Two Rigor States in Skinned Crayfish Single Muscle Fibers

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ABSTRACT We studied the tension and stiffness of crayfish skinned single muscle fibers during and after the induction of rigor by removal of MgATP (substrate). We found that the rigor state is not unique but depends on the condition of the muscle before rigor. Fibers induced into rigor with a minimum of activation (low rigor) develop a small tension and moderate stiffness, while those entering rigor during maximum activation (high rigor) maintain near peak tension (80%) and develop a high stiffness. These rigor states are insensitive to Ca addition or deletion but they are partially interconvertible by length change. Stiffness changes when the rigor muscle length is varied, a condition in which the number of attached cross-bridges cannot change, and high-rigor muscle becomes less stiff than low-rigor muscle when the former is brought to the same tension by length release. The sensitivity of low, high, or length-released high-rigor muscles to trace substrate concentration (<1 μM) differs, and rigor at lower strain is more susceptible to substrate.

INTRODUCTION

In the sliding filament model of skeletal muscle actin filaments are connected in series with myosin filaments by labile cross-links which in some as yet unknown way transduce chemical energy into a relative sliding of the filaments (for review, see A. F. Huxley, 1974). In active muscle myosin cross-bridges cycle through several states. For simplicity we may define three states: "broken" bridges or the dissociated form of actin and myosin, force-generating bridges which are ready to or are discharging the work, and rigor bridges (Lymn and Taylor, 1971) which are a product of the energy transduction step. When substrate (MgATP) is present, it dissociates the rigor bridges. It is, of course, possible to define more states for the cross-bridges on biochemical grounds (Lymn and Taylor, 1971; Eisenberg and Kielley, 1973). However, some of the states can be lumped together to give fewer states (e.g., see Julian et al., 1974) which are readily identifiable by mechanical measurements.

One can prohibit the dissociation step by deletion of the substrate which results in the accumulation of the cross-bridges in the rigor complex. This provides a good condition for mechanical studies of a single cross-bridge state, because in rigor little transition to other states is expected (Dos Remedios et al., 1972; Heinl et al., 1974) when the length of the muscle is changed, and because

all cross-bridges are believed to be in the "angled" configuration (Reedy et al., 1965; Reedy, 1968; H. E. Huxley, 1968). In this report we focus on the rigor complex induced in skinned fibers and report its characteristics in terms of muscle tension and stiffness when (*a*) the path to substrate deletion is varied, (*b*) the length of the muscle fiber is changed in rigor, and (*c*) when incremental amounts of substrate are added. Preliminary results were reported earlier (Kawai, 1972).

MATERIALS AND METHODS

Muscle Fiber Preparation

Single muscle fibers were isolated from the extensor or flexor muscles of crayfish (genus *Orconectes* or *Procambarus*) walking legs and placed in skinning solution (Table I), and the sarcolemma was removed mechanically (Reuben et al., 1971). One end of the fiber was attached by a Lucite clamp to the length driver, and the other end attached by a second clamp to a strain gauge mounted on a Narishige micromanipulator (Narishige, Tokyo, Japan) to adjust the gross length of the muscle fiber (Fig. 1). The presence of tendon did not affect the stiffness measurement since the tendon of this preparation is very rigid. The muscle was stretched by about 10% above the slack length and this generally achieved a sarcomere length of 7.0–8.2 μm (determined by optical diffraction; Kawai and Kuntz, 1973) which corresponds to the peak of the active length-tension diagram (April, 1969). Usually resting tension and stiffness at this sarcomere length are minimal in freshly skinned fibers (Kawai and Brandt, 1973). A dissecting microscope (Nikon Inc., Instrument Group, Garden City, N. Y.) was mounted over the muscle preparation for visual inspection, and no structural alterations were observed in any of the rigor states we studied. The muscle fiber was placed in an experimental chamber containing a bathing solution which was constantly stirred and maintained at 20 ± 0.5°C by either cooling or heating. Since the diameter was measured with an ocular micrometer in the dissecting microscope after skinning, it is overestimated in comparison to intact fibers because the fiber swells upon skinning (April et al., 1971). P_o induced by solution A (Table I) was used for tension normalization in a conventional manner and its mean value was 1.14 ± 0.11 × 10^6 dyn/cm^2 (SEM, N = 19) for the first contracture.

Solutions

Table I lists the composition of the solutions used in the present experiments. Na_2H_2ATP, H_4EDTA (ethylene diamine-N,N,N′,N′-tetraacetic acid), H_4EGTA (eth-

TABLE I

SOLUTIONS USED IN THE CURRENT REPORT (TOTAL CONCENTRATION)

Application	Symbol	Ca	Mg	ATP	EGTA	EDTA	Potassium propionate	Imidazole (pH 7.00)
		mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
Relaxing and skinning	R		1.12	2	5		173	5
Washing	W		1.12	2			189	5
Activating	A	5	1.02	5.2	5		161	5
KP wash	KP						200	5
Rigor	EDTA					10	170	5
Rigor	EGTA				5		185	5
pS series			Adjusted	5		3.3	160	5

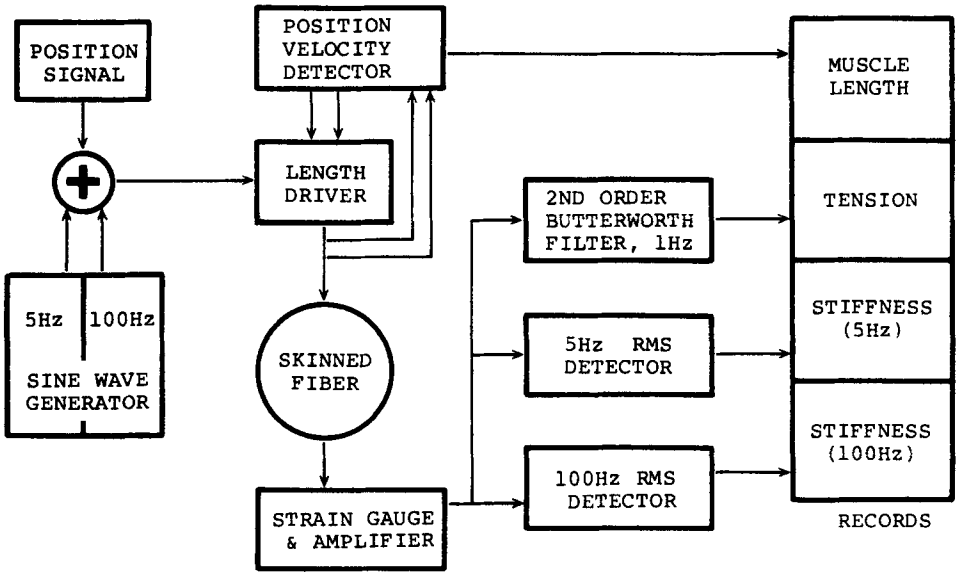

FIGURE 1. Schematic diagram of the experimental arrangement. The muscle length is commanded by the sum of two sine waves and a position signal via a servo-controlled length driver. Tension is detected by a Bionix F-100 strain gauge and its output is sent through a second-order Butterworth (low-pass) filter (cutoff frequency: 1 Hz) to a recorder. To obtain the stiffness information it is also sent to two rms detectors which include fourth order band-pass filters (5 Hz, 100 Hz; response time: 1 s, 50 ms, respectively), absolute value circuits, and second-order Butterworth filters. The tension and 5 Hz-stiffness signals are recorded on the two-channel Clevite Brush pen recorder (Mark 280), and the muscle length and 100 Hz-stiffness signals are read off directly from voltmeters. The signals of tension and stiffness are filtered appropriately so that the test oscillations are not superimposed on the records (Figs. 2, 3 A).

ylene glycol-*bis*-(β-amino-ethyl ether)N,N′-tetraacetic acid) were brought from Sigma Chemical Co., St. Louis, Mo. and $CaCO_3$, Mg acetate, propionic acid, potassium hydroxide, imidazole from Fisher Scientific Co., Pittsburgh, Pa. The pH of all solutions was 7.00 ± 0.02 and was buffered with 5 mM imidazole. Ionic strength was adjusted to 210 ± 10 mM by addition or deletion of potassium propionate. The concentrations of several ionic species, including pCa and pS (substrate)[1] were calculated after solving the multiple equilibria of two metals (Ca, Mg) and two ligands (EGTA or EDTA, ATP) by using the apparent association constants as follows (log values at pH 7.00): CaEDTA 7.38, CaEGTA 6.28, CaATP 3.70, MgEDTA 5.37, MgATP 4.00.

Length Driver

The length driver is constructed from two high compliance audio speakers (5-in diameter) and a position detector (200 DC-B Schaevitz Engineering, Pennsauken, N. J.) coupled by a rod. One speaker is used to drive the rod and the other to detect its velocity. The velocity signal is integrated to give a position signal at high frequencies because the

[1] pCa represents $-\log [Ca^{++}]$ where $[Ca^{++}]$ is the molar concentration of ionized Ca. Likewise pS represents $-\log [MgATP^{=}]$ where $[MgATP^{=}]$ is the concentration of Mg-chelated ATP.

position detector is limited in its band width. Both position and velocity signals are fed back to the driving speaker to improve frequency response (300 Hz) and linearity. The length of the muscle fiber was constantly oscillated at 5 Hz with an amplitude of 0.1–0.2% (peak to peak) of the total fiber length (L_o). Since 5 Hz may not be fast enough for significant stiffness measurements, a 100 Hz sine wave of the same amplitude was occasionally superimposed to allow simultaneous measurements at 5 Hz and 100 Hz. The constant oscillation at this amplitude did not affect the steady-state tension significantly.

Tension and Stiffness Detection

The signaling and detection system is depicted in Fig. 1. The tension is sensed by a two-element strain gauge (F-100 of Bionix, El Cerrito, Calif.) and a Clevite Brush carrier amplifier (Clevite Brush Instruments, Cleveland, Ohio). Including the Lucite clamps and the length driver, the overall compliance of the system was 8 nm/dyn. The 5 Hz and 100 Hz components of tension are separated by appropriate band-pass filters and their root mean square (rms) values detected. Since the band-pass filters were sufficiently narrow the two frequency signals did not interfere at all, and noise due to stirring or 60 Hz did not interfere either. Phase information was not collected primarily because the phase shift was constant for rigor conditions examined in the current report. The ratio of rms values of tension to length is taken as stiffness.

RESULTS

Rigor States

There appear to be at least two distinctive rigor states depending on whether the fiber is fully active before and during substrate removal. The muscle fiber used for obtaining the records in Fig. 2 A was in relaxing solution initially and then it was washed by 10 mM EDTA several times to reduce MgATP concentration. A small tension (10% P_o) developed and a slow and moderate increase in stiffness was observed. We term this the low-rigor state. The time to develop full rigor stiffness is 1.36 min on the average, but its variation is large (1.36 ± 0.75, SD, N = 90) and ranges from 0.5 to 3 min. Increasing EDTA concentration to 66 mM does not make an appreciable change in the time course or final level of rigor tension and stiffness. The muscle of Fig. 2 A was minimally activated as it went into rigor because the Ca concentration was kept low (pCa >9) and the fiber was not exposed for long to an intermediate substrate concentration in which it could develop tension (Reuben et al., 1971).

The fiber used to obtain the data in Fig. 2 B was first activated by solution A (pCa 5, pS 3), and then washed repeatedly with KP-rigor solution free of chelating agents for Mg or Ca. The addition of Ca to the KP-rigor solution makes no difference to the subsequent time course or steady level of rigor tension and stiffness, thus the muscle is fully activated with Ca as rigor is induced. The stiffness quickly increased and the tension decreased about 20% in 1 min as the fiber went into rigor. We term this the high-rigor state. The stiffness change was reversed as the activating solution was added again (Fig. 2 C). After the high-rigor state is fully developed, treatment of the fiber with Ca or Mg chelaters hardly affects tension or stiffness (Fig. 2 B). In both high and low rigor, tension slowly declines (see also Kuhn et al., 1972) while stiffness remains constant. Since the stiffness of the high rigor is reproducible and the largest for

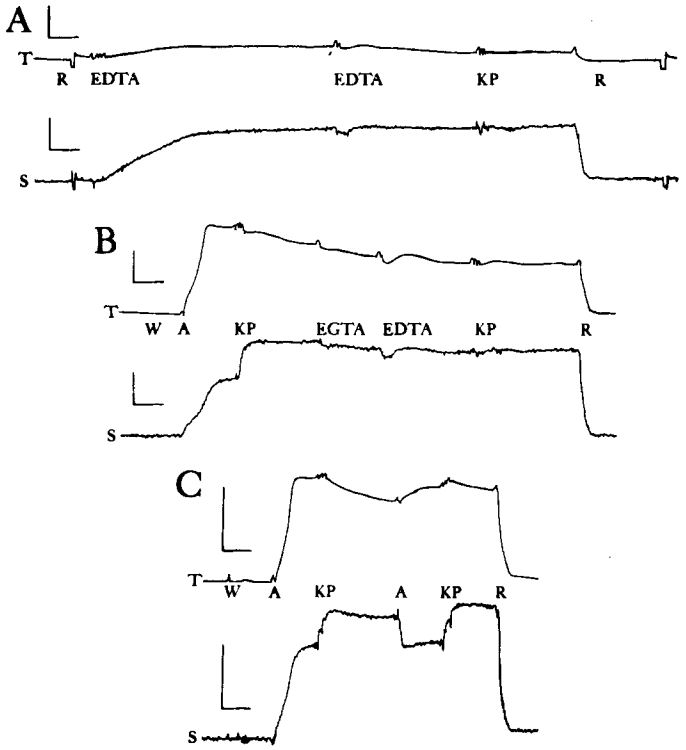

FIGURE 2. Records showing time courses of tension (T) and 5 Hz-stiffness (S) during initiation and maintenance of rigor. Each artifact on tension records corresponds with a solution change as noted (see Table I for solution composition). In A, B, an occasional slow wave after the solution change represents a temperature transient which slightly alters the length of the Lucite clamps. A, low rigor. Muscle length was released by 0.5 mm before and after rigor to find the tension base line. Note that after rigor is established, tension and stiffness are unaffected by further EDTA or potassium propionate (KP) washes. B, High rigor of the same preparation (1/14/74). After rigor is established stiffness is unaffected by further EGTA, EDTA, or KP washes, although tension declines slowly regardless of solution changes. C, showing reversibility in high-rigor and active muscle. Preparation of 7/23/73. Calibration bars: tension 200 dyn, stiffness 10^5 dyn/cm, and time 30 s. Traced from original records.

all conditions of skinned fibers, it is used to normalize other stiffness data. The corresponding Young's elastic modulus (Y_o) of the high-rigor muscle is $1.08 \pm 0.20 \times 10^8$ dyn/cm^2 (SEM, $N = 7$, initial high rigor) when measured at 5 Hz. Stiffness is nearly independent of frequency, and both amplitude and phase (~5°) of the complex stiffness (tension: length, see Kawai and Brandt, 1975; Kawai et al., 1976)[2] are approximately constant in the range of 0.25–133 Hz. More precisely, amplitude is linearly related to the *log* of frequency, and the

[2] Kawai, M., P. W. Brandt, and M. Orentlicher. 1976. The dependence of energy transduction in skeletal muscle on the time in tension. Manuscript in preparation.

ratio of stiffness at 100 Hz to that at 5 Hz is 1.15 ± 0.04 (SD, N = 11). This is the same as saying that there is no detectable first-order transition in rigor muscle in response to length change, which is consistent with the observation by Heinl et al. (1974), and it supports the conclusion of Dos Remedios et al. (1972) that myosin heads do not undergo passive rotation.

It is possible to produce rigor with tension and stiffness values intermediate between low and high rigor as is summarized in Table II. The ratios of tension to stiffness for these various rigors fall into two classes: if the rigor is induced from relaxed muscle the ratio is about 0.2, whereas if it is induced from active muscle the ratio is about 0.8. The ratio is calculated from values normalized to P_o and Y_o, and since $P_o/Y_o \simeq 0.01$ the ratio approximately represents the quick length release (in percent L_o) required to bring the tension to zero after extrapolation. At sarcomere length of 7.5 μm, a release of 75 Å or 300 Å per half-sarcomere is required to bring low- or high-rigor tension, respectively, to zero on the average.

Large External Length Displacements

It might be supposed that strain in cross-bridges would be different between low and high rigor simply because the muscle fiber is in a different stress. For this reason the muscle length was changed externally to see if elimination of stress decreases the apparent difference between two rigors. Fig. 3 A is a recording of the tension and stiffness of a high-rigor muscle as its length is released and it is then restretched in a stepwise manner. Both tension and stiffness drop simultaneously to length release, and they go through a small and slow transient even in the EDTA-rigor solution where no substrate is available. Similar transients were observed in the other rigor conditions and they were independent of rigor solutions (KP, EGTA, or EDTA). When the fiber is stretched again, tension and stiffness increase but along a different path (Fig. 3 B,C). This hysterysis disappears when the stiffness is plotted against tension (Fig. 3 D). From this plot it is clear that at the same tension, low rigor is stiffer than high rigor.

One other aspect of Fig. 3 D is that stiffness is roughly proportional to tension for muscle length decreases up to 2.3% L_o. This adds further importance to the ratio of stiffness to tension for the various rigors (Table II), and if the ratio is the same for two rigors, muscle length can be adjusted to match both tension and

TABLE II

TENSION AND STIFFNESS FOR VARIOUS RIGOR STATES

Prerigor condition	Procedure to rigor	Tension (% P_o)	Stiffness, 5 Hz (% high rigor)	Tension/stiffness	Comment
R	EDTA	10±5 (18)	56±11 (18)	0.18±0.10 (18)	Low rigor
R	EGTA	9 (2)	61 (2)	0.14 (2)	
R	KP	13±4 (4)	68±11 (3)	0.20±0.05 (3)	
A	EDTA	73±8 (4)			
A	EGTA	38 (1)	50 (1)	0.76 (1)	
A	KP	80±10 (53)	100 (Ref.)	0.80±0.10 (53)	High rigor

Tension is normalized to P_o (active tension with solution A), and stiffness to that of the high-rigor muscle. For symbols in columns 1 and 2, see solution Table I. Standard deviation is shown after (±) and the number of data points is in parentheses. Data of 1/73–1/74.

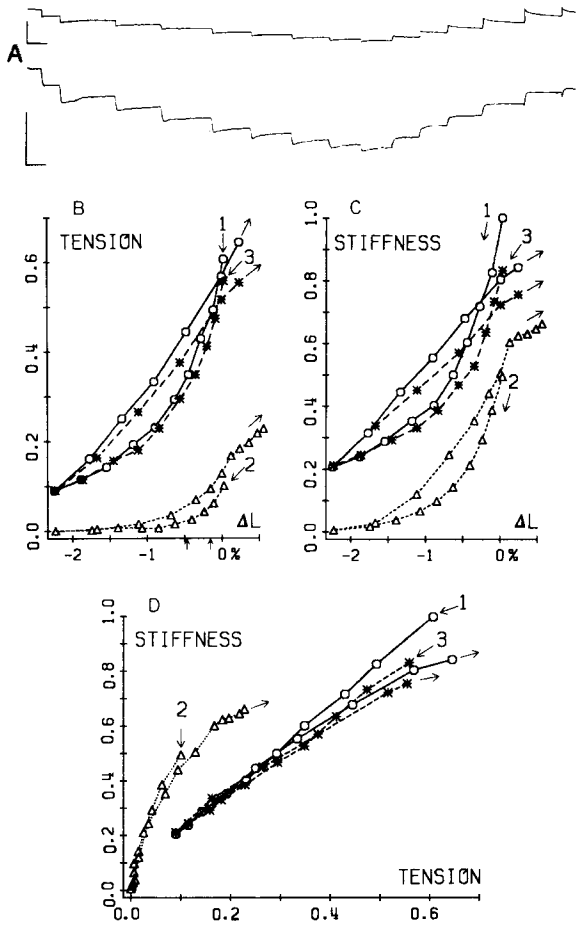

FIGURE 3. (*A*) Tension (upper trace) and 5 Hz-stiffness (lower trace) time courses for a high-rigor muscle as the length is released and restretched in steps. The fiber was activated with solution A, washed with KP to establish the high-rigor state, then put into the EDTA-rigor solution before the records of the figure were taken. Data are replotted as steady-state tension vs. length change (*B*), stiffness vs. length change (*C*), and stiffness vs. tension (*D*). Curve labeled 1 (—○—) is obtained from the record of *A* (high rigor), curve 2 (····△····) from the same muscle in low rigor, and curve 3 (---*---) after return to the same condition as curve 1. The time courses of curves 2 and 3 are not shown, but they are similar to traces in *A*. Arrows on curves indicate starting and end points for release and restretch cycle. In *B*, arrows on the abscissa indicate instantaneous length change (from L_0) required to bring the rigor tension to zero. These points were determined by extrapolating to zero tension based on tension and stiffness data. The arrow on the left is for high rigor, on the right for low rigor. The tension is normalized against P_0 (510 dyn), and the stiffness against the high-rigor stiffness (1.80×10^5 dyn/cm) of curve 1. The length of the muscle was 3.6 mm and the approximate diameter was 200 μm. Calibration bars in *A*: tension 200 dyn, stiffness 10^5 dyn/cm, and time 30 s. Experiment of 3/7/73.

stiffness, whereas if the ratio is different, length cannot be adjusted to match both.

The purpose of the experiment in Fig. 3 was to see whether the length change can compensate differences of stiffness and tension in the two rigor states. It should not be concluded from this experiment that 2.3% of quick length release is necessary to reduce tension to near zero, because muscle tension and stiffness go through slow transients and some 20 min were spent for the release and restretch cycle. As described above, if the muscle length is released quickly, 0.2% L_o (75 Å per half-sarcomere) is enough to bring low-rigor tension to zero. It is premature to conclude that all of this length change is absorbed by cross-bridges, since in series elements such as thin filaments could also be compliant. However, as A. F. Huxley (1974) has pointed out, a useful working hypothesis is that the cross-bridges are the major compliant elements.

Addition of Calcium to the Low-Rigor Muscle

It is possible that high rigor differs from low rigor because the former is initiated in saturating Ca, and possibly Ca remains bound to the fibers or induces some other change. To determine if there is a Ca effect on low rigor, a Ca-rigor solution (pCa 5.5, 5 mM total EGTA) was added. pCa 5.5 was chosen because with 1 mM substrate present we find tension is saturated. There is no change in tension or stiffness when Ca is added to low-rigor muscle in the absence of substrate (stiffness ratio before and after Ca addition is 0.98 ± 0.05, SD, $N = 6$).

Addition of Substrate to Low/High-Rigor and Released High-Rigor Muscles

Rigor is induced by the deletion of substrate. In terms of the three-state cross-bridge model described in the Introduction, cycling is blocked by a lack of substrate. There appears to be no mechanical way to interconvert low-rigor and high-rigor states, and it may be necessary for cross-bridge dissociation and subsequent cycling to occur for interconversion. We can ask how much substrate is necessary for interconversion to occur.

To both low- and high-rigor states substrate was added incrementally in the absence of Ca. In low-rigor muscle the tension progressively increased to reach a maximum at pS ~6, then declined with further increase in substrate (Fig. 4, solid line). The stiffness reached a maximum at a lower substrate concentration, and declines with further increases in substrate (Fig. 4 B, solid line). The decline of stiffness was parallel to the decline of tension.

Both tension and stiffness declined monotonically upon addition of incremental concentrations of substrate to high-rigor muscles (Fig. 4 A,B, dotted line). In order to test further the hypothesis, that the difference between low and high-rigor is due to internal strain, the length of the high-rigor muscle was released by 1.37% L_o to a tension and stiffness comparable to that of low-rigor muscle, then substrate was added as above. As shown in Fig. 4 C,D, there was not much difference between released high-rigor muscle (△) and low-rigor muscle (○, □): both tension and stiffness rose at low substrate concentration, and decreased again at high substrate concentration. There was no characteristic frequency dependence of stiffness measured at 5 Hz or 100 Hz in the above conditions.

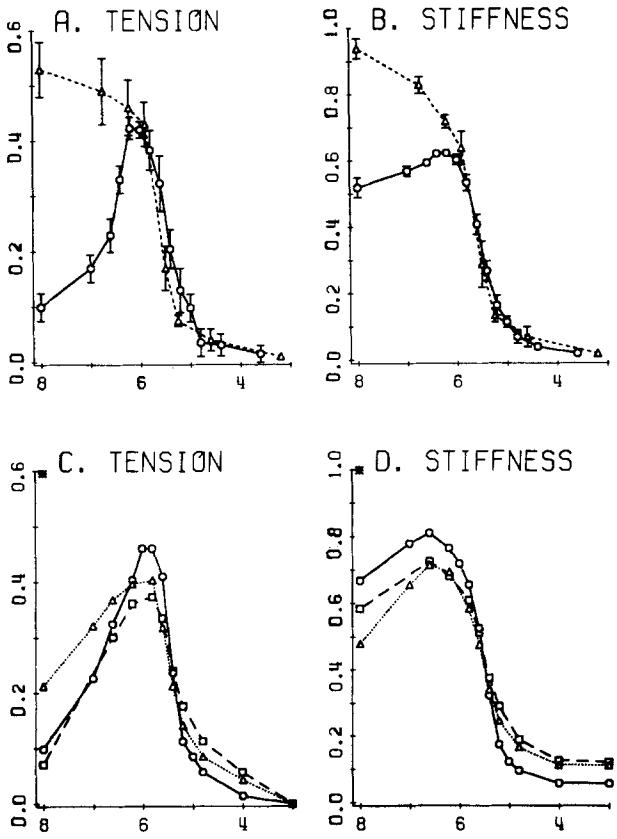

FIGURE 4. (*A, B*) Tension and stiffness when substrate (S = MgATP) is added incrementally to muscle fibers in low-rigor (—○—), and high rigor (---△---). Each point represents the mean of four to eight measurements, and the bars represent standard errors of the mean. Data collected between 2/73 and 6/74. (*C, D*) A comparison of "length-released" high rigor to low rigor. The experiments were done in the following order: (1) low rigor control at L_o to which substrate was added incrementally (—○—); (2) after high rigor was induced as in Fig. 2 B (point *) the fiber length was released by 1.37% L_o, and substrate added (····△····); (3) low-rigor was induced again at the released length, and substrate added as before (---□---). Experiment of 7/3/74. pS values (abscissa) were calculated by solving the multiple equilibria for Mg, ATP, and EDTA without accounting for Mg contamination. Since there are 0.5–0.85 μmol of Mg contamination per 1 mmol of ATP (Reuben et al., 1971) the actual value for pS 8 is close to 6.6. Other values are in parentheses: 7.0 (6.5), 6.6 (6.3), 6.4 (6.2), 6.2 (6.1). At pS values less than 6 there is no significant effect of Mg contamination. Although this correction changes the shape of the high pS side of the curve, it does not alter the difference between high and low rigor.

Effect of Sarcomere Length

It is possible that sarcomere length modifies the properties of the two rigor states. To test this possibility we studied rigor tension and stiffness at other

sarcomere lengths (6.2, 9.3 μm). We found both tension and stiffness decline as predicted by the sliding filament model (Gordon et al., 1966) applied to crayfish muscle (April, 1969), and the tension-to-stiffness ratios for high rigor and for low rigor were unchanged.

DISCUSSION

The rigor state is conventionally defined as the state of muscle in the absence of substrate (Bozler, 1956; White, 1970; Reuben et al., 1971; Bendall, 1973) and is characterized by high stiffness. Electron microscopic study by Reedy (1968) has shown that cross-bridges are made in the "angled" position with actin, and X-ray diffraction study has shown that the myosin heads shift from the thick to the thin filaments (Huxley and Brown, 1967) as rigor cross-bridges are formed. In reconstituted protein systems the rigor state corresponds to the association of myosin and actin (Bremel and Weber, 1972). Although these results are often interpreted to mean that there is a uniquely defined rigor state in the muscle, our physiological experiments show that the rigor tension and stiffness are not unique but depend on the state of muscle before rigor.

In this report we focus on two extreme conditions for generation of rigor: rapid substrate withdrawal from relaxed fibers (low rigor) and substrate withdrawal from activated fibers (high rigor). It is possible to produce intermediate rigors, but we assume for the present that the intermediate forms are mixtures of the two extremes. Low rigor is characterized by low tension and moderate stiffness, and high rigor is characterized by high tension and high stiffness. In both cases one can assume that cross-bridges are maximally interacting with actin based on the stiffness data: high rigor is the stiffest condition of the skinned muscle fiber we studied, and low rigor becomes comparably stiff if tension is increased by stretch.

The property of stiffness variation with tension is characteristic of the rigor muscle, and it must not be overlooked in studying the mechanical properties of both rigor and active muscles (cf. Podolsky and Nolan, 1973; Ford et al., 1974; Julian and Sollins, 1975). In elastic solids the stiffness is constant and the tension is proprotional to length change, but in rigor muscles tension and stiffness decline proportionately on small length release (Fig. 3). If the release is larger the muscle buckles and both tension and stiffness drop to zero. Since it is generally assumed that the number of cross-bridges is not changing in rigor, decrease in stiffness must be due to slackening of compliant in-series elements including cross-bridges.

It is useful to correlate the generation of two rigor states with known biochemical schemes such as those of Lymn and Taylor (1971), Inoue et al. (1973), or Weber and Murray (1973), although direct comparison of physiological data with that from reconstituted systems is still conjectural. In cycling cross-bridges the rigor configuration is assumed to exist transiently (Lymn and Taylor, 1971) following energy transduction, but before substrate dissociates cross-bridges. If substrate is removed abruptly all the bridge activities stop at the strained rigor configuration. They are strained because the mechanical energy, the outcome of energy transduction, accumulates in in-series elastic elements such as S-2 portion of cross-bridges. This mechanism can explain high tension in the high-rigor

condition. On the other hand, if substrate is removed quickly from resting muscle, it slowly goes into rigor probably because the high energy hydrolysis product slowly desorbs from myosin (Lymn and Taylor, 1970). Once myosin becomes free of nucleotides it combines with actin (Bremel and Weber, 1972) even if actin is turned off by the troponin-tropomyosin (Ebashi and Endo, 1968) system. This actin-myosin interaction cannot transduce energy because hydrolysis energy is not available and this accounts for the small tensions observed in low rigor. In our experiments the absence of the hydrolysis product is inferred from the absence of Ca sensitivity in low-rigor muscle.

There is evidence that cross-bridges under strain are less sentitive to substrate than are strain-free bridges. In a fiber initially in low rigor, steady-state tension rises as increments of substrate up to 1 μM are added to Ca-free rigor solution (Fig. 4 A, solid line). This phenomenon was first reported by Reuben et al. (1971) using crayfish muscle, and reproduced by Fabiato and Fabiato (1975) on rat ventricle muscle and frog semitendinosus muscle. We infer from the rise of tension that substrate dissociates low-rigor cross-bridges and its energy is transduced into mechanical work. We know the bridge cycling takes place because the muscle can shorten if allowed to do so. In contrast, when aliquots of substrate (up to 1 μM) are added to high-rigor muscle, tension and stiffness decrease slightly but never sufficiently to overlap values for the low-rigor muscles (Fig. 4 A and B, dashed line). We interpret the results of the experiments in Fig. 4 C and D to mean that strain in high-rigor muscle protects cross-bridges from the dissociating effect of substrate at this low concentration. In other words, the dissociation rate constant is strain sensitive and it is larger at lower strain. This could serve as a mechanism of stretch activation as discussed by Thorson and White (1969), and by White and Thorson (1972) in insect muscle[3]. It appears to us that the primary difference between high and low-rigor states resides in the strain in the cross-bridges. We observe an additional difference in the two rigor states: plots of stiffness vs. tension do not overlap for low and high rigor (Fig. 3 D). One possible explanation is that the degree of heterogeneity in the cross-bridge array differs between two rigor states, because the process of rigor bridge formation is different as discussed earlier. In substrate greater than 2 μM (pS <5.7) tension and stiffness of low- and high-rigor muscles, or strain-reduced high-rigor muscle, are the same (Fig. 4). From this observation we can conclude that this substrate level is sufficient to dissociate all rigor cross-bridge types, and allows high- and low-rigor muscles to equilibrate to the identical tension and stiffness.

White (1970) studied stiffness and tension in glycerinated insect and rabbit psoas muscles. The rigor state he studied appears to be comparable to our high-rigor state because both stiffness and tension were high, and they decreased monotonically on addition of ATP (in excess Mg). He demonstrated that hydrolysis as well as diffusion contributed to the development of rigor in his preparations. Our experiments have shown that it is the degree of activation before and

[3] We find the oscillatory work (the same component as stretch activation) is present in maximally activated crayfish muscle preparations (Kawai and Brandt, 1975; Kawai and Orentlicher, 1976; and footnote 2).

during induction of rigor which determines which rigor state predominates. Furthermore, we have shown that sensitivity to substrate is dependent on strain.

ADDENDUM

After the present work was submitted, Mulvany (1975) published on a similar subject, and he identifies two rigor conditions in iodoacetic acid (IAA) treated frog sartorius whole muscle. According to Mulvany these rigor conditions are interconvertible by addition or deletion of Ca, contrary to our finding. The Ca sensitivity of IAA-treated muscle can be understood if we note that 0.7 mM of ATP was still present, as Mulvany pointed out, in the IAA preparation. We do not find Ca sensitivity at substrate (MgATP) concentrations less than 1 μM, and Ca sensitivity appears at substrate concentration higher than ~3 μM (see Brandt et al., 1972), which is in good agreement with the biochemical observations (Weber, 1969).

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