlined above. Similarly, 80% (400 counted) of the desensitized molecules with clearly defined heads had distinctly rounded heads.

The change in appearance of the heads upon removal of the regulatory light chains is reversible. Re-addition of regulatory light chains restores calcium sensitivity to the myosin and the heads again appear pear-shaped. Intact myosin did not show any difference in appearance in the presence and absence of calcium. Myosin desensitized at 10°C is expected to retain, on average, one regulatory light chain per molecule (Chantler & Szent-Györgyi, 1980). This preparation shows a mixed population of sensitive and desensitized molecules (possibly because some exchange of light chains occurs in the solutions used for microscopy), as well as some molecules that appear as though only one head contains a regulatory light chain.

The heads are seen at a wide range of angles relative to the tail regardless of their light chain content. The tails of myosin appear extremely flexible and capable of bending at many points. The length of the tail is 1540 ± 90 Å (65 molecules) (Fig. 8). In some cases a sharp bend in the tail can be seen about a third of its length from the heads. Molecules were selected in which the angle made at the bend was greater than 90°; about 20% of the molecules were of this

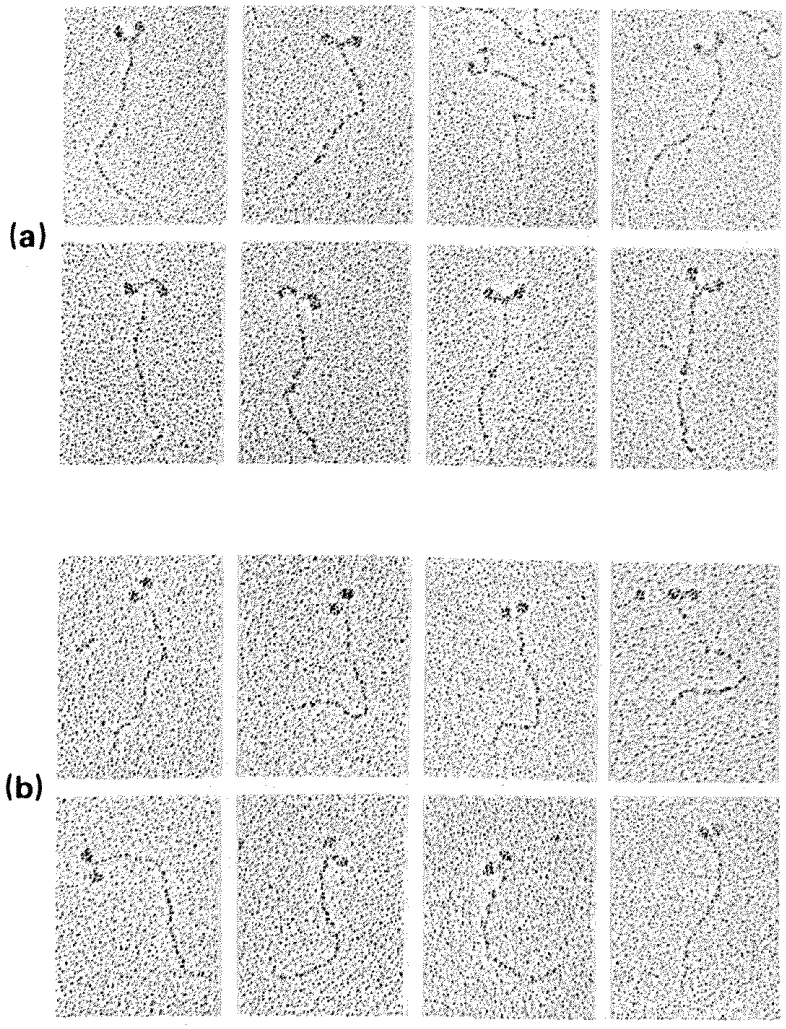

FIG. 6. Rotary-shadowed intact and desensitized myosin molecules showing a difference in appearance of the heads. Magnification, 130,000×. (a) The heads of intact myosin are widest at the end remote from the tail and taper to a narrow neck near their junction with the tail. (b) The heads of desensitized myosin (regulatory light chains removed) appear rounded. In some cases an apparent gap between the head and the tail can be seen.

TABLE 2

Dimensions of myosin heads and S-1s

	Length (Å)	Width (Å)
Sensitive myosin	195 (±30)	80 (±15)
Desensitized myosin	135 (±25)	85 (±15)
CaMg S-1	180 (±25)	80 (±15)
EDTA S-1	105 (±15)	85 (±15)

Histograms illustrating the distributions of lengths and widths are shown in Fig. 7. Errors quoted in parentheses are standard deviations.

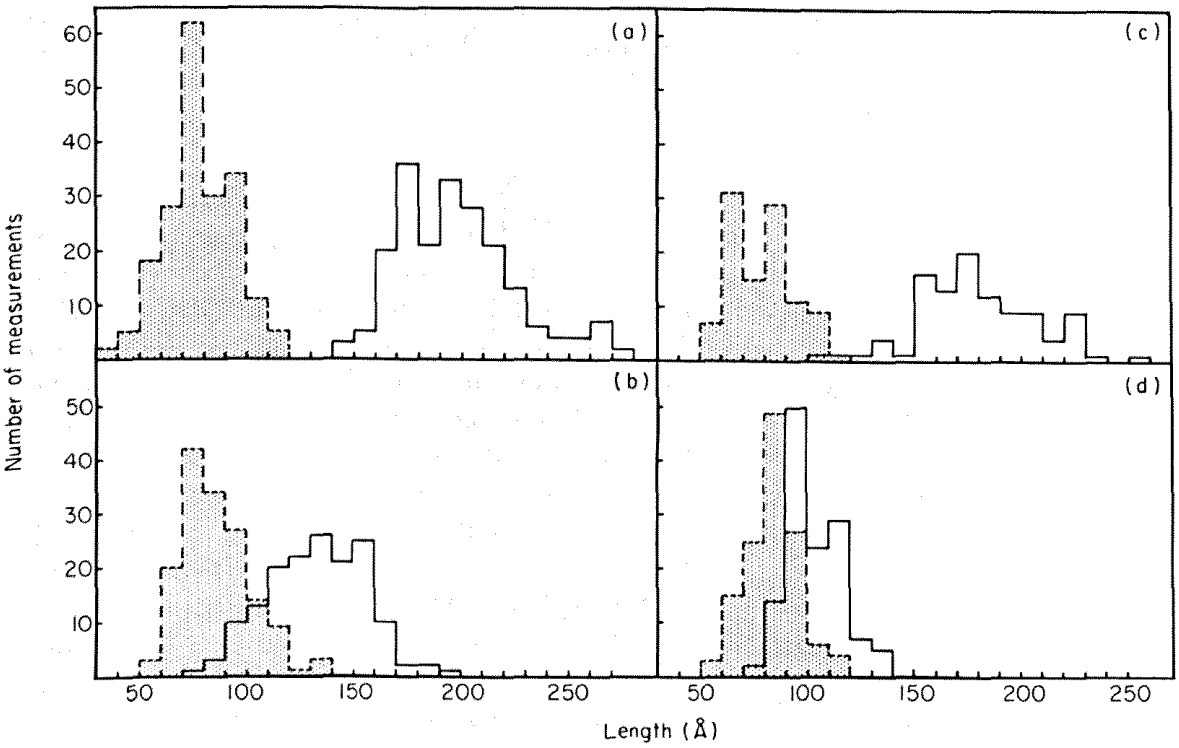

FIG. 7. Histograms showing the distribution of lengths (open) and widths (stippled) of (a) intact myosin heads; (b) desensitized myosin heads; (c) CaMg S-1; and (d) EDTA S-1. All measurements have been corrected for the thickness of the platinum shadow (see Experimental Procedures).

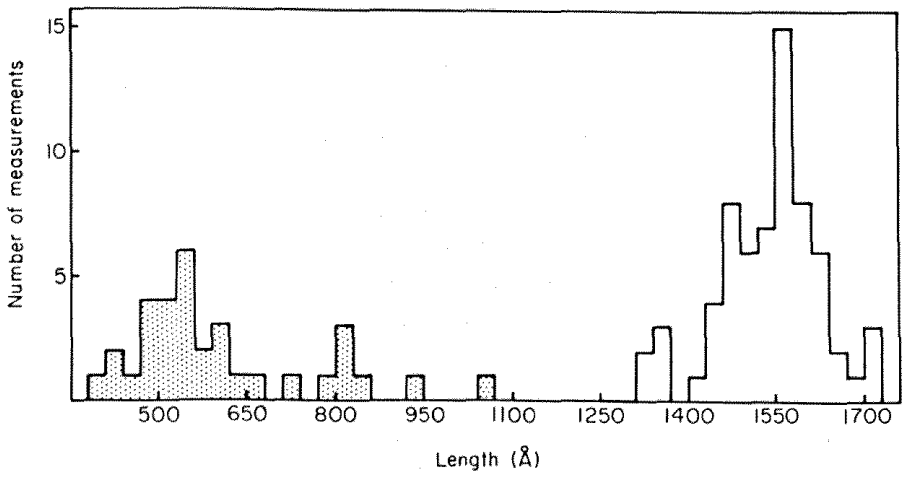

FIG. 8. Histograms showing the length of the myosin tail (open) and the location of sharp bends in the tail (stippled). The distance from the junction of the heads with the tails to bends greater than 90° was measured.

type. The distribution of the distance from the head/tail junction to the bend is shown in Figure 8. About half of the bends were located at 470 to 590 Å from the heads.

(b) *Subfragment-1 appearances*

Two types of S-1 that differ in their light chain content can be prepared from scallop myosin. Examination of these subunits showed that they too have different appearances that are correlated with light chain content (Fig. 9). CaMg S-1 contains a full complement of light chains (although the regulatory light chain lacks an approximately 1500 to 2000 M_r piece); its shape is similar to that of the intact myosin head; that is, it appears elongated, with a curved extended end. EDTA S-1, on the other hand, lacks the regulatory light chain and contains a fragmented essential light chain. Like the heads of desensitized myosin, it appears round and lacks an elongated end. About one-half (700 counted) of the CaMg S-1 molecules shows a clearly elongated shape: about one-third appears rounded like the EDTA S-1. However, the EDTA S-1s rarely (8% of 750 counted) exhibit an extended shape. The lengths of the S-1s are more difficult to estimate than those of the myosin heads, since there is no well-defined point like the head/tail junction to define one end. Nevertheless, the dimensions of the S-1s are the same, within experimental error, as those of native or desensitized myosin heads (Table 2).

(c) *Antibody labeling of myosin*

Specific antibodies directed against regulatory light chains or essential light chains provide a means of visualizing the location of light chains on myosin. Three classes of antibodies were used: one class specific for regulatory light chains and two classes specific for the essential light chain, which differ in their effect on the Ca^{2+}-sensitivity of myosin (Wallimann & Szent-Györgyi, 1981*b*).

The three types of Fab fragments attach to intact myosin in various positions. The anti-R-LC-Fab bound most often to the narrow portion of the head near its junction with the tail. Both populations of anti-SH-LC-Fab (desensitizing and non-desensitizing antibodies) also bound predominantly to the neck region of the head. Examples of anti-R-LC-Fab and anti-SH-LC-Fab bound to intact myosin in a range of locations are shown in Figure 10.

Antibodies were also incubated with desensitized myosin. Typically desensitized myosin had 95 to 97% of the R-LC removed, and indeed little binding of anti-R-LC-Fab was seen. Qualitatively, more desensitized myosin molecules appear to bind anti-SH-LC-Fab than do intact myosin molecules, in agreement with biochemical results (Wallimann & Szent-Györgyi, 1981*b*). However, the globular shape of both desensitized heads and Fab fragments made it particularly difficult to distinguish the head from the Fab. Therefore, no attempt was made to specify the binding site of anti-SH-LC-Fab on desensitized myosin.

About one-third of the myosin molecules incubated with Fab fragments showed bound antibody. This degree of binding of Fab to myosin was advantageous for interpretation of the images. Visualization of the bound Fab fragment requires

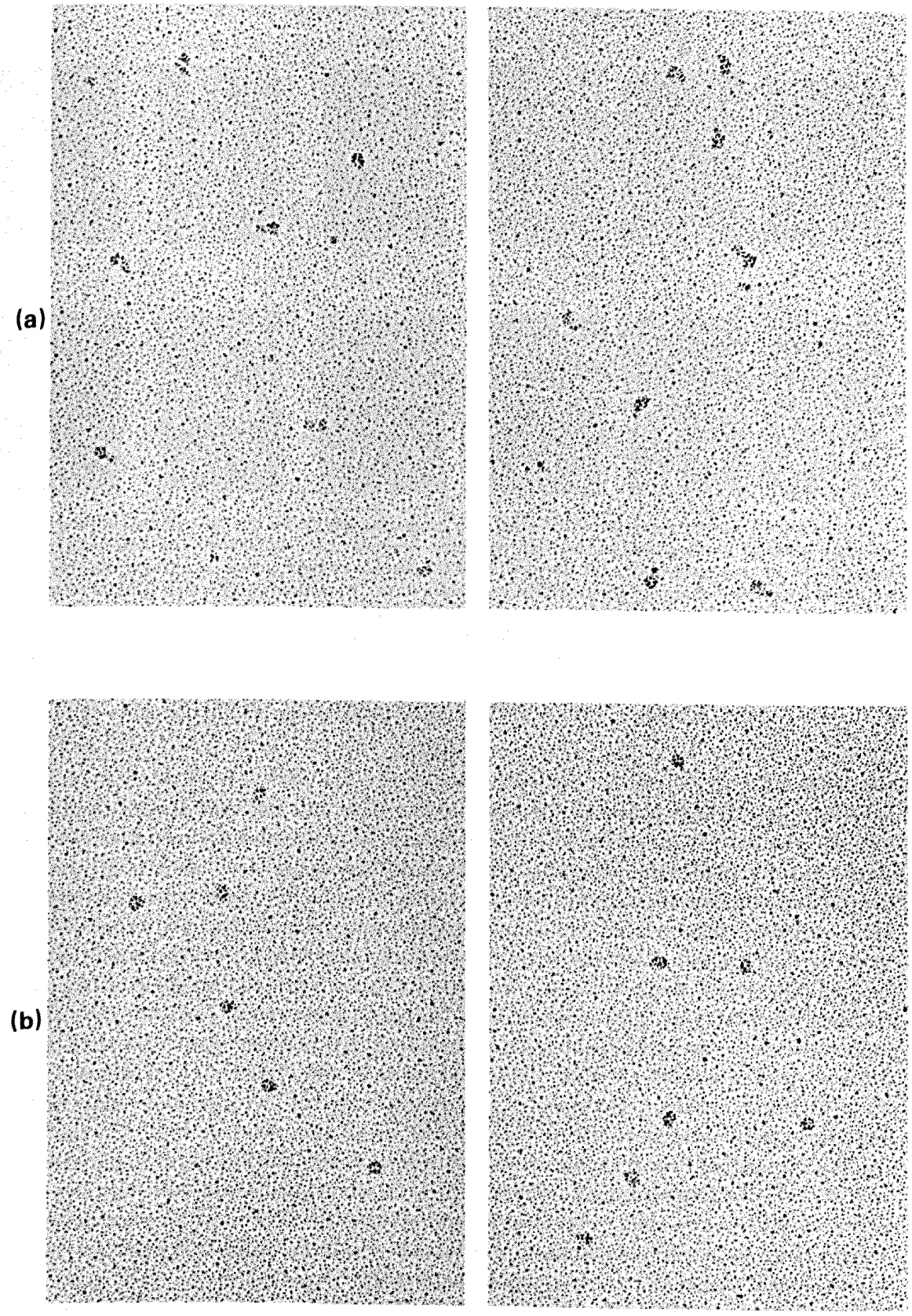

FIG. 9. Electron micrographs of CaMg S-1 and EGTA S-1. Magnification, 175,000×. (a) The elongated appearance of CaMg S-1, a wide domain and curved extended end, is similar to that of intact heads. (b) EDTA S-1 appears rounded.

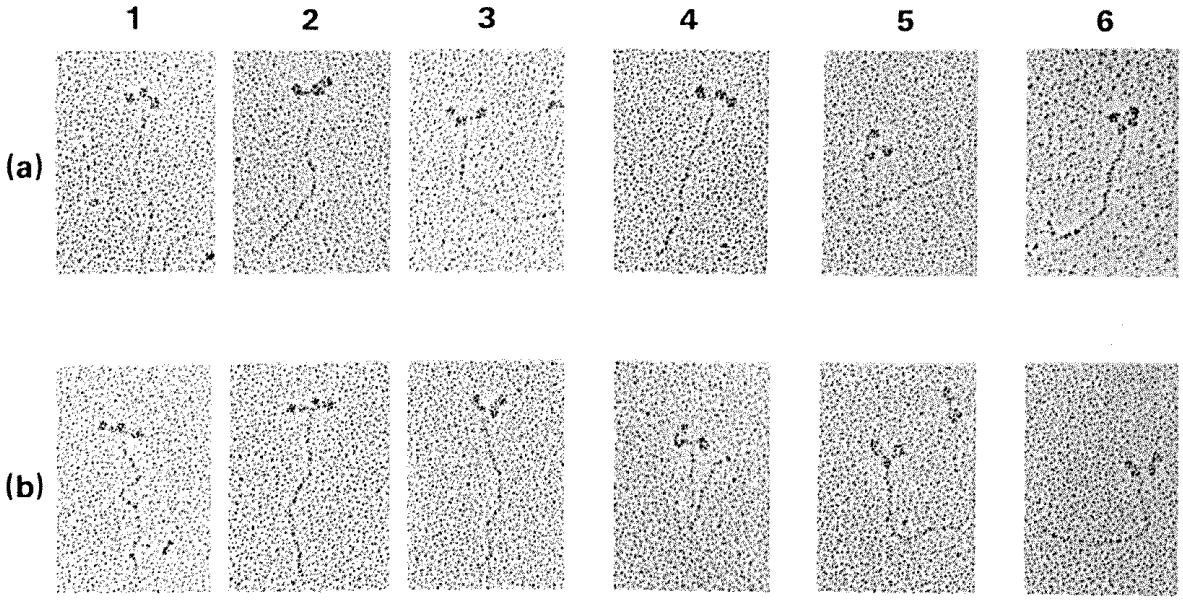

FIG. 10. Intact myosin molecules with bound Fab fragments specific for the light chains showing the range of apparent binding sites of the Fab fragments. Magnification, 125,000×. The Fab fragments bound to the myosin molecules in (a) are specific for the regulatory light chains. Fab fragments specific for the essential light chain are shown bound to myosin in (b). In columns 1 to 3, Fab appears to bind to the neck region of the head near its junction with the tail. In column 4, Fab fragments are seen bound to the widest part of the head away from the tail. In column 5, Fab apparently binds to the S-2 region of the tail; the junction of the heads and tail can be distinguished. In column 6 myosin molecules with 2 Fab fragments bound are shown.

that the binding site of the antibody to the myosin and the orientation of the myosin and antibody complex on the mica surface be such that the antibody is distinguishable. Two or more Fab fragments could sometimes be seen bound to a single myosin molecule (Fig. 10). In those instances, it was often difficult to distinguish the Fab fragments from the myosin heads. At the low ionic strength used, myosin is precipitated as filaments, which may have limited the accessibility of the myosin to Fab. Even after extensive washing of myosin pellets, where 90 to 95% of the excess antibody was removed, unbound antibody was visible in the electron microscope. This observation implies that some low affinity Fab fragments may have dissociated from myosin in the dilute solutions used for shadowing. The anti-R-LC-Fab dissociates some of the light chain (Wallimann & Szent-Györgyi, 1981*b*); therefore, a low percentage of myosin molecules with bound antibody is expected for experiments using this class of Fab. Unspecific binding of control Fab fragment was rarely seen. The advantages of our method include a low background of unbound Fab fragment and a limited number of myosins with more than one Fab fragment bound. The method chosen is a compromise between the limited extent of binding of Fab fragments and interpretability of the electron microscope images.

In order to specify the location of the binding sites of anti-light chain antibodies to intact myosin, the distance between the junction of the head with the tail and the center of the bound Fab fragment was measured. The precision of

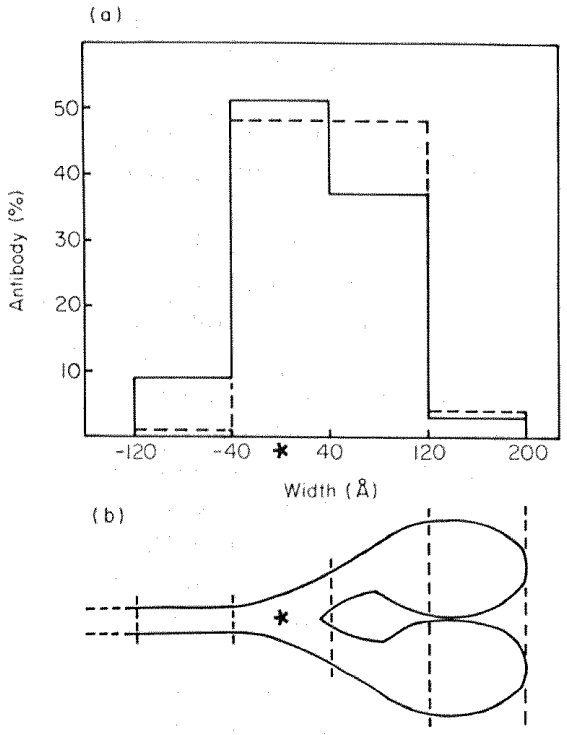

FIG. 11. (a) Histogram showing the distribution of anti-R-LC-Fab (continuous line) and one class of anti-SH-LC-Fab (broken line) on myosin molecules. The percentage of antibody seen bound to each of 4 regions of the myosin molecule is shown. (b) The diagram of a myosin molecule illustrates the domains selected; each is 80 Å wide, the widest dimension of the Fab fragment. The location of the antibody was measured from the estimated position of the head/tail junction (*) to the center of the Fab fragment.

the location is limited by the size of Fab fragment. We defined three domains on the myosin head and one domain on the tail within which a Fab molecule could bind (Fig. 11); each was 80 Å wide, the largest dimension of the Fab (Poljak *et al.*, 1973). Measurements of the positions of the two populations of anti-SH-LC-Fab showed their binding sites were indistinguishable at this resolution. A histogram of the distribution of Fab fragments specific for the regulatory light chain and one class of anti-SH-LC-Fab is shown in Figure 11. About 90% of the Fab fragments appeared in the neck region within 120 Å of the head/tail junction. Half of the anti-R-LC-Fab bound within 40 Å of the junction of the head and tail.

4. Discussion

All muscle myosins examined using rotary shadowing exhibit the same overall shape and dimensions (Elliott & Offer, 1978; Elliott *et al.*, 1976), further demonstrated here with scallop myosin. Elliott and Offer extended the earlier description of the shape of the myosin as comprising two globular domains and a rod-like tail (Slayter & Lowey, 1967) by showing more detail about the shape and size of the head and flexibility within the molecule. In particular, their images showed that the heads are wider at their ends than near the junction with the tail. The intact scallop myosin heads shown here display this same shape, and their

length (195±30 Å) is similar to the length of myosin heads reported by Elliott & Offer (1978). However, the width of the heads we measured (80±15 Å) is greater by 20 to 25 Å, suggesting that the myosin molecules were more flattened by our technique. In addition, the length of the tail (1540±90 Å) agrees with previous measurements from a variety of muscle myosins (Elliott *et al.*, 1976; Elliott & Offer, 1978).

Sharp bends have been reported in the tail regions of myosin from various sources, and are thought to have functional significance. Elliott & Offer (1978) found bends at a well-defined location about 430 Å from the head/tail junction in shadowed rabbit striated myosin, although Takahashi (1978) described a flexible region centered at 630 Å from the heads in similar molecules contrasted by negative stain. McLachlan & Karn's (1982) analysis of the amino acid sequence of the myosin rod is consistent with a hinge at about 470 to 485 Å from the NH_2 terminus of the rod. Our own observations suggest that scallop myosin is flexible over an extended region about 470 to 590 Å from the heads. This general region of the myosin rod has often been thought to contain a functionally important hinge that would permit the S-2 region to move away from the surface of muscle thick filaments during activity. The length of the heads (Table 2; see also Elliott & Offer, 1978; Vibert & Craig, 1982) relative to the filament separation suggests, however, that such movement of S-2 would not be required simply to bridge the interfilament gap; it may, nonetheless, be important in allowing the heads to make stereospecific contact with actin. (We thank R. Craig for a discussion of this point.) A bend with possible functional importance occurs in this region of the regulated myosins from some smooth and non-muscle cells, permitting the molecules to adopt a "hair-pin" conformation under certain conditions (Trybus *et al.*, 1982; Craig *et al.*, 1983). This hair-pin conformation has not, however, been identified for scallop myosin in any of the conditions we have studied.

We have analyzed the shape of scallop myosin molecules and proteolytic fragments of the head in some detail to define the location and structural role of the light chains. The major effect of removing the regulatory light chain is to change the shape and apparent length of the heads (Figs 6 and 9). A loss of mass occurs in the narrow "neck" region of the head near its junction with the tail, but the true length of the head is probably not altered. The heavy chain must pass through the neck of desensitized myosin; moreover, the mass of the heavy chains is similar in the two S-1 preparations. Antibody binding shows that the essential light chain also contributes to the neck. Thus, the apparent decrease in head length is due, at least in part, to the reduced visibility of the neck region when the regulatory light chain ($M_r \simeq 18{,}000$) is removed. There does, however, seem to be another effect of light chain removal: the denuded neck of desensitized myosin sometimes collapses (Fig. 6(b)), and in EDTA S-1 no sign of an extended neck is seen (Fig. 9(b)). Thus, the regulatory light chain may stabilize the structure of the heavy chain and the essential light chain in the neck region.

In addition to the altered regulatory light chain content, the two types of S-1 differ slightly in heavy chain mass, and the essential light chain is also reduced in mass in EDTA S-1. The dimensions of the CaMg S-1 and EDTA S-1s (Table 2) are very similar, however, to those of the sensitive and desensitized heads, suggesting

that the regulatory light chain is critical to the different S-1 appearances. Three-dimensional reconstruction of negatively stained thin filaments decorated with EDTA S-1 or CaMg S-1 also showed increased mass at the end of CaMg S-1 (Vibert & Craig, 1982). The contour lengths of EDTA S-1 and CaMg S-1 in the reconstructions, 135 Å and 175 Å, respectively, are comparable to the lengths reported here. Elliott & Offer (1978) compared measurements of their images of myosin molecules with the size of S-1 indicated by earlier three-dimensional reconstructions and hydrodynamic measurements, and suggested that S-1 from vertebrate skeletal muscle is only about 140 Å long, significantly less than the length of the myosin head. Our measurements of scallop EDTA S-1 indicate a similar length, and suggest that this is the apparent length of the head subfragment in the absence of a regulatory or DTNB-type light chain. When the full complement of light chains is present, however, the subfragment appears to correspond to an entire head and has a length close to 190 Å. In shadowed CaMg S-1 preparations some rounded heads are seen, although the majority of the heads are elongated. This variability may in part be due to different views of the same elongated structure, but it also suggests that the neck of scallop myosin is flexible even in the presence of the regulatory light chain.

Antibodies specific for the light chains demonstrate the position of the regulatory light chain and the proximity of the two types of light chain. The antibodies used for this study are not monoclonal and bind to a number of antigenic sites on the light chains (Wallimann & Szent-Györgyi, 1981*a*). Binding of antibody to peptide fragments of the essential light chain shows that antigenicity does not vary substantially along the chain; moreover, the accessibility of sites on both kinds of light chain is unchanged, whether the light chains are bound to myosin or free in solution. Isolated light chains are estimated to be about 100 Å long, and have a stable secondary structure (Stafford & Szent-Györgyi, 1978). Thus, the predominant binding of Fab fragments in the neck region (Fig. 11) probably indicates that both the regulatory and essential light chains occupy an approximately 100 Å-long region in the neck of the myosin molecule. (Following this interpretation, the small amount of binding recorded in the extremes of the histogram reflects distortion of the three-dimensional structure of the antibody–head complex that occurs during drying and shadowing. Cross-reactivity between light chain antibodies and the myosin heavy chain is very low (Wallimann & Szent-Györgyi, 1981*a*), and would be negligible at the antibody concentrations used for microscopy.) These results support other recent evidence for the proximity of the two types of light chain. The regulatory light chains protect the essential light chain from digestion by papain (Stafford *et al.*, 1979), or from alkylation of its thiols (Hardwicke *et al.*, 1982). In addition, regulatory and essential light chains can be cross-linked with bifunctional reagents about 6 to 8 Å long, and probably overlap for most of their length (Wallimann *et al.*, 1982; Hardwicke *et al.*, 1983). It appears that the light chains extend into and perhaps slightly beyond the junction of the heads and the tail (Figs 10 and 11). Interactions of the regulatory light chain with the S-2 region of myosin may account for biochemical evidence that has implicated the S-2 region in regulation (Stafford *et al.*, 1979).

These results help to define the structure and function of the neck region of the scallop myosin molecule. It is clear that three polypeptide chains, two light chains and the heavy chain, contribute to this region. Myosin heads adopt a wide variety of angles relative to the tail, both in isolated molecules and under physiological conditions, so that the region near the S-1/S-2 junction must include a very flexible hinge. Our results suggest that the regulatory light chain may pass through the hinge, but removal of the light chain does not alter the angles at which the heads are disposed in isolated molecules. On this evidence, regulation is unlikely to depend on changes in flexibility of the hinge. The actin-binding site, as seen by three-dimensional reconstruction of decorated thin filaments (Vibert & Craig, 1982), lies near the end of the head remote from the tail, so the regulatory light chain probably does not extend into the actin-binding site. Thus, regulation of scallop myosin is not likely to occur by direct physical interference of actin binding by the regulatory light chain. The location of the regulatory light chain in the neck instead suggests that more complex interactions may be involved in regulation. The amino-terminal region of the regulatory light chain moves relative to the essential light chain when the molecule is switched on (Hardwicke *et al.*, 1983). Ca^{2+}-induced changes in the interactions of the three polypeptides in the neck may be propagated from this domain to the actin-binding and nucleotide-binding domains further along the head, and thus influence both actin–myosin binding and events at the nucleotide site (cf. Chalovich *et al.*, 1983).

A number of biochemical studies indicate that co-operative interactions between the two heads of myosin also play a significant part in the regulatory mechanism. The form of the Ca^{2+}-dependence of the actin-activated ATPase of heavy meromyosin shows that neither head is activated until both have bound calcium ions (Chantler *et al.*, 1981). Moreover, the relationship of myofibrillar ATPase to light chain content indicates that two regulatory light chains are required to switch the molecule off (Kendrick-Jones *et al.*, 1976; Chantler & Szent-Györgyi, 1980). The finding that single heads (i.e. CaMg S-1) are incapable of being turned off, even when they contain intact regulatory light chains (Szent-Györgyi *et al.*, 1973; Stafford *et al.*, 1979), is consistent with this view. Single-headed myosin, however, retains its Ca^{2+}-sensitivity (Stafford *et al.*, 1979), implying that the critical region for regulation and co-operativity is near the S-1/S-2 junction. Our studies suggest a basis for this concept in that both the regulatory and essential light chains are components of this region. Moreover, the regulatory light chains in the two heads can be cross-linked by bifunctional reagents only 6 to 8 Å long (Wallimann *et al.*, 1982). Thus, we may picture that altered interactions between the regulatory chains and the essential and heavy chains near the S-1/S-2 junction could co-operatively affect the structures of both neck domains, and so modify the properties of the actin and nucleotide-binding domains in both heads.

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