Effects of EDTA Treatment upon the Protein Subunit Composition and Mechanical Properties of Mammalian Single Skeletal Muscle Fibers

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ABSTRACT Considerable interest has been focussed on the role of myosin light chain LC2 in the contraction of vertebrate striated muscle. A study was undertaken to further our investigations (Moss, R. L., G. G. Giulian, and M. L. Greaser, 1981, J. Biol. Chem., 257:8588-8591) of the effects of LC2 removal upon contraction in skinned fibers from rabbit psoas muscles. Isometric tension and maximum velocity of shortening, Vmax, were measured in fiber segments prior to LC2 removal. The segments were then bathed at 30°C for up to 240 min in a buffer solution containing 20 mM EDTA in order to extract up to 60% of the LC2. Troponin C (TnC) was also partially removed by this procedure. Mechanical measurements were done following the EDTA extraction and the readditions of first TnC and then LC2 to the segments. The protein subunit compositions of the same fiber segments were determined following each of these procedures by SDS PAGE of small pieces of the fiber.

Vmax was found to decrease as the LC2 content of the fiber segments was reduced by increasing the duration of extraction. EDTA treatment also resulted in substantial reductions in tension due mainly to the loss of TnC, though smaller reductions due to the extraction of LC2 were also observed. Reversal of the order of recombination of LC2 and TnC indicated that the reduction in Vmax following EDTA treatment was a specific effect of LC2 removal. These results strongly suggest that LC2 may have roles in determining the kinetics and extent of interaction between myosin and actin.

The role(s) of the low molecular weight subunits, or light chains, of myosin in the contraction of vertebrate skeletal muscles have not been clearly resolved. The great majority of the work that has been done to investigate this problem has involved in vitro biochemical studies of the isolated contractile proteins, actin and myosin. Results obtained using this approach have generally been unable to indicate specific functions for the light chains. Removal of the so-called alkali light chains with NH4Cl has been found to result in the loss of actomyosin ATPase activity (11, 15); however, these extraction conditions may denature the remainder of the myosin molecule, rendering it inactive (13). More recently, Wagner and Giniger (26) and Sivaramakrishnan and Burke (22) have shown that significant myosin ATPase activity remains even after the total removal of all light chains. Removal of up to 50% of the LC2 light chain with dithionitrobenzene (DTNB) has been found to have little effect upon actomyosin ATPase activity (16), though recent evidence (21) indicates that LC2 may play a role in modulating the ATPase activity of myosin and regulated actin during Ca2+ activation.

Examination of the mechanical properties of single muscle cells in which the light chain (LC) composition could be manipulated and quantitated would seem to be a useful approach to the study of the physiological function(s) of these subunits. Work in this laboratory has shown that when LC2 was partially extracted from single skinned muscle fibers (to levels that were ~70% of control) the maximum velocity of shortening (Vmax) measured during maximal Ca2+ activation was reduced by about 40% (18). This phenomenon has been investigated further in the present study. LC2 extraction conditions have been varied to yield LC2 compositions within individual fiber segments that were 40-95% of control values measured in the same fibers. Vmax was found to decrease as the LC2 content of the segment was reduced. EDTA treatment of...
the fiber segments also resulted in reversible reductions in tension due mainly to the loss of troponin C (TnC). $V_{\text{max}}$ was unaffected by decreases in tension per se, down to tension values as low as 30% of control values. Both $V_{\text{max}}$ and tension could be restored by readdition of LC2 and TnC respectively. These results suggest that LC2 may have a modulatory role in the interaction of actin and myosin in skeletal muscle. A brief report of these results was presented at the Meetings of the Biophysical Society (19).

MATERIALS AND METHODS

**Preparation:** Male New Zealand rabbits (2.5-3.5 kg body wt) were sacrificed by cervical dislocation. The psoas muscles were quickly excised and placed in a cold solution containing, in mM: KCl, 100; MgCl$_2$, 1; ATPNa$_2$, 4; EGTA, 5; imidazole, 10; pH 7.00 ± 0.01. Small fiber bundles were dissected from the muscles, tied with surgical silk to capillary tubes, and stored in a cold solution containing 50% (vol/vol) glycerol at -22°C for 3-14 d before use. On the day of its use each bundle was bathed for 30 min in cold solution containing 0.5% (wt/vol) Brij 58, and single fibers were then pulled free. Fiber segments were transferred to the muscle chamber containing relaxing solution (below) and mounted with small connectors (17) to wires extending between the motor arm and the force transducer. A segment of fiber between 2.0 and 5.0 mm in length was always present in the relaxing solution. The segments were in each case observed and photographed through a light microscope in order to obtain a measure of sarcomere length (17) both while relaxed and during Ca-activation. Initially, the end-to-end length of each segment was adjusted so that the mean sarcomere length was 2.7 μm.

**Solutions:** The solutions that were used to relax and activate the fiber segments have been described previously (17). Relaxing solution, containing, in mM: KCl, 100; EGTA, 2; MgCl$_2$, 1; ATP Na$_2$, 4; imidazole, 10; pH 7.00. The composition of the activating solution was identical to that of relaxing solution except that the concentration of EGTA was 4.0 mM and CaCl$_2$ was added to yield a concentration of 3.8 mM. Assuming an apparent stability constant of 10$^{-5}$ for the Ca$^{2+}$-EGTA complex, the free Ca$^{2+}$ in the activating solution was 10$^{-9}$M (6). All reagents were obtained from the Sigma Chemical Co. (St. Louis, MO).

The composition of the solution used to partially extract protein subunits from the fiber segments was in most cases 20 mM EDTA, 50 mM KCl, 5 mM phosphate buffer, pH 7.0 (i.e., LC$_2$ extracting solution). TnC was isolated from rabbit skeletal muscle using the method of Greaser and Gergely (4) with the following modifications: (a) inclusion of 0.1 mM phenylmethylsulfonyl fluoride and 1% Triton X-100 in the washes of the muscle mince and (b) chromatography of troponin on DEAE cellulose. Total myosin light chains were removed from myosin using 4 M urea dissociation (16). The individual LC$_1$ and LC$_2$ preparations were obtained by chromatography on DEAE cellulose in 6 M urea containing 15 mM $\beta$-mercaptoethanol, at pH 6.0, using a 10 mM to 125 mM potassium phosphate gradient. The proteins were dialyzed versus 0.2 M KCl, 20 mM imidazole (pH 7.0), and were subsequently mixed with EGTA, MgCl$_2$, and ATP, and dialyzed to yield salt concentrations identical to those of the relaxing solution.

**Physiological Apparatus:** The mechanical system was similar to the one described previously (17). In the present study, the experimental troughs were made of stainless steel rather than polymer-coated aluminum. The scanning motor (Model 300 s Cambridge Technology, Cambridge, MA) that was used was controlled so that the mean sarcomere length was 2.7 μm. The temperature of the bathing solution was lowered to 15°C and control measurements of $V_{\text{max}}$ and tension ($P_o$) were made during maximal Ca-activation. The force transducer was connected to the motor arm and the force transducer. A segment of fiber between 2.0 and 5.0 mm in length was always present in the relaxing solution. The segments were in each case observed and photographed through a light microscope in order to obtain a measure of sarcomere length (17) both while relaxed and during Ca-activation. Initially, the end-to-end length of each segment was adjusted so that the mean sarcomere length was 2.7 μm.

**Experimental Protocol:** Following dissection, each muscle fiber was divided into three segments between 3 and 5 mm in length. These segments were immediately dissolved in sample buffer (described below) for subsequent analysis by PAGE. Another segment was mounted into the apparatus and was used to make mechanical measurements. The third segment was stretched to the length at which it was just taut and then tied at both of its ends to the wire extending from the motor arm. Thus, although mechanical measurements were not made on the third segment, this segment was exposed to the same bathing solutions as the second segment, on which mechanical measurements were made.

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was accomplished in 10% (vol/vol) glutaraldehyde solution for 30 mins, followed by overnight washing in deionized water, using a continuous water flow system. Staining was done in a freshly made ammonium silver solution, which was made by first adding 31.5 ml of 90 mM NaOH to 2.1 ml of cold 14.8 M NH₄OH, and then mixing in 6.0 ml of 1.14 M AgNO₃. The solution was brought to a final volume of 150 ml with deionized water. The gel was bathed in this solution for 3.25 min with mild agitation on a rotary shaking table (modified Model G-2; New Brunswick Scientific, Edison, NJ). The gel was then washed twice for 1.0 min in deionized water and developed in a freshly prepared solution of 0.005% (wt/vol) citric acid and 0.019% (wt/vol) formaldehyde. Development of the protein bands was stopped by transferring the gels to pure water, and the gel slabs were then dried. The slabs were scanned using a laser scanning densitometer (Model SL-504-XL; Bio-Med Instruments, Chicago, IL). The areas under the protein peaks were measured from the output of the integrator section of the densitometer. The relative amount of LC2 present in each segment was expressed as a fraction of the total alkali LCs present in the same segment (8). This ratio, LC2/(LC1 + LC3), was calculated as the area under the peak on the densitometric tracing corresponding to LC2 divided by the sum of the areas corresponding to LC1 and LC3. The silver stain was linear for the LC subunits in the range of fiber lengths that were used (Giulian, Moss, and Greaser, manuscript submitted for publication).

RESULTS

Mechanical Characteristics of Untreated Fiber Segments

The acceptability of a particular fiber segment for use in this study was judged primarily on the basis of striation pattern uniformity at rest and during maximal activation. The mean sarcomere length in the relaxed segments was 2.72 ± 0.08 μm (n = 68), as measured from light photomicrographs (Fig. 1 a). Maximal activation with Ca²⁺ usually resulted in a decrease in sarcomere length in the central portions of the segments (Fig. 1 b), due primarily to a slight yielding of the segment ends at the points of attachment (17). Activated segments in which sarcomere length was found to decrease by 10% or more of the control value were discarded, as were any segments in which gross striation non-uniformities were observed. Nearly 50% of all segments tried were rejected for these reasons.

The maximum Ca-activated tension developed by the fiber segments averaged 1.34 ± 0.21 kg/cm, which is in good agreement with the value of 1.61 ± 0.77 kg/cm reported by Julian et al. (8) for rabbit psoas fibers at 15°C. A mean Vmax of 2.48 ± 0.38 muscle lengths per second (ML/s) (n = 75) was measured in the fiber segments prior to extraction with EDTA and encompassed a range of 1.86–3.45 ML/s. Addition of 15 mM creatine-P₄ and creatine-phosphokinase (1 mg/ml) resulted in no significant change in either Vmax or isotropic tension in 11 fiber segments that were tested, indicating that at 15°C the ATP supplies within the segments were sufficient to sustain contraction.

Effects of EDTA Treatment on the Light Chain Composition of the Fiber Segments

Fiber segments that were freshly dissected from stored fiber bundles were found to have LC2/(LC1 + LC3) ratios that averaged 0.81 ± 0.08 (n = 16), and lay within the range 0.71–0.96 (see example, Fig. 2 a). A variety of solution protocols was employed in an attempt to optimize the conditions for removal of LC2 from the segments. By bathing segments in a solution containing 10 mM EDTA, 50 mM KCl, and 5 mM phosphate, pH 7.0 (23) at 37°C for periods of up to 120 min, it was possible to reduce the LC2/(LC1 + LC3) ratio to values as low as 0.07 (Fig. 2 b). At this temperature, the LC2/(LC1 + LC3) ratio was found to decrease by >50% even with a 15-min incubation in the EDTA-containing solution.

Bathing the segments at 30°C for 120 min in the LC2 extracting solution (see Materials and Methods) was found to reduce the LC2/(LC1 + LC3) ratio to values as low as 40% of control (also, see Moss et al. [18]). Increasing the duration of the treatment to 240 min had little additional effect upon this
ratio. However, by decreasing the duration to 15 min, it was possible to reduce the amount of LC₂ extracted, in that the value of LC₂/(LC₁ + LC₃) was found to be 0.72 ± 0.09. It is likely that TnC was also extracted by this treatment, since Ca²⁺-activated tension was found to be greatly reduced, as discussed below. The amount of TnC actually lost in each case was difficult to quantitate due to its relatively low staining intensity in the silver system.

Additional experiments were done in which segments were bathed for up to 120 min at 25°C in the LC₂ extracting solution. In these cases, only slight decreases in the amounts of LC₂ and TnC present were seen. The LC₂/(LC₁ + LC₃) ratio was in all cases within 10% of control values.

**Effects of EDTA Treatment on Resting Tension**

The extractions performed at 37°C were usually accompanied by large increases in the tensions exerted by the fibers while in relaxing solution (Fig. 3). These tensions were in some instances as great as 25% of the maximum Ca-activated tensions (P₀) developed by the same segments prior to treatment, though the relative amounts of tension exerted in relaxing solution varied greatly in different fiber segments. The molecular basis for such increases is not presently known. Resting tensions exerted by the segments following the extractions at 30°C were increased by small amounts for soak durations of 90 and 120 min but were not significantly different from control values for shorter soak times. The amount of resting tension did not exceed 10% of P₀ in any instance, and was found in most cases to be <5% of P₀. Extractions done at 25°C resulted in no significant changes in resting tension even after incubations as long as 120 min.

**Active Tension Development following EDTA Extraction**

The steady isometric tension developed by the EDTA-treated fiber segments was measured at 15°C in a solution containing maximally activating levels of Ca²⁺, i.e., pCa 5.49 (8). In the segments that had been bathed in EDTA at 37°C, large decreases in Ca-activated tension were observed. The magnitude of this decrease was not greatly influenced by the duration of the EDTA soak, though slightly lower active tensions were seen at longer times. After 60 min of EDTA treatment at this temperature, tension was found to range between 8 and 26% of control values. The observed decreases in tension were reversed only slightly by soaking the segments at 15°C in a relaxing solution containing 1 mg/ml of TnC (Fig. 4 a), even though TnC re-uptake by the segments was apparent (Fig. 2 c). Subsequent soaks in relaxing solution containing 0.5 mg/ml of LC₃ (Fig. 4 b) had no further effect on the tension developed by the segments. Re-uptake of LC₃ is demonstrated in the gel of Fig. 2 d. In no instance was the amount of tension recovery following the addition of these subunits >20% of the control value. The total tension developed by these segments (i.e., the sum of the resting and Ca-activated tensions), never exceeded 40% of P₀.

The losses in active tension that followed extractions with EDTA at 30°C (Fig. 5) were consistently smaller than those seen when the extractions were done at 37°C, though in every instance tension was found to be less than the value obtained prior to the EDTA treatment. Generally, longer duration soaks...
Addition of TnC to these segments usually, but not always, resulted in small increases in \( V_{max} \) relative to the same segments just following the EDTA extractions. Finally, recombination of LC2 into the segments (Fig. 7) resulted in increases in \( V_{max} \) to values near control. The level to which \( V_{max} \) recovered following LC2 addition appeared to be independent of the duration of the EDTA soak.

The Relationship between \( V_{max} \) and Light Chain Content

\( V_{max} \) is plotted, as percent control, in Fig. 9 vs. the \( LC_2/(LC_1 + LC_3) \) ratio obtained from gel analysis of the same fiber segments. This plot includes data only from segments in which the control value of \( LC_2/(LC_1 + LC_3) \) was greater than 0.70. This criterion was used in order to avoid possible errors in quantitation of the LC ratio due to ineffective staining of the gels with silver. A clear correlation between \( V_{max} \) and the \( LC_2/(LC_1 + LC_3) \) ratio is apparent for both the EDTA treated segments and following readdition of LC2, with \( V_{max} \) decreasing as the light chain ratio was reduced. However, the conclusion that there is a functional relationship between \( V_{max} \) and the LC content of a fiber segment does not necessarily follow from this data, since active tension is also greatly reduced by the EDTA treatments (Fig. 5). Thus, the decline in \( V_{max} \) following EDTA extraction may, at least in part, be attributable to the concomitant fall-off in tension. For example, the presence of a substantial internal load within skinned fibers, which has been suggested previously (24), might result in progressively decreasing \( V_{max} \) values as tension declined. Possible interactions between tension changes and EDTA treatment to alter \( V_{max} \) have been investigated for the fiber segments that were extracted for \( 30-120 \) min at \( 30^\circ C \). Under these conditions, EDTA treatment resulted in decreases in tension to \( 46 \pm 10\% \) control values and in \( V_{max} \) to a value that was \( 55 \pm 9\% \) of control (Fig. 10). Addition of TnC to the treated segments resulted in an increase in tension from \( 46 \pm 10\% \) to \( 80 \pm 12\% \) of control. The mean value of \( V_{max} \) on the other hand, remained virtually unchanged, though in individual instances, small increases in

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**FIGURE 5** Relative active tension versus the duration of EDTA treatment at \( 30^\circ C \). At each time point, the tension was measured just following the soak (diagonal cross-marking), and following the TnC (clear bars) and LC2 (stippled bars) recombinations. Tensions were in each case expressed as a percent of the active tension measured in the same fiber before treatment, and are reported here as the mean \( \pm 1 \) SD \( (n = 4-6) \).

**FIGURE 6** Slack test determinations of \( V_{max} \) prior to EDTA treatment (○) and following 60 min of extraction at \( 30^\circ C \) (○). The actual force records, with expanded scales, obtained under each condition are shown adjacent to each set of experimental points. \( V_{max} \) prior to treatment was \( 2.43 \) ML/s and following treatment was \( 1.66 \) ML/s. ML = 2.50 mm; sarcomere length = 2.57 \( \mu \)m. Fiber #11161.

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**V\(_{max}\) following EDTA Extraction**

Treatment of the fiber segments with EDTA resulted in a decrease in the measured value of \( V_{max} \) (see example, Fig. 6). EDTA extraction at \( 37^\circ C \) resulted in \( V_{max} \) values that in most segments ranged between 11 and 34\% of control values. In several additional segments, the shortening velocity was too low to be measured accurately. Recombinations of TnC and especially LC2 into these segments were not complete and were usually without effect with regard to \( V_{max} \), though in three instances recoveries of \( V_{max} \) to within 30–41% of control were observed. The \( 30^\circ C \) extraction in EDTA (Fig. 7) led to decreases in \( V_{max} \) that were dependent on the duration of the soak (Fig. 8). Small though significant decreases in \( V_{max} \) to values that were \( 75 \pm 6\% \) \((n = 4)\) of control, were observed following 15 min of extraction at this temperature; while at 120 min, \( V_{max} \) had decreased to \( 48 \pm 4\% \) \((n = 6)\) of control values. Addition of TnC to these segments usually, but not always,
FIGURE 7  SDS-polyacrylamide gels and corresponding densitometric scans of segments of the same fiber at different stages of the LC2 extraction protocol at 30°C. (a) An untreated control segment, LC2/(LC1 + LC3) = 0.83. (b) A segment following LC2 extraction and TnC readdition, LC2/(LC1 + LC3) = 0.61. (c) A segment following LC2 readdition, LC2/(LC1 + LC3) = 0.89. The abbreviations used are listed in the legend of Fig. 2.
$V_{\text{max}}$ of $<10\%$ were frequently observed. Subsequent addition of LC$_2$ to these segments resulted in a small, though significant ($P < 0.01$; paired Student's $t$ test), additional increase in the mean developed tension, to $91 \pm 13\%$ of the control values; however, $V_{\text{max}}$ was found to increase substantially, to $92 \pm 8\%$ of control. For these extraction conditions, then, the observed decreases in Ca-activated tension appear to be due mainly to the loss of TnC, with smaller additional decreases due to the partial extraction of LC$_{2\alpha}$, while alterations in $V_{\text{max}}$ resulted from reductions in the LC$_2$ content of the segments.

For further investigation of the relationships between the mechanical properties of the fiber segments and protein subunit composition, the order of TnC and LC$_2$ recombinations was reversed in several cases. Results from one fiber segment are shown in Fig. 11. In this particular case, the decline in $V_{\text{max}}$ following EDTA extractions was nearly completely reversed by readdition of LC$_{2\alpha}$, while at the same time active tension increased from $44\%$ of control immediately following LC$_2$ extraction to $53\%$ of control following LC$_2$ readdition. Subsequent addition of TnC to the fiber segment had little further effect on $V_{\text{max}}$ but resulted in virtually complete recovery of tension. Qualitatively similar results were obtained in three additional fiber segments.

**Effect of LC$_1$ Addition on the Mechanical Properties of EDTA-treated Segments**

Recent work (27) has shown that, at $37^\circ$C, solutions containing $10$ mM EDTA will partially extract the alkali light chains...
from skeletal muscle myosin. Thus, it seemed possible that EDTA extractions done at 30°C may remove small amounts of LC1 and/or LC2. Since LC1 is present in the LC2 addback solution (Fig. 4b), it is conceivable that the reversible decrease in Vmax seen following EDTA treatment may be related, at least in part, to the partial loss of alkali LCs. To test this possibility, several fiber segments, following the EDTA treatment at 30°C and the subsequent TnC recombination, were bathed in a relaxing solution containing purified LC1 (Fig. 4c) prior to the LC2 recombination. Following this treatment, no uptake of LC1 into the fiber segments was apparent from the gels of the segments, and there was no effect on either Vmax or isometric tension. Upon bathing the segment in relaxing solution containing LC2, Vmax was restored to approximately the control value. This result indicated that if some portion of the alkali light chains was lost from the segments during the EDTA treatment, the amount lost was not sufficient to affect the mechanical properties of the segments.

(Note: The ordinal intercept of the straight line fitted to the slack test data is a measure of the extension of series elastic elements during tension development [7]. Thus, in Fig. 11, the data obtained prior to EDTA treatment and that obtained following TnC readdition yielded greater intercepts that the data obtained following EDTA treatment and LC2 readdition. These differences are due to the greater tensions developed by the segment in the former two cases. This same argument applies to the observed difference in ordinal intercepts in Fig. 6.)

Addition of LC2 and TnC to Untreated Control Fibers

In several instances, untreated fibers were bathed in relaxing solution containing either TnC or LC2. This was done in order to determine whether the mechanical properties of this particular skinned fiber preparation were somehow limited by an incomplete complement of either of these protein subunits. Diminished amounts of these subunits could occur due to losses during storage in the glycerol-relax solution. Also, the relative amounts of the subunits present might vary between animals. In five fiber segments from two different rabbits, Vmax following the TnC soak was 101 ± 6% of the pre-soak value, while active tension was 100 ± 5% of control. In an additional five fiber segments, the LC2 soak resulted in tension and Vmax values that were 102 ± 5% and 102 ± 3%, respectively, of the control values. Thus, in no case was a significant difference found upon addition of LC2 and TnC to segments that had not been previously extracted with EDTA.

Effect of Trifluoperazine upon Mechanical Properties of EDTA-treated Fiber Segments

In light of the recent report by Guerriero et al. (5) that myosin LC kinase appears to be bound within the I-bands of myofibrils isolated from skeletal muscle, it was conceivable that some part of the observed mechanical changes following the LC2 extraction and readdition procedures were due to changes in myosin LC kinase activity, and hence in the degree of LC2 phosphorylation, in these fiber segments. As a test of this idea, a specific kinase inhibitor, trifluoperazine (TFP; Smith-Kline and French, Philadelphia, PA), was applied to the fiber segments in concentrations of up to 50 μM (10). TFP had no effect upon either tension or Vmax in the EDTA-treated segments during maximal Ca2+-activation or in the same segments following readdition of LC2. This result indicates that the mechanical effects of LC2 extraction and subsequent readdition of LC2 are unlikely to be due to differences in the levels of LC2 phosphorylation.

DISCUSSION

The main results of this study indicate clearly that the Vmax of maximally Ca-activated skinned skeletal muscle fibers decreases as the LC2 content of the fibers is reduced by extraction with EDTA. This effect of LC2 extraction can be reversed by readdition of LC2 to the fiber segments (18). The procedure used to remove LC2 also resulted in the dissociation of a significant amount of TnC from the segments, as reported previously (18), which mechanically was reflected in the much lower Ca-activated tension developed by the EDTA-treated fiber segments. Readdition of TnC to the segments resulted in the recovery of most but usually not all of the control tension. Subsequent recombination of LC2 into these fibers led to an additional smaller recovery of tension. A conclusion to be drawn from these results is that myosin LC2 in skeletal muscle is involved in the interaction between actin and myosin, and appears specifically to have roles in determining the kinetics and, to a lesser degree, the extent of this interaction.

Biochemical measurements have shown that the ATPase activity of myosin and regulated actin in the presence 10−6 M Ca2+ was reduced by about one-half following the extraction of ~1 mol LC2/mol myosin (21). These results are consistent with the effects on Vmax following of LC2 removal from skeletal muscle fibers reported here. The present study extends the earlier biochemical findings by indicating that the modulation of the actomyosin ATPase by LC2 occurs in a preparation in which the thick and thin filament lattices were maintained intact and the cross-bridges were under load during mechanical measurements, conditions that are difficult to simulate in solutions of actin and myosin.

The effect of LC2 removal upon Vmax appears to be unrelated to removal of alkali light chains by the EDTA treatment. Wikman-Coffelt et al. (27) have found that, at 37°C, 5–10% of the alkali light chains are removed following a 10-min incubation in a solution containing 10 mM EDTA. Wagner and Giniger (26) reported significant reductions in the actin-activated ATPase activities of myosins from which most of the alkali LCs had been removed, though it is unclear whether such reductions were the result of LC removal per se or instability of the alkali LC−deficient myosin. In the present study, addition of LC1 to TnC-treated, EDTA-extracted segments resulted in no significant changes in Vmax or tension. Recovery of Vmax in these segments occurred only after readdition of LC2. Thus, if alkali light chains were extracted in the EDTA solutions, the amounts removed were apparently insufficient to affect mechanical properties.

Extraction conditions similar to those of the present study have been used previously to remove so-called regulatory LCs from scallop myosin (9, 23). At 0°C, incubations in solutions containing 10 mM EDTA resulted in the loss of ~50% of the normal 2 mol regulatory LC/mol myosin (9). At the same time, the Ca regulatory mechanism for the interaction of scallop myosin with actin was lost. At higher temperatures, 25°C or 35°C depending on the species of scallop, treatment with 10 mM EDTA led to the complete dissociation of the regulatory LCs (3). The effect of the removal of these LCs on the actomyosin ATPase in the presence of Ca2+ were qualitatively similar to the effects on Vmax reported in the present study. Removal of the regulatory LC resulted in a decrease in the ATPase to ~20% of the value obtained for intact myosin.
Dependence of $V_{\text{max}}$ on LC2 Content

The correlation between relative $V_{\text{max}}$ and LC2 content of the fiber segments is striking (Fig. 9). Tension also decreased with EDTA treatment (Fig. 5), due mostly to the loss of TnC and in part to the removal of LC2. In some cases, tension in the EDTA-treated segments recovered to near the control value when TnC was added, with little or no change in $V_{\text{max}}$. Thus, there is no appreciable dependence of $V_{\text{max}}$ on isometric tension, at least following extractions at 30°C, and decreases in $V_{\text{max}}$ following EDTA treatment therefore appear to be causally related to the reduction in the LC2 content of the segments.

EDTA extractions at 37°C led to losses in both tension and $V_{\text{max}}$ that were largely irreversible upon the readdition of TnC with EDTA treatment (Fig. 5), due mostly to the loss of TnC to the small sample size, i.e., <0.1 μg total protein. A good indication of this ratio in EDTA-treated segments probably overestimate the amounts of LC2 present, the values of this ratio in EDTA-treated segments probably overestimate the amounts of LC2 present. The extraction procedure likely removes small amounts of the alkali light chains (27), which will tend to increase the calculated ratio. On the other hand, expression of the absolute amounts of LC2 present, as a function of the total protein, are not presently feasible due to the small sample size, i.e., <0.1 μg total protein. A good indication that substantial amounts of alkali light chains were not removed by this procedure is that the ratio of the areas under the LC4 and actin peaks changed little in most segments relative to control following the EDTA soak.

The role of LC2 in Skeletal Muscle Contraction

The effect of LC2 removal to alter $V_{\text{max}}$ indicates that this LC may modulate the interaction kinetics of actin and myosin. Biochemical evidence consistent with such a suggestion has only recently begun to emerge. Lehman (14) has reported that carefully prepared myofibrils, from which troponin and tropomyosin had been removed with low salt solutions, have an ATPase activity that is $\text{Ca}^{2+}$ sensitive. Furthermore, Pemrick (21) has reported that, in solutions of myosin and regulated actin present, the concentration of LCs present, the values of this ratio in EDTA-treated segments probably overestimate the amounts of LC2 present. The extraction procedure likely removes small amounts of the alkali light chains (27), which will tend to increase the calculated ratio. On the other hand, expression of the absolute amounts of LC2 present, as a function of the total protein, are not presently feasible due to the small sample size, i.e., <0.1 μg total protein. A good indication that substantial amounts of alkali light chains were not removed by this procedure is that the ratio of the areas under the LC4 and actin peaks changed little in most segments relative to control following the EDTA soak.