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Contraction characteristics and ATPase activity of skeletal muscle fibers in the presence of antibody to myosin subfragment 2

(antibody to subfragment 2/muscle fiber stiffness/force–velocity relation/ATPase activity in muscle)

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ABSTRACT **To investigate the role of the myosin hinge region in muscle contraction, we examined the contraction characteristics and Mg-ATPase activity of glycerinated muscle fibers prepared from rabbit psoas in the presence and absence of polyclonal antibody directed against the subfragment 2 (S-2) region of myosin. The antibody-induced reduction of Ca^{2+}-activated isometric force was always accompanied by a parallel decrease of muscle fiber stiffness, so that the stiffness versus force relation remained unchanged by the antibody treatment. Force–velocity relations of the fibers, obtained by applying ramp decreases in force at steady isometric forces, indicated that the antibody had no effect on maximum shortening velocity or on the shape of force–velocity curves. Simultaneous measurements of Mg-ATPase activity and Ca^{2+}-activated force showed that Mg-ATPase activity of the fibers remained unchanged despite the antibody-induced reduction of isometric force even to zero. These results indicate that when anti-S-2 antibody attaches to the S-2 region of myosin molecules, their heads still hydrolyze ATP but no longer contribute to both force generation and muscle fiber stiffness.**

Muscle contraction results from alternate formation and breaking of cross-links between the myosin head (subfragment 1; S-1), extending from the thick filament and a neighboring thin filament (1, 2). The energy for contraction is supplied by ATP hydrolysis. Since the ATPase activity and actin binding site are localized in the S-1 region of myosin, S-1 is commonly believed to play a major role in muscle contraction. Recent *in vitro* motility assays have shown that S-1 alone is sufficient to produce force and move actin filaments (3, 4), but it is not clear whether the ATP-dependent actin–myosin sliding observed in the assay systems is the same as that actually taking place in living muscle.

On the other hand, it has been proposed that melting and shortening in the proteolytically sensitive hinge region lying between the short subfragment 2 (S-2) and light meromyosin segments of the myosin tail contribute to force generation in muscle (5–8). In support of this hypothesis, polyclonal anti-S-2 antibody has been shown to reduce Ca^{2+}-activated isometric force in glycerinated skeletal muscle fibers, while ATPase activity of the fibers and the initial unloaded shortening velocity of isolated myofibrils undergo little change (9, 10). More recently, it has been shown that, in the presence of antibody directed against a 20-amino acid peptide segment within the hinge region of cardiac myosin, movement of actin filaments in an *in vitro* motility assay is suppressed, while ATPase activity of myofibrils and purified S-1 remained unchanged (11).

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The present experiments were undertaken to further investigate the effect of anti-S-2 antibody on the contraction characteristics and ATPase activity of glycerinated muscle fibers prepared from rabbit psoas. It will be shown that anti-S-2 antibody produces a parallel decrease of muscle fiber stiffness and Ca^{2+}-activated isometric force development, while it has no effect on the maximum shortening velocity, the shape of force–velocity (*P–V*) curves, and Mg-ATPase activity of the fibers. Our results indicate that Ca^{2+}-activated force and muscle fiber stiffness are uncoupled from Mg-ATPase activity in the antibody-treated fibers, implying an essential role for the myosin hinge region in muscle contraction.

MATERIALS AND METHODS

Muscle Fiber Preparation and Experimental Setup. Strips of rabbit psoas muscle (diameter, ≈2 mm) were dissected and tied to a glass rod and kept in a 50% (vol/vol) glycerol solution containing 50 mM KCl, 4 mM $MgCl_2$, 4 mM EGTA, and 20 mM Tris maleate (pH 7.0) at 0°C overnight. After a change of the solution, they were stored at −20°C for 1–3 weeks before use. Single muscle fibers (diameter, 40–80 μm) were carefully dissected from the glycerinated muscle strips and mounted horizontally in an experimental chamber (0.1 ml) between a force transducer (with a compliance of 0.1 mm/N and a resonant frequency of 5 kHz; Akers, Horten, Norway; AE801) and a servomotor (controlled by JCCX101 control unit; General Scanning, Watertown, MA; G100PD) by glueing both ends to the extension of the transducer and the servomotor with collodion. The servomotor contained a displacement transducer (differential capacitor) sensing the position of the motor arm. The compliance of the motor arm (length, 10 mm) at the point of attachment of the fiber was ≈0.2 mm/N when the servomotor system was operating in the length clamp mode. The sarcomere length of the fiber was measured by use of optical diffraction with HeNe laser light. Unless otherwise stated, the fiber was kept at its slack length (≈3 mm) with sarcomere lengths of 2.2–2.3 μm. Relaxing solution contained 125 mM KCl, 20 mM Pipes (pH 7.0), 4 mM $MgCl_2$, and 4 mM EGTA. Contracting solution was prepared by adding 4 mM $CaCl_2$ to relaxing solution. All experiments were performed at room temperature (18°C–20°C).

Stiffness Measurement. Muscle fiber stiffness was continuously determined by applying small sinusoidal length changes (1 kHz) of fixed peak-to-peak amplitude (≈0.1% of fiber length) with the servomotor and measuring the amplitude of resulting force changes. The sinusoidal voltages were

Abbreviation: S-2, myosin subfragment 2.

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produced with a waveform generator (Wavetek, San Diego; model 164). The force signal consisted of muscle fiber force and a superimposed sinusoidal force component. The sinusoidal component was separated from muscle fiber force with a high-pass filter with a cut-off frequency of 20 Hz, and its amplitude was measured with an absolute value converter constructed by one of us (T. Kobayashi). The force and stiffness changes were simultaneously recorded in a digital wave memory of a digital oscilloscope (Nicolet; model 3091), transferred to a microcomputer (IBM; model PC-AT) for data processing and analysis, and displayed on an *X–Y* plotter (Hewlett–Packard; model 7470A). The values of force and stiffness in the presence of antibody were expressed relative to the control values.

Determination of Force–Velocity Relation. The servomotor system operated either in the length control mode or in the force control mode (12). First, the system was in the length control (length clamp) mode so that the fiber contracted isometrically when it was maximally activated in contracting solution. After the fiber developed steady isometric force, the servomotor system was switched to the force control mode, and a ramp decrease in force (= load) from the steady force to zero was applied by feeding a ramp force decrease signal from the waveform generator to the servomotor system. The resulting shortening of the fiber was recorded in the digital wave memory together with the ramp decrease in force, and force–velocity (*P–V*) relation was obtained and displayed on the *X–Y* plotter after data processing with the microcomputer. The rate of force decrease was ≈5.5 mN/s.

Measurement of ATPase Activity During Force Development. Mg-ATPase activity of the fibers during Ca^{2+}-activated isometric force development was recorded by the decrease of NADH during cleavage of ATP (13–15). A small fiber bundle consisting of two or three muscle fibers was mounted horizontally between the force transducer and a stainless-steel rod in the sample compartment (≈0.36 ml) of a dual-wavelength spectrophotometer (model 156; Hitachi) with a sample monochrometer at 340 nm and a reference monochrometer at 400 nm, so that the decrease of NADH was measured from the difference in absorbance between 340 and 400 nm. To both relaxing and contracting solutions, 0.25 mM NADH/1.25 mM phospho*enol*pyruvate/pyruvate kinase (50 units/ml)/lactic dehydrogenase (50 units/ml)/10 mM NaN_3/50 μM quercetin/oligomycin (1 μg/ml) was added. The light path length through the sample compartment was 10 mm, and solutions in the compartment were constantly stirred with a magnetic stirrer (16). The outputs of the spectrophotometer and the force transducer were fed to the digital oscilloscope and displayed on the *X–Y* plotter.

Preparation of Anti-S-2 Antibody. Polyclonal antibody directed against the long S-2 region of rabbit psoas muscle myosin was produced in goat (9). Long S-2 (61 K) was prepared and purified as described (17).

RESULTS

Effect of Anti-S-2 Antibody on Muscle Fiber Stiffness and Isometric Force. Single glycerinated fibers were maximally activated (each time for ≈40 s) with Ca^{2+} (≈0.1 mM) in contracting solution before and 30, 60, and 90 min after administration of anti-S-2 antibody. In each experiment, the fiber was first activated to contract without antibody and made to relax with relaxing solution containing anti-S-2 antibody (1.5 mg/ml). Then the fiber was repeatedly activated and relaxed by alternate application of contracting and relaxing solutions, both containing the same concentration of the antibody. Typical muscle fiber stiffness and isometric force records in response to contracting solution are shown in Fig. 1 (*Insets*). After each application of contracting solution, the fiber could be made to relax completely with relaxing solution, so that both stiffness and force records always started to change from "zero" baselines. Muscle fiber stiffness and isometric force always increased in parallel with each other, while steady isometric force attained was markedly reduced with time after administration of the antibody.

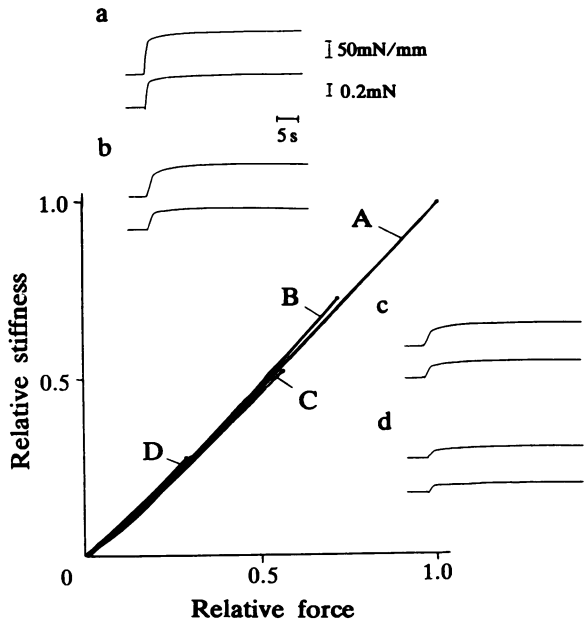

FIG. 1. Relation between muscle fiber stiffness and isometric force during Ca^{2+}-activated force development of a single muscle fiber before (curve A) and 30 (curve B), 60 (curve C), and 90 (curve D) min after administration of anti-S-2 antibody (1.5 mg/ml). Both stiffness and force are expressed relative to control values in the absence of the antibody. (*Insets*) Stiffness versus force curves A, B, C, and D were obtained from stiffness (upper traces) and force (lower traces) records a, b, c, and d, respectively.

As shown in Fig. 1, the stiffness versus force relation during isometric force development was almost linear and did not change appreciably in the presence of the antibody. Fig. 2 summarizes the stiffness versus steady force relation obtained from seven different fibers. The data points fall on the straight line starting from the origin to the control data point, indicating that muscle fiber stiffness and isometric force decrease in parallel with time in the presence of the antibody, while the stiffness versus force relation remains unchanged.

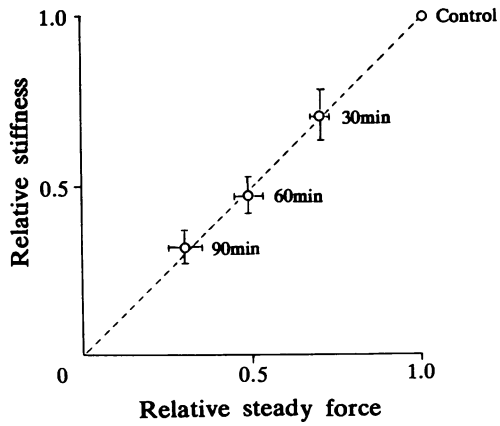

FIG. 2. Stiffness versus force relation at steady Ca^{2+}-activated isometric forces of a single fiber before (control) and 30, 60, and 90 min after administration of anti-S-2 antibody (1.5 mg/ml). Each data point represents the mean of seven different experiments with different fibers. Vertical and horizontal bars indicate SD of stiffness and of force, respectively.

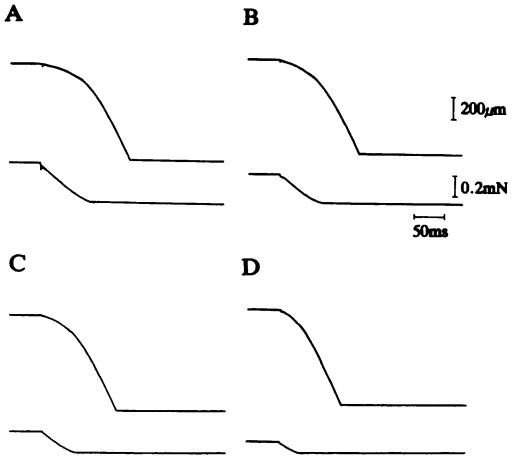

FIG. 3. Changes of fiber length (upper traces) and force (lower traces) of a single fiber in response to ramp decreases in force applied at steady Ca^{2+}-activated forces before (*A*) and 30 (*B*), 60 (*C*), and 90 (*D*) min after administration of anti-S-2 antibody (1.5 mg/ml).

Effect of Anti-S-2 Antibody on Force–Velocity Relation. The *P–V* relations in maximally activated fibers were determined in the absence and presence of antibody by applying ramp decreases in force from steady isometric forces to zero. Typical fiber length and force records in response to applied ramp force decrease are shown in Fig. 3. Since the force (= load) on the fiber was continuously changing with time, shortening velocity was determined by averaging the first-time derivative of fiber length record for each consecutive time segment (duration, 1 ms) during the course of fiber shortening (12). As shown in Fig. 4*A*, the *P–V* curves thus obtained at various levels of steady isometric force were double hyperbolic in shape as with the *P–V* curve of single frog muscle fibers (12, 18), and the maximum shortening velocity remained unchanged in spite of the marked antibody-induced reduction of steady isometric force to <30% of control value. The *P–V* curves were found to be identical in shape when velocities were replotted against forces expressed relative to their respective steady isometric forces (Fig. 4*B*), indicating that the *P–V* curves were scaled in proportion to steady isometric forces at which ramp force decreases were applied.

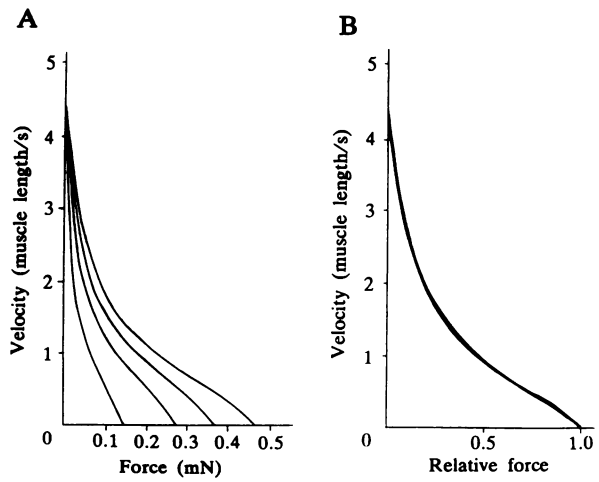

FIG. 4. Effect of anti-S-2 antibody on *P–V* relation in a Ca^{2+}-activated single fiber. (*A*) *P–V* curves obtained before (control) and 30, 60, and 90 min after administration of anti-S-2 antibody. Both velocities and forces are expressed in absolute values. Note that maximum shortening velocity remains unchanged in spite of marked reduction of steady isometric force. (*B*) The same *P–V* curves in which forces are expressed relative to their respective steady initial forces. Note that the curves are identical in shape. The curves were obtained from the results shown in Fig. 3.

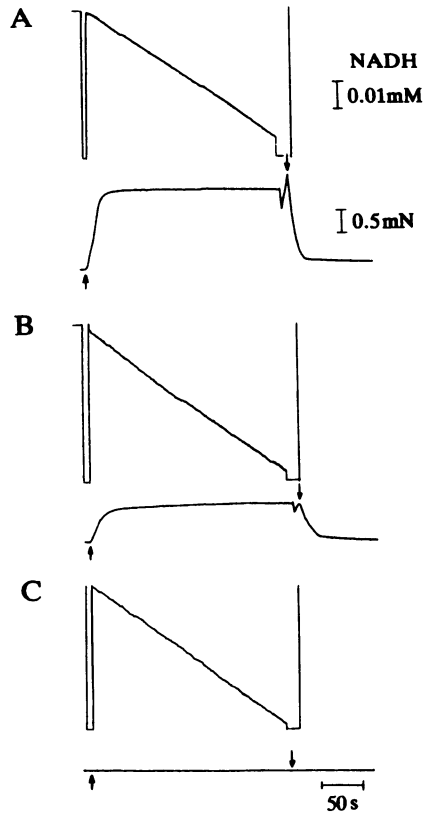

FIG. 5. Simultaneous recordings of Mg-ATPase activity (upper traces) and Ca^{2+}-activated isometric force development (lower traces) of a small fiber bundle consisting of three fibers before (*A*), and 100 (*B*) and 150 (*C*) min after administration of anti-S-2 antibody (1.5 mg/ml). Note that the slope of ATPase records does not change appreciably, even when Ca^{2+}-activated force development is reduced to zero (*C*). Decrease of ATPase trace shows decrease of NADH absorbance. Times of application of contracting solution and relaxing solution are indicated by upward and downward arrows, respectively.

Effect of Anti-S-2 Antibody on ATPase Activity During Isometric Force Development. Typical examples of simultaneous recordings of Mg-ATPase activity and isometric force development of Ca^{2+}-activated fibers are presented in Fig. 5. In both the absence and presence of anti-S-2 antibody, Mg-ATPase activity of relaxed fibers was very small and was not significantly different from the rate of spontaneous decrease of NADH concentration in the sample compartment without the fibers (≈0.02 μM/s) (16). Therefore, the result that the slope of ATPase records showed no appreciable changes when Ca^{2+}-activated force development was reduced even to zero indicates no appreciable effect of anti-S-2 antibody on Mg-ATPase of Ca^{2+}-activated fibers. Similar results were obtained with five different fiber bundles.

A few experiments were also performed in which the fibers were activated in the absence of the antibody at various initial lengths. In agreement with a previous report (19), Mg-ATPase of the fibers decreased roughly in proportion to the decrease of isometric force development as a result of decreased amount of overlap between the thick and thin filaments.

DISCUSSION

The present experiments have shown how anti-S-2 antibody affects contraction characteristics of glycerinated single fibers. The antibody-induced reduction of Ca^{2+}-activated iso-

metric force development was always accompanied by a parallel decrease of muscle fiber stiffness (Fig. 1), so that the stiffness versus force relation did not change with the antibody not only at steady isometric forces but also during the course of force development (Figs. 1 and 2). In the contraction model of Huxley (1), muscle fiber stiffness is a measure of the number of myosin heads attached to actin on the thin filament at the time of stiffness measurement. On this basis, the time-dependent reduction of force development in the presence of the antibody may be due to a progressive decrease in the number of myosin heads involved in isometric force generation. This implies that, if the antibody attaches to the S-2 region of a myosin molecule, its heads can no longer contribute to both force generation and muscle fiber stiffness.

The idea described above is consistent with the result that the maximum shortening velocity remains unchanged in the presence of the antibody despite the marked reduction of isometric force (Fig. 5). This follows since the maximum shortening velocity is not influenced by a decrease in the number of myosin heads interacting with actin (1, 20), provided internal resistance against actin–myosin sliding remains unchanged within a muscle fiber. The shape of the *P–V* curve reflects kinetic properties of cyclic actin–myosin interaction (1, 16). The *P–V* curves, which were scaled in proportion to steady isometric forces in the presence of the antibody (Fig. 4), can also be taken to indicate that only "native" myosin molecules are involved in force generation and shortening. Thus, the present *P–V* data in the presence and absence of the antibody indicate that the heads of myosin molecules, with the antibodies attached to their S-2 region, neither contribute to muscle fiber stiffness nor provide any appreciable internal resistance against Ca^{2+}-activated actin–myosin sliding.

In the present study, we have simultaneously recorded Mg-ATPase activity and Ca^{2+}-activated isometric force development, and we have shown that the ATPase activity of the fibers remained virtually unchanged even when Ca^{2+}-activated force development was reduced to zero in the presence of the antibody (Fig. 5). This result is in accord with the previous reports that anti-S-2 (9) and anti-hinge (11) antibodies have little or no effect on the ATPase activity of skeletal and cardiac muscle myosins. The antibody-induced dissociation of Ca^{2+}-activated force generation from Mg-ATPase activity of the fibers implies, together with the stiffness and *P–V* data, that the heads of myosin molecules, with anti-S-2 antibody attached to the S-2 region, still hydrolyze ATP but no longer contribute to both Ca^{2+}-activated force generation and muscle fiber stiffness.

In this connection, it is of great interest that anti-S-2 antibody causes uncoupling of both Ca^{2+}-activated force generation and muscle fiber stiffness from Mg-ATPase activity. According to current biochemical schemes (21, 22), muscle contraction is produced by actin–myosin interactions, which include both weak and strong binding states. A question, which arises at present based on the biochemical schemes, is how antibody attached to the S-2 region of a myosin molecule can exert an allosteric influence on its two head regions, so that their ATPase reactions proceed without involving "strong" actin–myosin binding, which can be detected by the stiffness measurement.

In summary, it now seems clear that the myosin hinge plays an essential role in muscle contraction, and much more attention should be paid to the function of this region as well as its long-range effect on the mechanochemical properties of the myosin head.

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