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STUDIES ON THE ROLE OF MYOSIN LIGHT CHAIN-LC_2 IN TENSION GENERATION

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ABSTRACT Myosin deficient in light chain LC_2 was prepared and tension generation by actomyosin-threads composed of skeletal muscle actin, myosin, and light chain-(LC_2)-deficient myosin was measured. No difference was observed between myosin complexed with LC_2, and myosin deficient in LC_2, with respect to velocity of contraction and tension generation. These studies demonstrate that LC_2 has no specific role in the tension generated by actomyosin threads.

INTRODUCTION

Skeletal muscle contains four low molecular weight subunits which are thought to play a functional role in the enzymatic activity of myosin. The "alkali" light chains, also known as, LC_1 and LC_3, increase the affinity of myosin for actin (1), and may be involved in actin-myosin interaction (2,3). Light chain LC_2 contains the high affinity binding site for divalent cations (4,5). The discovery that the binding of calcium ions to the homologous light chains of molluscan skeletal muscle myosin regulates the myofibrillar ATPase activity (6) has prompted speculation that LC_2 may have some regulatory role in addition to that derived from the regulatory components of troponin and tropomyosin on the thin filaments (7). Myosin-linked regulation has been shown to be operating in vertebrate smooth muscle (8). However, there is still no evidence supporting a direct involvement of LC_2 in the regulation of skeletal muscle contraction. Partial removal of the DTNB light chain, LC_2, does not affect the ATPase activity of myosin (9). There have been several reports in the past which showed that Ca^{2+}-regulation of actin-activated ATPase activity of myosin deficient in LC_2 was impaired (10). Pemrick (11) has reported that LC_2 of rabbit skeletal muscle myosin stabilizes a particular

ABBREVIATIONS: Pi: Phosphate; DTT: Dithiothreitol; EDTA: Ethylenediamine tetracetic acid; PAGE: Polyacrylamide gel electrophoresis; SDS: Sodium dodecylsulfate; TES: (N-tris(hydroxymethol)methyl 2-aminoethane sulfonic acid)

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conformation which enhances actin-interaction as A·M·ADP·P* complex, thereby increasing the calcium affinity of troponin. This ternary complex is important for force generation.

Since the above studies investigated only biochemical properties of the actomyosin interaction, we decided to determine whether LC_2 has a role in the tension generated by synthetic actomyosin threads. Such a technique has been successfully employed in this laboratory (12). The present study also describes the removal of more than 50% LC_2 of skeletal muscle myosin without an appreciable loss of "alkali" light chains

EXPERIMENTAL PROCEDURES

Purifications. Myosin was purified from rabbit skeletal muscle as described earlier (13). Actin was purified by the method of Spudich and Watt (14).

Dissociation of Myosin LC_2 Myosin (0.2 mg/ml) was incubated for 5 min at 35°C in a solution containing 0.65 M KCl, 5 mM EDTA, 1 mM DTT, 3 mM ATP (disodium salt) and 0.1 M Tris·HCl, pH 7.0. At the end of the incubation period, myosin was immediately precipitated with 45% $(NH_4)_2SO_4$, stirred for 5 min in the cold (0-4°C) and then centrifuged immediately. After centrifugation, myosin was solubilized in 0.05 M Tris·HCl, pH 7.5, containing 0.5 M KCl, and 1 mM DTT. The above method of incubation and precipitation was repeated. The protein concentration was measured by Biuret Method (15).

Polyacrylamide Gel Electrophoresis, Analyses and Quantification Polyacrylamide gradient slab gels containing sodium dodecylsulfate were prepared as described in earlier reports (16). For determination of protein concentration present in Coomassie-blue-stained polyacrylamide gels, the dye was removed with 25% pyridine, analysed at the maximum absorption wavelength (E_{max}) of 605 nm as described earlier (17) and protein concentration was determined.

ATPase Activity of Myosin Actin-activated myosin ATPase was assayed in 0.02 M imidazole·HCl, pH 7.0, 1 mM $MgCl_2$, 0.03×10^{-4} M $CaCl_2$, and 0.8 mM ATP at 35°C. $EDTA/K^+$ -ATPase of myosin was measured in a mixture containing 0.1 M Tris·HCl, pH 7.8, 1 mM EDTA, 0.65 M KCl, and 5 mM ATP. Calcium-activated myosin ATPase activity was measured in 0.2 M Tris·maleate, pH 6.5, 10 mM $CaCl_2$, and 5 mM ATP. The protein concentration and time were chosen so that less than 15% ATP was hydrolysed at any one time. Pi was estimated by the method of Fiske and SubbaRow (18).

Measurement of Tension Generation by Actomyosin Threads Actomyosin was prepared as described earlier (12). In brief, threads were formed by extruding the concentrated solution of actomyosin comprising different ratios of actin to myosin through a syringe into a trough that contained buffer (50 mM KCl, 5 mM $MgCl_2$, 10^{-5} M $CaCl_2$, and 20 mM TES, pH 7.0). We used a 20 gauge needle and the ends of the needle were squared off and the outside edges were beveled to produce a lamilar flow around the end of the needle. The temperature of the buffer was 0 + 1°C.

The threads were removed from the trough and mounted on the tensiometer with a curved dissecting needle. The details of the tensiometer have been described earlier (12). The threads were

glued to the mounting rod with a drop of a solution of acetone and plexiglass (10 mg plexiglass/ml). They were then immersed in 2.5 ml of above buffer maintained at 24 ± 2°C containing 4 mM phosphocreatine and 0.4 mg/ml creatine kinase. Approximately 5 min was allowed for the equilibration of threads with solution. At this point the tensiometer was in the isometric mode, the threads were in rigor and the tension was small. When ATP was added the tension increased. After the tension reached its maximum, the tensiometer was switched momentarily into the isotonic mode and the position of the arm was recorded on an X-Y recorder. The tensiometer was returned to the isometric mode and the thread was allowed to return to its initial length before a subsequent velocity was recorded at a different tension. Using this method an entire force-velocity curve was produced in about 2 min. The area of the thread was measured (12) and data normalized accordingly.

RESULTS

A single 5 min incubation of myosin in the presence of the chelating agent, EDTA, caused a 50% release of light chain LC_2, whereas 2 consecutive treatments resulted in a 70% dissociation of LC_2 (Figure 1 (+EDTA)). Two such treatments in the absence of EDTA resulted in a 7-10% light chain LC_2 dissociation (Figure 1 (-EDTA)). The released light chains were recovered in the 80% $(NH_4)_2SO_4$ saturation fraction (Figure 1 (LC)). Procedures for the release and recovery of light chains were similar to those reported earlier (19). In both types of treatment, i.e., in the presence or absence of EDTA, there was a small loss of "alkali" light chains, LC_1 and LC_3 (Table I). In both cases there was no significant decrease in the $EDTA/K^+$ or Ca^{2+} -stimulated ATPase activities (Table I) as reported earlier (19) for cardiac myosin, although a 10-15% decrease in actin-

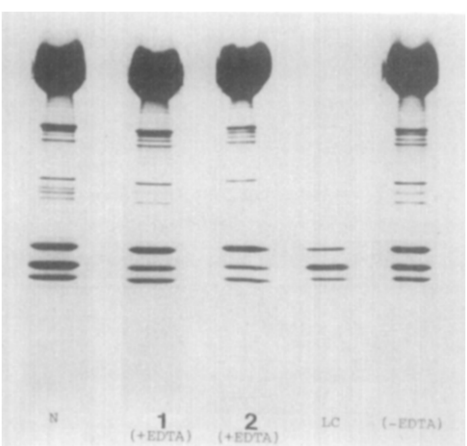

Figure 1 SDS-PAGE Electrophoretograms of skeletal muscle myosin after treatment under different experimental conditions as described in text. 'N' is normal myosin, i.e., no treatment; '1' and '2' (+EDTA) are 1 and 2 treatments with EDTA; 'LC' are the light chains released ; and '(-EDTA)' is treatment in the absence of EDTA (Control).

TABLE I

MYOSIN ATPase ACTIVITY AND PERCENT LIGHT CHAIN DISSOCIATION UNDER VARIOUS EXPERIMENTAL CONDITIONS*

Dissociation Conditions	ATPase Activity % Control				% Light Chains Dissociated	
	K^+	Ca^{2+}	Mg^{2+}	Actin+Mg^{2+}	$C_1 + C_3$	C_2
(+EDTA)	103	104	98	89	9	70
(-EDTA)	100	101	105	88	7	8

Normal values for myosin ATPase activity assayed at 37°C are the following: (K^+) 4.82; (Ca^{2+}) 1.10; (Mg^{2+}) 0.04; (Actin + Mg^{2+}) 0.60 μmoles Pi/mg·min.

*Data are for two consecutive (5 min) dissociations in the presence (+EDTA) and absence (-EDTA) of the chelating agent, EDTA, as described in Procedures. The degree of light chain dissociation was determined as described in Procedures.

activated myosin ATPase activity occurred (Table I), similar to that reported for cardiac myosin (20).

We then studied the tension generated by actomyosin threads formed of skeletal muscle actin and myosin. Actomyosin (LC_2-deficient) threads formed with myosin treated in the absence of EDTA (10% LC_2-deficient) served as a control for the threads formed with 50% and 70% LC_2-deficient myosin. We did not observe any significant change in the pattern of force-velocity measurements as shown in Figure 2A between native, and 10, 50, and 70% light chain LC_2-deficient myosin. (The concentration of protein was approximately 55 mg/ml). Tension increased linearly when plotted as a function of actin·myosin concentration. The values for the slopes of native, 10%, 50%, and 70% LC_2-deficient myosin were 0.23 x 10^{-9} g/cm^2 M^2, 0.16 g/cm^2 M^2, 0.16 g/cm^2 M^2, and 0.15 g/cm^2 M^2, respectively (Fig. 2B). Thus there was no significant difference among threads from the 10%, 50%, and 70% -LC_2-deficient myosins. However, there was a significant difference between the native myosin where none of the 3 light chains were released and the 10, 50, and 70% LC_2-deficient myosins, when data were expressed as $(P_o/(A)(M)) = g/cm^2\ M^2$.

DISCUSSION

Although myosin light chain LC_2 may be important to the structure of myosin (21) our studies indicate that light chain LC_2 has no role in tension generation of actomyosin threads. The difference in tension observed between native myosin, and the 10, 50

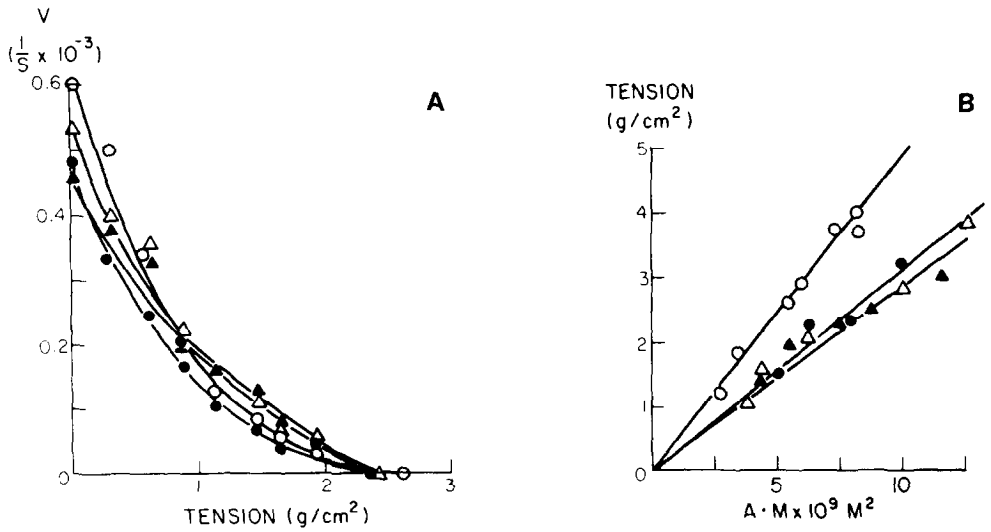

Figure 2a Force velocity curves of actomyosin threads. Threads were formed from the solution that contained myosin and actin (55 mg/ml) in the ratio of 1:2. Normal (0-0); Control (●-●); 50% LC_2-deficient (Δ-Δ); 70% LC_2-deficient (▲-▲). Tension was initiated by adding ATP (25 μM).

Figure 2b Isometric tension as a function of protein concentration. Isometric tensions are plotted as a function of the actin concentration times myosin concentration, in the solution from which the threads were were formed. Generation of tension was initiated by addition of ATP (25 μM). Normal (0-0); Control (●-●); 50% LC_2 deficient (Δ-Δ); 70% LC_2-deficient (▲-▲). The values for slopes of normal, control, 50% and 70% LC_2-deficient myosin were: 0.23 x 10^{-9} M^2, 0.16 x 10^{-9} M^2, 0.16 x 10^{-9} M^2, and 0.15 x 10^{-9} M^2, respectively.

and 70% LC_2-deficient myosin may be due to denaturation or loss of light chains LC_1 and LC_3. The loss of tension was similar to the loss in activity of actin-activated myosin ATPase. It did not correlate with the loss of light chain LC_2. Because myosin that was 10%, 50% and 70% deficient in LC_2, all generated the same tension we concluded that LC_2 is not playing a role in tension generation. There have been several studies in the past implicating directly or indirectly the role of light chain LC_2 in the structure and function of myosin. For example divalent cations bind with LC_2 and prevent cleavage of the hinge region of myosin (the junction between the S_1 and S_2 fragment) (22). Light chain LC_2 appears to bridge this junction in the presence of divalent cations, thereby conferring a conformation on myosin which makes this region less susceptible to proteolytic cleavage. From centrifugal, and viscosity measurements, Morimoto and Harrington (4) indicated that changes in Ca^{2+} concentration of 10^{-7} to 10^{-5} M caused the S_1 moiety to fan out radially, thus suggesting a role for LC_2 in the radial

movement of S_1 based on the premise that Ca^{2+} binds only to LC_2. Mendelson and Cheung (23), however did not observe such Ca^{2+} -induced radial fanning of crossbridges. The role of light chain LC_2 was further assessed using myosin in the soluble state. Pemrick (11) showed that in the troponin-tropomyosin system LC_2 enhanced the ability of myosin to interact with the thin filaments in the presence of Ca^{2+}. Recently, Malhotra et al (24) reported an LC_2-enhanced cardiac actomyosin ATPase activity after complete removal of light chain LC_2.

In contrast to the above studies where myosin was analyzed enzymatically in solution, myosin studied here was in a polymerized form, as actomyosin threads. Although the thread tension is several-fold smaller than that of the intact muscle, several lines of experimental evidence suggest that it is still the result of an actomyosin interaction, similar to that of muscle (12,25). Possible reasons for the decreased tension in threads may be due to 1) lack of an ordered filamentous array, and 2) low protein concentration. In spite of these uncertainties the threads provide a quantitative method for measuring mechanical aspects of actomyosin interaction.

Our results show that skeletal muscle myosin light chain LC_2 has no role in the tension generation by threads formed of skeletal actin and LC_2-deficient myosin though it could be expected from the work of Pemrick (11) and Morimoto and Harrington (4). Furthermore, light chain LC_2 does not appear to be important for either myosin or actomyosin ATPase activity when myosin is assayed in the soluble form.

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