Tension Generation by Threads of Contractile Proteins

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ABSTRACT Threads of contractile proteins were formed via extrusion and their isometric tensions and isotonic contraction velocities were measured. We obtained reproducible data by using a new and sensitive tensiometer. The force-velocity curves of actomyosin threads were similar to those of muscle, with isometric tensions of the order of 10 g/cm² and maximum contraction velocities of the order of 10⁻² lengths/s. The data could be fitted by Hill's equation. Addition of tropomyosin and troponin to the threads increased isometric tension and maximum contraction velocity. Threads which contained troponin and tropomyosin required Ca⁺⁺ for contraction and the dependence of their isometric tension on the level of free Ca⁺⁺ was like that of muscle. The dependence of tension or of contraction velocity upon temperature or upon ionic strength is similar for actomyosin threads and muscle fibers. In contrast, the dependence of most parameters which are characteristic of the actomyosin interaction in solution (or suspension) upon these variables is not similar to the dependence of the muscle fiber parameters. The conclusion we have drawn from these results is that the mechanism of tension generation in the threads is similar to the mechanism that exists in muscle. Because the protein composition of the thread system can be manipulated readily and because the tensions and velocities of the threads can be related directly to the physiological parameters of muscle fibers, the threads provide a powerful method for studying contractile proteins.

INTRODUCTION

In a muscle fiber the proteins exist in a highly organized array. It is thought that force is produced in this array by the interaction of actin, myosin, and ATP. When ATP is split, work is performed with high efficiency and the amount of ATP utilized is determined by the amount of work that is performed (Mommaerts, 1969; Curtin et al., 1974). This shows that the biochemical events and the mechanical events of the actomyosin interaction are closely coupled. The actomyosin interaction is an important energy-transducing mechanism in a wide variety of biological systems, and this interaction has been extensively studied in intact muscle fibers, in glycerinated and skinned muscle fibers, and in solutions or suspensions of extracted contractile proteins. (For reviews see Huxley, 1973, and Tonomura, 1973.) Contraction velocity and the generation of force can be studied directly by using muscle fibers. However, the protein composition of the muscle cannot be easily controlled by the experimenter. Thus, most biochemical studies of the actomyosin interaction are facilitated by extracting the proteins.
and performing the experiments in solution where the composition of the system can be modified and controlled. One problem of working in solution is that tension is not generated and some of the coupling of the biochemical and mechanical events must therefore be lost. Although much valuable information has been obtained with extracted muscle proteins, the exact relationship of the biochemical parameters of the solution assays (ATPase, rate of superprecipitation, etc.) to the physiological parameters of muscle (force, velocity, etc.) is not known. Some studies have found a correlation between the biochemical and the physiological parameters while other studies have found a lack of correlation. Thus it is difficult to assess which aspects of the actomyosin interaction have been lost or altered by working in solution.

One can obtain additional information about the actomyosin interaction if one reconstitutes a contractile system in vitro. Portzehl and Weber (1950) were the first to reconstitute such a system. (For a review of this work, see Weber and Portzehl, 1954.) They extruded a concentrated solution of natural actomyosin through a syringe into a solution of low ionic strength in which the proteins precipitated, forming a thread. These threads generated tension in the presence of ATP. Hayashi (1952) formed contractile threads from protein monolayers and measured their ability to perform work. Although the existence of contractile protein threads has been known for over 25 yr, these systems have rarely been used to answer questions concerning the actomyosin interaction. This is because the tensions generated were small and hard to measure, and because the results obtained were not very reproducible.

The present report describes a system in which the contractile properties of actomyosin threads are measured accurately and reproducibly. The major improvement that has been introduced is a new type of tensiometer that was specifically designed for making measurements on the threads. Using this tensiometer, we have measured not only the isometric tensions, but also force-velocity relationships of actomyosin threads, and we have found that they resemble the force-velocity curves of muscle. In a number of experiments in which the protein concentration, temperature, ionic strength, etc., were varied, the contractile mechanism which operates in the threads appeared similar to the mechanism which operates in muscle. In contrast, the actomyosin interaction that occurs in solutions or suspensions of contractile proteins appears to be different from the interaction which exists in muscle fibers and in the threads.

**METHODS**

**Protein Preparation**

Myosin was prepared by the method of Tonomura et al. (1966) with the following modifications. The minced muscle fibers were extracted for 2 hr with 0.6 M KCl, 5 mM MgCl₂, 1 mM ATP, and 0.1 M potassium phosphate, pH 6.8. After each high-speed centrifugation the lipid that floated to the top of the tube was carefully removed with a transfer pipette fitted to an aspirator. ADP (100 μM) and MgCl₂ (1 mM) were added to the myosin to retard denaturation during storage. Actin was prepared by the method of Spudich and Watt (1971). Troponin and tropomyosin were prepared by the method of Greaser and Gergely (1971, 1973). Myosin subfragment 1 was made by the method of
Cooke (1972). Creatine phosphokinase was purchased from Boehringer, Mannheim, W. Germany.

**Thread Formation**

In order to achieve the high protein concentrations necessary to produce tension in the threads, the proteins (in 0.4–0.6 M KCl) were precipitated by being diluted to 0.1 M KCl and collected by centrifugation at 30,000 g for 15 min. The pellet was weighed and 3 M KCl was added to bring the KCl concentration to 0.5 M in a minimum volume; dithiothreitol (0.1 mM) and NaN₃ (3 mM) were added to preserve the sample. The viscosity of the concentrated solution was decreased by addition of 1 mM MgCl₂ and 0.2 mM PP₁. This facilitated mixing of the protein solution. The sample was mixed with a spatula or by repeated suction into a transfer pipette. Bubbles were then removed by low-speed centrifugation. We incorporated troponin and tropomyosin into the threads by adding them to the actomyosin solution (in 0.6 M KCl) before precipitation and centrifugation.

We studied the composition of the concentrated protein solutions and the threads. These solutions were analyzed by densitometry of sodium dodecyl sulfate-polyacrylamide gels stained with Coomassie blue. A small amount of protein (5%) was lost in the supernate of the centrifugation step. At ratios of actin to myosin greater than two, the loss of this protein did not measurably affect the ratio of actin to myosin in the pellet. At low ratios of actin to myosin the loss of actin in the supernate was significant, so these actomyosin solutions were used without the concentration step. The composition of the threads was found to be identical to that of the solution from which they were formed. The diameter of the threads was close to the inner diameter of the syringe needle from which they were extruded, indicating that they did not shrink after extrusion. Protein determinations on threads of known volume confirmed that there was no loss of protein during the formation of the threads.

We experimented with threads formed from monolayers and with threads formed by extrusion before deciding that the extrusion method was capable of giving more reproducible results. In all of the experiments reported here, threads were formed by extruding the concentrated solution of actomyosin through a syringe and into a trough that contained buffer 1 (50 mM KCl, 5 mM MgCl₂, 10⁻⁵ M CaCl₂, 0.1 mM dithiothreitol, and 20 mM N-Tris [hydroxymethyl]methyl-2-aminoethane sulfonic acid [TES] pH 7.0). The protein concentration in the actomyosin solution was varied from 15 to 45 mg/ml. We used needles with gauges from 20 to 25 to extrude the threads. The ends of the needles were squared off and the outside edges were beveled to produce a laminar flow around the end of the needle during extrusion. The syringe was mounted on an arm that rotated in a horizontal plane, with the needle extending into the solution in the trough. The needle was bent so that the axis of the needle was aligned with the direction of motion as the needle was swept across the trough. The movable arm was swept manually across the trough at the same time that the plunger of the needle was slowly depressed to extrude the actomyosin solution. With a little practice, we could form straight threads with a uniform diameter. We determined thread diameters with a dissecting microscope.

The threads were removed from the trough and mounted on the tensiometer with a curved dissecting needle. The thread mounts on the tensiometer consisted of two thin horizontal glass rods (Fig. 1). The lower rod was attached to a micromanipulator so that its height could be adjusted. The upper rod was attached to the arm of the meter movement which was used to measure force. The threads were glued to the mounting rod with a small drop of a solution of acetone and plexiglass (18 mg plexiglass/ml). They were then immersed in 2.5 ml of an appropriate experimental solution. The solution was stirred at 100–200 rpm with a small magnetic stirring bar. The temperature of the solution could be regulated to within ±0.1°C, but was left at room temperature except where specified.
Tensiometer

Fig. 2 shows a schematic diagram of the tensiometer. The thread is attached to the arm of a meter movement which is used both to apply and to measure force. When the thread contracts, it tends to depress the arm. As the arm is depressed its position is sensed by a photodiode system. An opaque shield attached to the end of the arm allows more light from a light-emitting diode to strike the photodiode. The photodiode current, which is linearly proportional to the vertical position of the arm, is amplified, filtered, and fed back through the coil of the meter movement, applying a torque to the arm. The circuit acts in a negative feedback mode so that an increase in the photodiode signal will tend to raise the arm. In this mode the position of the arm is approximately constant and the tension of the thread is proportional to the current in the coil. The tensiometer can be switched from the constant length mode to a constant tension mode by switching the relay shown at the bottom of Fig. 2. In the constant tension mode the relay is in the other position and the feedback is broken so that the current in the coil, and thus the tension on the thread, is determined by the variable voltage source. If the applied tension is less than the isometric tension of the thread, the thread will contract. The signal in the photodiode is recorded as a function of time; the slope of the function is proportional to the velocity of contraction and can be measured at any preset tension.
The photodiode (PIN 2DP, United Detector Technology Inc., Santa Monica, Calif.) has two sensitive elements. The light intensity on the reference element is constant, while the light intensity on the signal element is obstructed by the opaque shield. The ratio of the signal current to the reference current is measured by a ratio amplifier (Analog Devices no. 521, Analog Technology Corp., Pasadena, Calif.) operated in the current detection mode. This signal passes through two operational amplifiers where it is amplified and filtered. The time constant of the filter varied between 0.1 and 2 s. Switching between modes was accomplished by a 4pdt relay which, in addition to the two functions shown in Fig. 2, switched the filter system off in the constant tension mode. This was accomplished in such a manner that the tension decreased rapidly (10 ms) on switching from the constant length mode to the constant tension mode, while the increase in tension occurred slowly (2 s) when the tensiometer was switched back to the constant length mode. The slow rise in tension was necessary to avoid damaging the thread. Details of the tensiometer circuit will be provided on request.

In the constant length mode the compliance of the instrument is 0.5 μm/mg. The drift of the instrument is less than 0.1 mg/h. Noise levels are also of the order of 0.1 mg with a time constant of 0.5 s. Since the length of the threads is of the order of 5 mm, a typical tension of 5 mg will result in a change of less than 0.1% in the length of the thread during the measurement of isometric tension. The great advantage of this tensiometer is that the tension can be controlled electronically. This allows one to switch rapidly and reversibly between isometric and isotonic modes in a way that does not harm the thread.

In most of the following experiments the threads were immersed in buffer no. 1 containing 4 mM phosphocreatine (PC) and 0.4 mg/ml creatine phosphokinase (CPK). Approximately 5 min were allowed for the PC and CPK to diffuse into the thread. One can calculate that the diffusion of PC and of CPK to the center of a thread with a diameter of 0.4 mm would require approximately 2 min and 8 min, respectively. At this point the tensiometer was in the isometric mode, the thread was in rigor, and the tension of the
thread was small (about 1 g/cm²). 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 as a function of time on either an x-t recorder or an oscilloscope. The tensiometer was returned to the isometric mode and the thread was allowed to return to its initial length before a subsequent isotonic velocity was recorded at a different tension. Using this method, we could produce an entire force-velocity curve in about 2 min.

Calcium Dependence of Thread Tensions

In experiments designed to measure isometric tensions produced by actomyosin threads containing troponin plus tropomyosin, a calcium-EGTA buffer system was employed so that the tensions produced could be related to the free calcium ion concentration. Initially, the buffer contained 20 mM KCl, 5 mM MgCl₂, 4 mM PC, 0.4 mg/ml CPK, 1 mM EGTA, and 50 mM TES, pH 7.05. Each experiment was initiated by adding ATP to a final concentration of from 50 to 200 μM. Calcium concentrations were adjusted by adding serial aliquots of 20 mM CaCl₂. Isometric tension was measured after each addition of CaCl₂. The relative tension reported for a given calcium concentration was calculated as the ratio of the absolute tension at the given calcium concentration to the maximal absolute tension produced by the thread at a saturating calcium concentration (1.1 mM).

The free calcium concentration (pCa) present in the buffer after each addition of calcium was computed by using the association constants reported by Portzehl et al. (1964), and the equations of Reed and Bygrave (1975). It was found that the pH of the buffer decreased by 0.1 pH unit when the total calcium concentration was raised from 0 to 1 mM. Since the calcium-EGTA buffer system is quite sensitive to pH, this pH change was accounted for in the calculation of pCa. It was also necessary to correct the pCa for the presence of magnesium ion. This was done by the iterative method of Portzehl et al. (1964). An excess of magnesium ion, relative to ATP, allowed us to ignore the effect of ATP upon magnesium ion concentration.

Miscellaneous

Protein concentrations were determined by the method of Lowry et al. (1951). ATPase was measured in a pH-stat (Radiometer, Denmark) equipped with a regulated water bath at 25°C. Superprecipitation was monitored in a Bausch & Lomb dual beam spectrometer (Bausch & Lomb Inc., Rochester, N.Y.) which we modified to permit stirring of the sample. Turbidity was measured at 350 nm in a 1-cm cuvette. The temperature was unregulated at approximately 23°C. The velocity of superprecipitation was taken as (t₁/₂)⁻¹, where t₁/₂ is the time required for the optical density to reach one-half maximum.

RESULTS

Properties of Actomyosin Threads

Threads of purified actin and myosin can produce force. In the absence of ATP the threads are stiff and a small extension will result in a large rigor force. The addition of 0.5 mM PPI relaxed this rigor force to about 25% of its original value. Upon addition of ATP to the medium (buffer no. 1), the tension of the threads increased. Maximum tensions were produced by concentrations of ATP in excess of 0.5 mM. The requirement for high ATP concentrations is due to the slow diffusion of ATP into the threads. This problem was avoided through the use of a creatine phosphokinase (CPK)-phosphocreatine (PC) "feeder system" to
maintain the concentration of ATP and to avoid ADP accumulation. In the presence of this feeder system, half-maximal tension was reached at about 8 μM ATP and maximum isometric tension was obtained at less than 50 μM ATP. The maximum tensions generated in the presence of the feeder system were two to three times greater than those generated by ATP alone. By using the feeder system, full tension was reached within 2 min after the addition of ATP. CPK and PC comprise the physiological feeder system in living muscle. In order to ask whether the CPK system was interacting directly with the actomyosin system in the threads, we tested a second type of ATP-regenerating system. In the presence of 4 mM phosphoenolpyruvate and 0.4 mg/ml pyruvate kinase, the rate and extent of isometric tension development were similar to rates and extents found with the use of the CPK system.

We studied the dependence of the isometric tension on the concentration of actin and myosin in the solution from which the threads were formed (Fig. 3). If either actin or myosin was omitted from the solution the threads were very fragile and did not generate tension upon addition of ATP. The protein concentration of the threads was varied in two ways. We varied the protein concentration at a fixed ratio of actin to myosin (4:1 mol/mol) and found that the isometric tension was proportional to the square of the protein concentration. In the second procedure we kept the myosin concentration fixed (approximately 40 μM) and varied the actin concentration from 20 μM to 160 μM. In these experiments the isometric tensions were linearly proportional to the actin concentration. Isometric tensions obtained by either method fell on a straight line when plotted against the product of the actin and myosin concentrations, as shown in Fig. 3. If the molar ratio of actin to myosin were increased to about 5, the measured tension was less than that expected from Fig. 3. It is possible that all of the actin cannot interact with myosin filaments at this ratio. At molar ratios of actin to myosin <0.5 threads were fragile and easily broken. The protein concentrations in the uncontracted threads were determined as outlined in Methods. However, the threads contracted laterally after the addition of ATP. The diameters of threads measured after isometric contraction were 30-40% less than before contraction. Thus the protein concentration in the contracted threads is greater than that of the original solution.

The threads could contract to less than 50% of their original length. As the length decreased, the isometric tension also decreased, as shown in Fig. 4. During the re-extension of shortened threads, the tension increased with little hysteresis. Contraction and extension cycles could be repeated many times with little loss in tension, and threads could maintain tension for at least 1 h.

The threads tended to break when the concentration of ATP was raised above 500 μM in the presence of the feeder system. A similar effect was seen for single glycerinated muscle fibers. The fibers broke when the level of ATP exceeded 200 μM, even in the absence of a feeder system. The level of ATP above which the threads break varies from one myosin preparation to another and is less for freshly prepared myosin than for myosin that is more than 1 wk old. If fresh myosin is allowed to stand for 1 h at room temperature before preparation of the actomyosin solution from which the threads are produced, the tendency of the threads to break when exposed to high ATP concentrations (up to 2 mM) is
Figure 3. Isometric tension as a function of protein concentration. Isometric tensions are plotted as a function of the protein concentration in the solution from which the threads were formed. Both the total protein concentration and the ratio of actin to myosin were varied as described in the text. The buffer solution contained buffer no. 1 plus 4 mM phosphocreatine and 0.4 mg/ml creatine phosphokinase. The generation of tension was initiated by addition of ATP to 160 μM. Tensions generated by threads from a given solution were reproducible to within ± 10% of the mean value.

Figure 4. The isometric tension of an actomyosin thread as a function of length. The myosin and actin concentrations in the solution used to make the threads were 30 mg/ml and 4 mg/ml, respectively. The conditions used were the same as in Fig. 3. The isometric tension of the thread was first measured at the length at which it was formed (length = 100%). It was then allowed to contract. When contraction was stopped, the isometric tension was noted and the process was repeated. The thread was re-extended after shortening to 90% of its original length. Approximately 1-5 min were required for the tension to reach a steady value after each length change.
eliminated. This "curing" process decreased the actin-activated ATPase of the myosin by about 30%, but did not change the Ca**+-activated ATPase in 0.6 M KCl. Changes in the myosin that result from curing may be related to a side-by-side aggregation of myosin molecules which occurs with a similar time course (Johnson and Rowe, 1961).

Properties of Threads which Contain Relaxing Proteins

Typical force-velocity curves for actomyosin threads and for threads that also contain relaxing proteins are shown in Fig. 5. Addition of tropomyosin to the actomyosin solution before the formation of the threads caused a slight increase in both the isometric tension and the maximum contraction velocity of the threads. Addition of both tropomyosin and troponin to the actomyosin caused an even larger increase in these two parameters.

When purified troponin and purified tropomyosin are added to the solution of actomyosin from which threads are prepared, the isometric tensions produced are dependent upon the level of free calcium ion in the buffer solution. The method of determining the calcium dependence of thread tensions is important. The results which are shown in Fig. 6 were obtained by measuring the isometric tension at a high pCa, and then adding calcium to obtain measurements at successively lower pCa values until the tension reached a maximum.

![Figure 5. Force-velocity curves for actomyosin threads. Threads were formed from solutions which contained: 28.5 mg/ml myosin plus 2.6 mg/ml actin, —O--; actomyosin as above plus 0.6 mg/ml tropomyosin, —□--; actomyosin and tropomyosin as above plus 1.0 mg/ml of troponin, —Δ—. Experimental conditions were the same as in Fig. 3. The lines on the plot represent fits of the data to the Hill equation.](image-url)

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The ratio of the isometric tension in the presence of saturating calcium ion to the isometric tension in the absence of calcium ion (1 mM EGTA) was greater than 100. The ratio of the actomyosin ATPase activity in the presence of saturating calcium ion to the ATPase activity in 1 mM EGTA was only about 5, however. Thus the relaxing proteins provide better control in the threads than in the ATPase assay. For reasons which are not clear, efforts to relax the threads by addition of EGTA were only partly successful. Addition of EGTA to a thread contracting in Ca^{++} relaxed the tension to about one-half of its value in about 10 min.

We found that threads containing troponin and tropomyosin will develop tension in the absence of calcium when the ATP concentration is less than 50 μM. This dependence of actomyosin regulation upon ATP concentration is similar to that which has been reported for a variety of other muscle systems (for review see Weber and Murray, 1973). Changes in the free calcium ion concentration from pCa 9 to pCa 5 did not have any effect on the isometric tension generated by threads of purified actomyosin or of purified actomyosin plus tropomyosin. Calcium sensitivity was observed only when troponin was included.
in the threads. This argues against a mechanism in which the binding of calcium to myosin plays a large part in the regulation of the actomyosin interaction.

Concentration of KCl

One method of determining the relationship between a physiological parameter measured by using a muscle fiber and a biochemical parameter measured in solution is to perturb both systems with an agent that modifies the interaction of interest and then to determine whether both parameters respond in the same manner. In an effort to determine whether the parameters of the threads resemble those of other commonly used muscle assay systems, we have perturbed the threads by changing the concentration of KCl. We compared the behavior of the thread parameters with the behavior of the parameters of glycerinated muscle fibers as well as with the behavior of a number of parameters from other in vitro assays of the actomyosin interaction. The KCl concentration in the following experiments was varied from 0 mM to 160 mM. The results are plotted as a function of the square root of the total ionic strength of the solution because the Debye-Huckel theory predicts that the activity coefficient of a charged molecule should vary linearly with this parameter. Although the approximations of the Debye-Huckel theory are not valid at the ionic strengths used and its application to proteins is not straightforward, the data still fall on nearly straight lines.

The ATPase activities of both actomyosin and actosubfragment 1 (actoSF-1) were measured. Double reciprocal plots of the actoSF-1 ATPase activity vs. the actin concentration were fit by linear regression analysis. Two parameters were obtained; the 1/actin intercept was termed \( K_{\text{app}} \), and the ATPase at infinite actin (\( V_{\text{max}} \)) was determined from the 1/ATPase intercept. The actomyosin ATPase was measured at a fixed concentration of both actin and myosin. The log of the actomyosin ATPase activity and the \( K_{\text{app}} \) of actoSF-1 ATPase are plotted as a function of the square root of the ionic strength in Fig. 7. The values of both parameters decrease precipitously as the ionic strength increases. The velocity of superprecipitation also decreases sharply as the ionic strength increases, in a manner similar to that observed for \( K_{\text{app}} \) and for the ATPase of actomyosin. The velocity of superprecipitation appears to be inversely related to the square root of the ionic strength up to velocities of 0.22 s\(^{-1}\). Above this velocity the measured rate was determined by the time constants of the apparatus, and this rate became constant as the ionic strength was lowered. This is shown by the point at the lowest ionic strength in Fig. 7a. Superprecipitation was also investigated at a variety of initial ATP concentrations. At lower ATP concentrations the line in Fig. 7a was shifted to the left with no change in the slope. Two parameters, \( V_{\text{max}} \) and the extent of superprecipitation, decreased by about 30% as the ionic strength was increased over the range studied.

The isometric tensions of actomyosin threads and bundles of glycerinated muscle fibers were determined as a function of the KCl concentration. As shown in Fig. 8a, the value of these parameters decreased only slightly as the ionic strength increased. We determined the maximum velocity of contraction for the threads by extrapolating the force-velocity data to zero force using a linear form of the Hill equation (Murphy and Beardsley, 1974). The maximum velocities of
contraction appear to be almost independent of ionic strength over the range studied (Fig. 8b).

Temperature

The maximum contraction velocity decreased with a Q_{10} of about 2, as the temperature was reduced from 30°C to 5°C. The Arrhenius plot (log maximum velocity vs. T^{-1}) was slightly curved, yielding activation enthalpies of 50 kJ/mol from 5°C to 15°C and 33 kJ/mol from 15°C to 30°C. The isometric tension was constant above 15°C and decreased sharply, with a Q_{10} of around 4, as the temperature was lowered from 15°C.

**DISCUSSION**

There have been several previous attempts to reconstitute a contractile system from isolated muscle proteins. Boehm and Weber were the first to form threads of contractile proteins by extrusion and Szent-Gyorgyi showed that these threads
would shorten after addition of ATP (see Weber and Portzehl, 1954). Portzehl and Weber (1950) dried the threads to increase the protein concentration and found that these threads could produce large tensions (up to 200 g/cm²). Hayashi (1952) formed threads from protein monolayers and demonstrated that these threads could lift loads, thus performing work. These systems have not been commonly used for investigating the contractile mechanism because a number of problems have prevented the acquisition of quantitative and reproducible data.

We have introduced several modifications which improved the quality of the data. The most important improvement was the construction of a tensiometer that has great sensitivity and low drift. The tensiometer allows the force on a

![Diagram](image-url)
thread to be controlled electronically. This allows the isotonic velocity to be measured without stressing the fragile thread. A second crucial improvement is the inclusion of a feeder system to maintain the level of ATP. Without the use of a feeder system, the rate-limiting step in the generation of tension is the diffusion of ATP and ADP through the threads. Information on the performance of the proteins can be obtained only in systems where an adequate supply of ATP is maintained. These two improvements have allowed us to measure the small tensions of threads which have low protein concentrations and to measure complete force-velocity curves. We have thus eliminated the drying step used by Portzehl and Weber (1950) to concentrate the proteins in their threads. This makes our procedure simple to use and our results more reproducible. Thread tensions are directly related to the concentrations of the proteins in the threads and these concentrations are easily controlled in our system.

Many properties of the threads resemble those of muscle. After addition of ATP, the threads will generate tension and contract against a load. The threads will produce tension for up to 1 h. The force-velocity curves of the threads resemble those of mammalian muscle fibers (Gulati, 1976) and the data fall approximately on curves generated by using Hill's equation:

\[(P_0 - P)b = (P + a)V,\]

where \(P_0\) is the isometric tension, \(P\) and \(V\) are the tension and velocity, and \(a\) and \(b\) are experimental parameters. However, the isometric tensions and the maximum contraction velocities of the threads are small relative to muscle. There are two obvious reasons for which smaller values should be found for the threads. First, the protein concentration in the threads is less than that in muscle. If the line in Fig. 3 is extrapolated to \([\text{actin}] \cdot [\text{myosin}] = 1.2 \times 10^7 \text{ M}^2\), a protein concentration which has been estimated for muscle,\(^1\) the tension would be about 250 g/cm\(^2\) for a pure actomyosin thread and about 340 g/cm\(^2\) for the threads that have relaxing proteins. Second, the filament array in the threads is not as well ordered as the array of muscle. The structure of threads which were similar to those used here has been examined in the electron microscope (D'Haese and Kommick, 1972). Before the addition of ATP, actin filaments decorated by myosin were seen to have little orientation. After the addition of ATP, both actin and myosin filaments were seen, and the filaments were aligned with the long axis of the thread. Although this shows that order exists in the contracting threads, they do not have the precisely ordered array of muscle. In particular, the organization of skeletal muscle guarantees that the interaction of actin with myosin occurs with the correct polarity. If the filaments of the threads are aligned along the axis of the threads but the interactions between filaments are random, then only half of the actomyosin interactions will have the correct polarity for the generation of force. Consequently, the extrapolated thread tensions should be less than the tensions measured for muscle by a factor of two. The above arguments show that most of the difference between the thread tensions and those of muscle fibers can be reasonably explained and that the tension per filament in the threads is high. Therefore, the tensions measured

\(^1\) The concentration of myosin in the myofibril has been estimated to be between 120 and 170 \(\mu\text{M}\) and the molar ratio of actin to myosin in the myofibril is 5.7 (Tregear and Squire, 1973).
are probably the result of an actomyosin interaction which is similar to that operating in muscle. The high tension per filament in the threads implies that some continuity exists to transmit the tension from one filament to the next. If one constructs models with bipolar myosin filaments and unipolar actin filaments, it is easily seen that some mechanism is required to provide continuity between actin filaments. We do not know what mechanism provides this continuity.

Previous investigators have shown that threads of natural actomyosin require calcium ion for contraction and that removing the relaxing proteins renders these threads insensitive to calcium (Wideman et al., 1970). We have corroborated and extended these results by working with threads reconstituted from purified proteins. When the relaxing proteins are present in the threads, the dependence of isometric tension on calcium ion concentration resembles the calcium dependence of muscle (Fig. 6). At low concentrations of ATP the threads will contract in the absence of calcium ion. This effect, which is also seen in muscle, has been explained by the formation of rigor bridges which force the tropomyosin into the groove of the actin helix. The movement of tropomyosin exposes the actin sites, which can then interact with myosin to produce tension (for review see Weber and Murray, 1973). At higher ATP concentrations the threads do not generate tension in the absence of calcium ion. Both the pCa at which half the maximum tension is generated and the range of pCa values over which the switch is turned on are the same for threads which contain the relaxing proteins, for skinned muscle fibers (Hellam and Podolsky, 1969), and for the ATPase of myofibrils (Weber and Bremer, 1971).

The addition of the relaxing proteins to the threads increases the isometric tension by 40% and the maximum contraction velocity by 80%. The greater increase in the velocity suggests that this is due to an effect on the contractile interaction and not to an enhancement of the stability of the actin polymer. It is interesting to note that the addition of relaxing proteins also increases the ATPase of actoHMM or actoSF-1 (Eisenberg and Kielley, 1970; Bremer et al., 1972).

We took data on the effects of changing the temperature and KCl concentration in an attempt to understand how the tensions and velocities of the threads relate to the tensions and velocities of muscle. Temperature and KCl concentration were chosen as variables because they can be controlled easily both in solution and in muscle fibers, and because their effects can be attributed to a perturbation of the actomyosin interaction in solution or of the contractile apparatus in muscle fibers and in the threads. We have repeated some experiments on the effects of ionic strength which were previously done by others. We did this so that we would be able to make a direct comparison of the effects of ionic strength upon the several assay systems.

Results obtained by others have shown that increasing the KCl concentration decreases the isometric tension and does not greatly affect the maximum contraction velocity of both living (Gordon and Godt, 1970; Lannergren and Noth, 1973) and skinned muscle fibers (Gordon et al., 1973; Thames et al., 1974; Gulati and Podolsky, 1976). Fig. 8 shows that the isometric tensions and the maximum velocities of the threads are only slightly affected by changes in the ionic
strength. This behavior parallels that of muscle fibers. In contrast, our work and that of others (Burke et al., 1974; Rizzino et al., 1970; Moos, 1972; Tada, 1967) have shown that many parameters of the actomyosin interaction measured in solution or suspension depend very strongly on the KCl concentration. This behavior is probably related to the fact that the association of actin with myosin heads decreases strongly as the KCl concentration is raised (Highsmith, 1976). In agreement with this hypothesis the extrapolation of actoHMM ATPase to infinite actin concentration gives a $V_{\text{max}}$ that is independent of ionic strength. The fact that the isometric tensions and the maximum contraction velocities of both muscle fibers and the threads do not have a strong dependence on ionic strength indicates that the steps which are rate limiting for these processes are not very sensitive to the strength of the association of actin with myosin.

Both the isometric tension and the maximum contraction velocity of the threads decreased as the temperature was lowered. The activation enthalpy associated with the maximum contraction velocity (35–50 kJ/mol) resembled the enthalpies measured for frog muscle (40–60 kJ/mol) by Hill (1938) and those measured for rat muscle (45 kJ/mol) by Close (1965). Below 15°C the isometric tensions of the threads decreased with a $Q_{10}$ of about 4. Similar behavior was observed for rabbit skeletal muscle fibers by Gulati (1975) who found a $Q_{10}$ greater than 4 below 15°C. Above 15°C the isometric tensions of the threads and the fibers appear independent of temperature. The sharp break in the temperature dependence of contraction at 15°C was also observed with rabbit psoas myofibrils (Varga, 1950). Thus the temperature dependence of the thread parameters resembles that of muscle. In contrast, none of the parameters of the actomyosin interaction measured in solution display a temperature dependence that parallels that of the muscle fiber parameters. The activation enthalpies of actomyosin ATPase and of the $V_{\text{max}}$ for actoHMM ATPase vary from 105 to 125 kJ/mol (Barany, 1967; Moos, 1972). The activation enthalpy associated with the rate of superprecipitation varies from 50 to 210 kJ/mol, and the extent of superprecipitation (at a rate-limiting concentration of ATP) actually increases with decreasing temperature (Levy and Fleisher, 1965).

Although the interactions of actin and myosin have been studied extensively in solution, there have been few investigations which have directly correlated the parameters measured in solution with those measured by using muscle fibers. The correlations that were made have yielded mixed results. A positive correlation between the ATPase of actomyosin and the speed of the muscle from which the actomyosin had been extracted was found by Barany (1967). Another positive correlation is provided by studies which have shown that the relaxing proteins are able to regulate all of the assay systems with which we have been concerned, although some are regulated better than others. However, there was a poor correlation between the rates of nucleotide hydrolysis and the stimulation of myofibril contraction when a series of modified nucleotides was added to myosin, actomyosin, and myofibrils (Tonomura, 1973). As discussed above, the parameters of the solution assays are much more sensitive to changes in temperature or ionic strength than are the parameters of muscle. Our results suggest that there are differences as well as similarities between the actomyosin interaction which occurs in solution and the actomyosin interaction which occurs in
muscle. The exact nature of these differences is not clear, but at the very least the rate-limiting steps which govern the interaction in solution must be different from those which govern the tension or contraction velocity of a muscle fiber. There is a good correlation between the results we have obtained using the actomyosin threads and the results obtained by ourselves and by others with muscle fibers. This suggests that coupling between the biochemical and mechanical events has been effectively reconstituted in the threads. Therefore, the threads can provide a powerful tool for studying the mechanical aspects of the actomyosin interaction. The protein composition of the threads can be manipulated, as in the biochemical assay systems, and the measurable parameters of the threads may be sensitive to facets of the muscle protein interactions which are not evident in solution.

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