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Active tension generation in isolated skeletal myofibrils

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Summary

Single or double myofibrils isolated from rabbit psoas muscle were suspended between a fine needle and an optical force transducer. By using a photodiode array, the length of every sarcomere along the specimen could be measured. Relaxed specimens exhibited uniform sarcomere lengths and their passive length–tension curve was comparable to that of larger specimens. Most specimens could be activated and relaxed four to five times before active force levels began to decline; some specimens lasted for 10–15 activation cycles. Active tension (20–22°C) was reproducible from contraction to contraction. The contractile response was dependent on initial sarcomere length. If initially activated at sarcomere lengths of $\geqslant 2.7$ μm, one group of sarcomeres usually shortened to sarcomere lengths of 1.8–2.0 μm, while the remaining sarcomeres were stretched to longer lengths. Myofibrils that were carefully activated at shorter initial sarcomere lengths usually contracted homogeneously. Both homogeneous and inhomogeneous contractions produced high levels of active tension. Calcium sensitivity was found to be comparable to that in larger preparations; myofibrils immersed in pCa 6.0 solution generated 30% of maximal tension, while pCa 5.5–4.5 resulted in full activation. Active tension at full overlap of thick and thin filaments ranged from 0.34 to 0.94 N mm^{-2} (mean of 0.59 N mm^{-2} ± 0.13 SD. $n = 65$). Even allowing for a maximum of 20% nonmyofibrillar space in skinned or intact muscle fibres, the mean tension generated by isolated myofibrils per cross-sectional area is higher than by fibre preparations.

Introduction

The mainstay of experimental research on mechanics and energetics of striated muscle has long been the single muscle fibre. Left intact or skinned, the muscle fibre can be treated as a coherent unit and is therefore useful for studying the mechanism of contraction. Unfortunately, fibres also have attendant disadvantages. Their length makes it impractical to investigate dynamics of all sarcomeres in series. Their width raises the issue of uneven tension distribution among constituent myofibrils, resulting in potentially misleading conclusions about the relation between tension and sarcomere length. In the case of skinned fibres, whose width typically ranges between 40 and 200 μm, there is the added question of diffusional limitations of critical metabolites (Hellam & Podolsky, 1969; Lin *et al.*, 1988; Elzinga *et al.*, 1989). Thus, studies employing intact or skinned fibres are subject to certain interpretational ambiguities that can be resolved only by using more diminutive preparations.

One response to such limitations has been the development of the *in vitro* motility assay (Spudich *et al.*, 1985; Harada & Yanagida, 1988; Toyoshima *et al.*, 1988; Kron *et al.*, 1991). In this assay, single fluorescently-labelled actin filaments are observed to move over a myosin coated surface. The elegance of the *in vitro* assay is that it allows contractile proteins to be purified and modified by the experimenter. However, observations made using this technique are sometimes difficult to interpret. For example, the geometric relation between the constituent proteins is unknown, and F-actin appears to be buried in a randomly oriented 'forest' of myosin molecules (Harada *et al.*, 1990), thereby limiting one's ability to draw firm conclusions about the mechanism and amount of force developed during translation. Furthermore, translation of actin filaments is affected by solution composition differently in the *in vitro* assay than in intact sarcomeres; motility appears to be favoured at ionic strengths and ATP concentrations very different from those favoured in skinned fibre experiments (Homsher *et al.*, 1992). These features of the *in vitro* assay complicate its relation to *in vivo* motility and

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restrict the conclusions that can be drawn from such experiments.

The single myofibril, on the other hand, retains the fundamental sarcomeric unit of *in vivo* muscle. It can be isolated by simple tissue homogenization procedures and offers many advantages over larger preparations. Isolated myofibrils are thin enough to preclude the development of chemical gradients between the core of the specimen and the bathing milieu, while being short enough to allow length measurement of every sarcomere within the specimen. As all sarcomeres bear the same tension, tension can be related unambiguously to sarcomere length. Development of the single myofibril technique has been long in coming because instrumentation used for whole muscle fibres is not easily scaled down. Iwazumi, early on, recognized the advantages of such a preparation and developed an impressive apparatus to measure tension and sarcomere length in isolated myofibrils (Iwazumi, 1987a). Preliminary results suggested that very high levels of tension could be generated by myofibrils (Iwazumi, 1987b) but these results were not reported in enough detail to stir broad interest in the muscle community. Another apparatus with more limited capability has been developed by Ishiwata and colleagues but has been used primarily to measure sarcomeric oscillations (Ishiwata *et al.*, 1991) rather than mechanics.

We report here the results of the first comprehensive study of passive and active tension generation by isolated

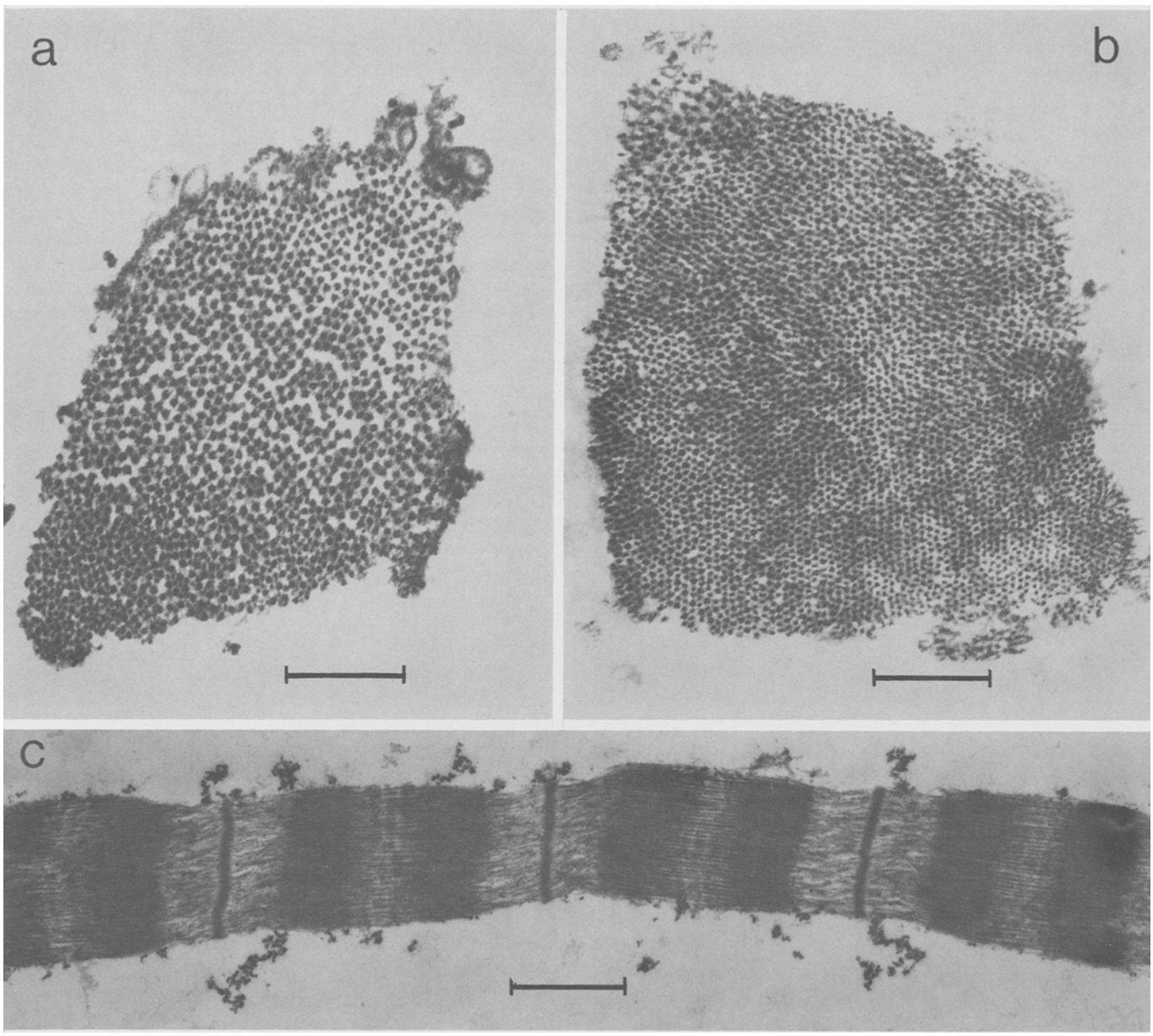

Fig. 1. Electron micrographs of fixed and embedded, isolated myofibrils. (a) transverse section through H-zone; (b) transverse section through overlap zone; (c) longitudinal thin section of myofibril fixed in rigor. Bars = 0.25 μm in a and b; 1.0 μm in c.

myofibrils. Myofibrils from rabbit psoas muscle were used in order to compare tension levels with published reports using skinned rabbit psoas fibres. At sarcomere lengths of 1.8–2.2 μm we obtained reproducible levels of active tension. Mean force per unit area generated by myofibrils was larger than reported for skinned fibres (Nosek *et al.*, 1990) and greater even than reported for intact fibres (ter Keurs *et al.*, 1978).

Materials and methods

Isolation of single myofibrils

Small strips of rabbit psoas muscle (0.5 × 4–5 cm) were tied to sticks and skinned in a 1% Triton/rigor buffer for 1 hr. Throughout all solution exchanges the muscle was kept on ice. After the first skinning, the muscle strips were soaked in: 50/50 by volume glycerol/rigor solution for 2 hr followed by 1 hr 1% Triton/rigor; again 50/50 glycerol/rigor overnight; and then placed in a freezer for a minimum of 2 weeks at −20°C. Myofibrils were obtained by shearing minced, glycerinated muscle in a blender (Sorvall Omni Mixer) for 5–10 s. A small drop of this suspension was placed in the sample chamber and myofibrils were allowed to settle and stick lightly to the chamber bottom where they could later be picked up by microneedles. Excess rigor solution and extraneous tissue were washed out of the chamber using several rinses of relaxing solution. Figure 1 shows electron micrographs of fixed and embedded myofibrils prepared in this manner.

Solutions

Experiments were performed at room temperature (20–22°C). All solutions were 200 mM total ionic strength in a MOPS buffer (Fabiato & Fabiato, 1979; Martyn & Gordon, 1988), pH 7.0 and contained 4 mM Na_2ATP without a regeneration system. Methane sulphonate was used as the major anion (Andrews *et al.*, 1991). Calcium concentration was controlled by an EGTA (15 mM) buffer system and 3 mM Mg. In addition, solutions contained leupeptin (20 μg ml^{-1}), aprotinin (5 μg ml^{-1}) and beta-mercaptoethanol (5 mM) as protease and oxidation inhibitors. PEG 8000 (4%, by weight) was added to some solutions as an osmotic compressive agent.

Force transducer

We have developed a sensitive force transducer to measure tension generation in single myofibrils (Fearn *et al.*, 1993). It is a modification of an optically-based transducer first described by Tung (1986). Figure 2 shows schematically how the transducer operates. An optical fibre (70 μm diameter) is used as a stiff, displaceable beam of known length and compliance. A cone of light emitted by the cleaved fibre illuminates two larger fibres. When force is exerted on the beam by the attached (glued) myofibril, this force deflects the optical fibre. Beam deflection is monitored by differentially measuring

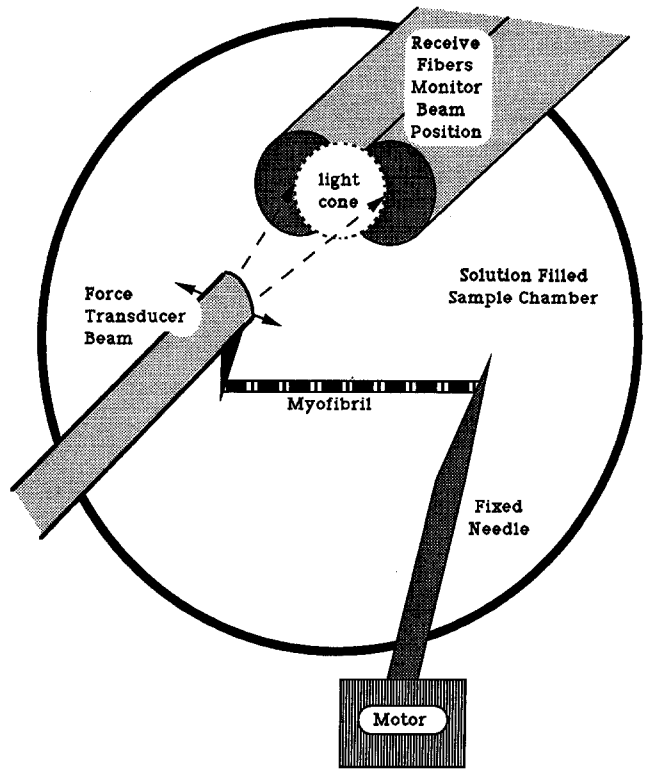

Fig. 2. Schematic of optical force transducer. Myofibril is suspended between flexible transducer beam and stiff motor needle. Deflection of the transducer beam is differentially measured by changes in illumination received by the two, larger optical fibres, giving a signal proportional to tension.

the light received by the two opposing optical fibres. Changes in output voltage are linear over a range of beam deflection more than three times that generated by typical specimens. The effects of temperature on measured tension were minimized by controlling the temperature of the specimen chamber and of the added solutions.

The force transducer was calibrated either by deflecting the beam a known distance or by applying a known force generated by a modified ammeter. Changes in output voltage could be correlated to a known change in beam position or to applied force. Utilizing the beam stiffness, length and change in measured voltage as a function of deflection or force, the absolute force generated by the attached myofibril could then be calculated (McLaughlin, 1977). Both methods of calibration gave the same results to within 10%. With a beam length of 6 mm, the resonant frequency of the transducer was typically 500 Hz and its compliance was approximately 16 nm μg^{-1} force).

Specimen mounting and control of myofibril length

The specimen was held in a temperature-controlled chamber built on a Zeiss Axiovert-35 inverted microscope. Two Narishige hydraulic micromanipulators, one controlling position of the force transducer and the other controlling position of a glass needle, were used to pick

up and hold the specimen. A piezoelectric motor, also mounted on one micromanipulator, held the stiff glass needle. Precise axial specimen movement could then be achieved by applying voltage ramps to the motor via a digital-to-analogue interface with a Macintosh II computer. A water-curing, silicone adhesive (Dow 3145 RTV) was used to glue the ends of the myofibril to the tip of the force transducer and glass needle. During all force measurements the myofibril was suspended between the force transducer and motor needle, a few micrometres above the chamber surface.

Specimen imaging

Phase-contrast optics were used to generate an image of the dark A-bands and lighter I-bands of the suspended myofibril. Z-lines were also visible. A Plan-Neofluor 100X, 1.3 NA objective was used to project the myofibril image onto a high-resolution CCD video camera (Sony XC-77RR), or a 512-element linear photodiode array (E.G. & G. Reticon Electronics, model no. RL0512K). The microscope was supported by an air suspension table to minimize the effects of extraneous vibration. A Macintosh II computer and MacAdios analogue-to-digital board were used to dynamically collect tension measurements. The striation pattern was also digitally stored if the linear photodiode array was used. When the two-dimensional CCD camera was used, the myofibril image data was stored on a S-VHS recorder (Mitsubishi BV-1000).

Sarcomere length and myofibril width were measured from data collected either by the CCD camera or the linear array. No significant differences were found between the two devices, although the linear array allowed us to increase signal integration time and thereby improve sarcomere length spatial resolution. Specimen width was determined by plotting the intensity profile across the fibril; then, pixel positions at half the maximum peak height were taken to define the two edges of the myofibril. As a control for this technique, A-band length was measured in a similar manner and found to be consistently overestimated (1.8–1.9 μm optically, in contrast to 1.65 μm using EM) (Page & Huxley, 1963).

To further characterize this overestimate of myofibril width, we compared phase contrast measurements of the average diameter of myofibrils with electron microscopy measurements of the average diameter of myofibrils in cross-sections of fixed and embedded fibres. Muscle fibres prepared for electron microscopy were from the same glycerinated muscle tissue as that used to generate myofibrils. Phase contrast measurements gave an average diameter of 1.28 ± 0.12 μm ($n = 36$) for single myofibrils, while measurements employing electron microscopy led to an average diameter of 0.83 ± 0.12 μm ($n = 25$). The value found using electron microscopy probably underestimates the true, physiological diameter because of preparation induced shrinkage. Therefore, a method to correct for the effects of shrinkage was adopted. Again using electron microscopy, we measured the average spacing between the myosin filaments and found this to be 34.6 nm. If it is assumed that X-ray diffraction of relaxed, intact muscle fibres reveals the true value of interfilament spacing, the spacing we measured should be scaled up from 34.6 nm to 42 nm (Elliot *et al.*, 1967; Schiereck *et al.*, 1992). The average myofibril diameter will similarly be scaled up to 1.01 ± 0.15 μm. Diameters of 1 μm are typical of values cited for rabbit psoas myofibrils. Comparison of this value with that measured by phase contrast shows that the optical technique overestimates myofibril width by 28%.

Passive tension measurement

Specimens were slowly stretched in relaxing solution to a selected length. They were allowed to relax for 3 min before sarcomere length and tension levels were recorded. Immediately after each measurement, the stretched myofibril was released to below rest length to re-check baseline tension of the force transducer. Each specimen could be reproducibly stretched beyond rest length many times to give an entire passive length–tension curve. However, if sarcomere length within the specimen exceeded about 3.8 μm at any time, further passive tension measurements no longer fell on the same exponential length–tension curve.

Activation protocol

Temperature changes of the bathing solution could affect measured tension in an artefactual manner. This was probably due to temperature induced variations in the dimensions and alignment of the force transducer's transmit and receive optical fibres. Protocols that minimized temperature changes during activation were used to control for this effect. Activation was performed at room temperature (20–22°C) to minimize the potential for temperature differences between added solutions and current bathing solutions. The myofibril image and generated tension were collected throughout the activation procedure. To minimize any temperature differences between the sample chamber and the incoming solutions, the relaxing solution that had been in the chamber for several minutes was replaced by fresh relaxing solution. This fresh relaxing solution and subsequent activating solution came from syringes that had equilibrated, side-by-side, to room temperature. Next, relaxing solution was exchanged for activating solution and active tension generated by the myofibril(s) was measured. Finally, activating solution was exchanged for relaxing solution and baseline tension before and after activation were compared.

Results

Specimen viability

Myofibrils prepared as described in the Materials and methods section appeared to function normally by

several criteria: the phase-microscope image of A- and I-bands exhibited good contrast; thick, thin and connecting filaments were present and well organized when examined using electron microscopy (Trombitás *et al.*, 1991); low passive tension was found in the relaxed state; and when the myofibrils were activated, high levels of force could be generated.

In some specimens, repetitive waves of contraction occurred during initial exchange of relaxing for activating solution. These oscillations occurred most frequently when the change from relaxing to activating solution was done slowly and the specimen spent more time at submaximal calcium levels. Activation with a solution of pCA 6.0 almost invariably led to such oscillations. Ishiwata and colleagues (1991), and also Fabiato & Fabiato (1978), have extensively studied this interesting phenomenon and have named it spontaneous oscillatory contractions (SPOCs). We are in agreement with these authors (Linke *et al.*, 1993) that SPOCs are not dependent on a functional sarcoplasmic reticulum but are generated by the contractile proteins themselves. Addition of 4% PEG to our solutions suppressed SPOC activity, allowing measurement of steady force generation at all calcium concentrations.

Myofibrils did exhibit some deterioration with repeated cycles of activation and relaxation. Contrast in the striation pattern decreased and sarcomere length inhomogeneity increased. The largest observable changes occurred during the first activation (discussed below). These types of deterioration are comparable with those seen in skinned fibres, as is the progressive decrease in active tension with repeated exposure to high calcium solutions. Brief ramp releases and re-stretches (Brenner, 1983) between activations appeared to minimize

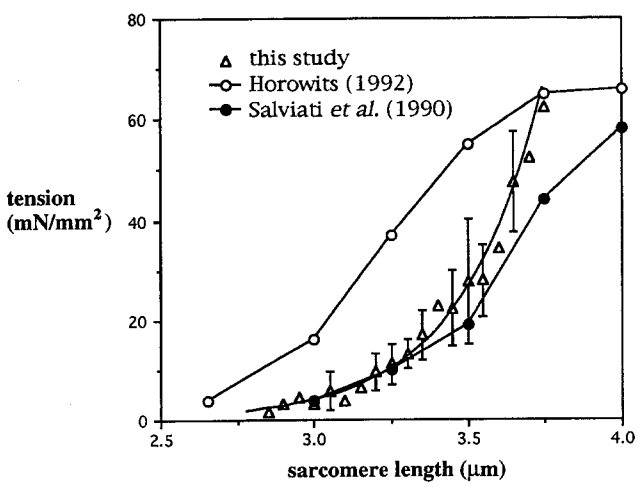

Fig. 3. Passive length-tension curve comparing measurements in myofibrils with measurements from whole skinned fibres. (△) tension in relaxed, isolated myofibrils after 3 min equilibration at each measured length; (○), (●) tension of relaxed, skinned fibres from rabbit psoas muscle.

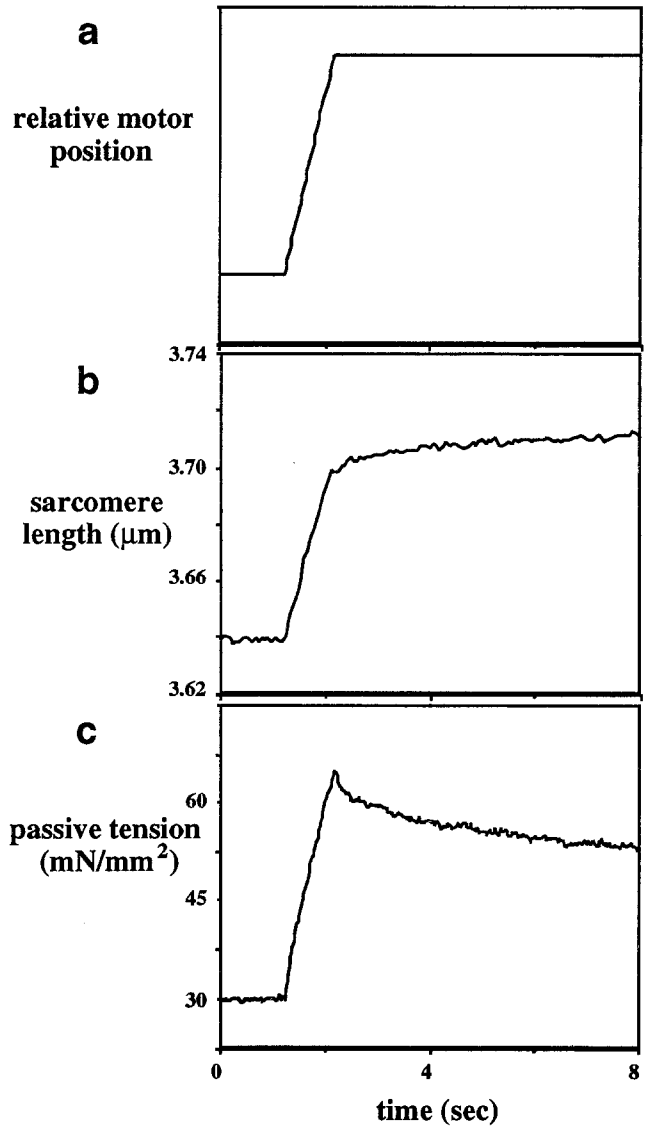

Fig. 4. Illustration of stress relaxation in relaxed, isolated myofibril. (a) Time course of imposed ramp and hold; (b) change in sarcomere length; (c) change in passive tension.

progressive sarcomere-length inhomogeneity. Uniform sarcomere lengths along the entire myofibril were usually found during the plateau of tension development when the specimen was activated at initial sarcomere lengths of 2.0–2.6 μm. Activation at longer sarcomere lengths, or repeated activations, usually led to progressive sarcomere length inhomogeneity during the active tension plateau.

Passive tension

The length–tension curve in relaxed myofibrils is shown in Fig. 3. Relatively low levels of passive tension were found for sarcomere lengths from 2.2 to 3.6 μm. The shape of the curve was similar to that reported for skinned fibres (Horowits, 1992; Salviati *et al.*, 1990). Tension levels were reproducible as long as sarcomeres were not stretched beyond about 3.8 μm. When sarcomeres were highly stretched, subsequent measurements

of passive tension at sarcomere lengths of 2.2–3.6 μm no longer fell on the same curve. No clear pattern of higher or lower passive tension was found after the myofibril had been subjected to extreme stretch.

Measurements of dynamic changes in sarcomere length and force were carried out in relaxed myofibrils. As observed in whole fibre preparations, quick ramp lengthening of the myofibril induced stress relaxation (Fig. 4). This phenomenon is characterized by a rapid increase in passive force during stretch, followed by a slow decay (10–30 s) to the expected, isometric level of passive tension. Sarcomere length changes closely

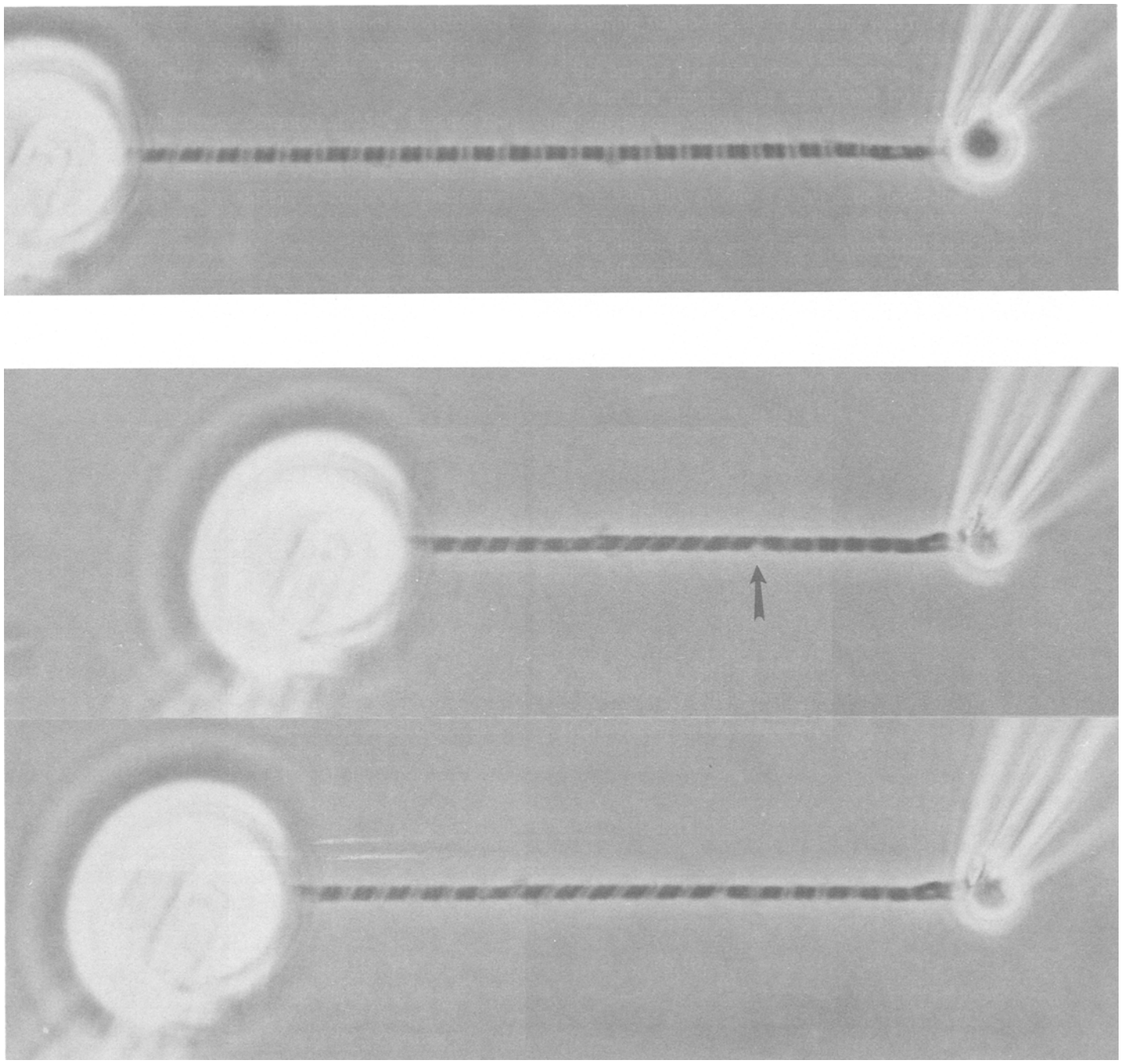

Fig. 5. Photomicrograph of single myofibril held between rigid motor needle and force transducer. (a) Relaxed myofibril that has been stretched; (b) activated myofibril (from shorter sarcomere length than in a). Note nick in centre (arrow) that apparently led to slight skewing of central sarcomeres but does not affect overall sarcomere length homogeneity; (c) same myofibril as in b, now relaxed. Sarcomere length is now slightly longer as compliance in the force transducer re-stretches specimen.

followed the imposed ramp stretch (Fig. 4a and b); then, while specimen length was held constant after stretch, sarcomeres lengthened slightly (< 15 nm/sarcomere) and tension decreased by 20–25%. The 15 nm increase in sarcomere length was caused by compliance in the force transducer, which had to move slightly, lengthening the specimen, in order to register the large decrease in tension during stress relaxation.

Active tension

All reported tension values are from specimens that exhibited a uniform striation pattern along their length of 20–30 sarcomeres in the relaxed state (Fig. 5a). We discarded myofibrils whose sarcomere lengths varied by > 10%, assuming that such variation indicated damage to the specimen during isolation or mounting. Specimens were stretched in the relaxed state so that all sarcomeres were in the range of 2.2–2.8 μm. Because some or all sarcomeres visibly shortened to 1.8–2.4 μm on activation (Fig. 5b and c), tension generation measured in myofibrils was assumed to correspond to maximal overlap of thick and thin filaments. Maximally activated (pCa 5.5 to 4.5) tension levels ranged from 0.34 to 0.94 N mm^{-2}, with a mean value of 0.59 ± 0.13 N mm^{-2} (13 specimens, 65 activations).

When myofibrils were activated, the contractile response was dependent on initial sarcomere length. If initially activated at sarcomere lengths of ⩾2.7 μm (Fig. 6), one group of sarcomeres usually shortened to sarcomere lengths of 1.8–2.2 μm, while the remaining sarcomeres were visibly stretched to variable lengths (Fig. 6b and c). During the plateau of active tension no further changes of sarcomere length were measurable (Figure 6d–f). Once the specimen was again relaxed, the group that had shortened during activation re-stretched but to only about 2.4 μm sarcomere length instead of 2.5–2.8 μm (Fig. 6g and h). In later activation cycles the same group of sarcomeres consistently shortened each time, retaining a clear striation pattern that was particularly visible when the specimen was relaxed. Sarcomeres that had been stretched during activation often had a skewed or over-stretched appearance both in the relaxed (after the first activation, Fig. 6a) and activated state. With repeated activations, the group of 'actively shortening' sarcomeres exhibited shorter and shorter sarcomere lengths when relaxed, while the stretched sarcomeres slowly lengthened; when this redistribution of sarcomere lengths became pronounced, active tension decreased.

Myofibrils that were activated at sarcomere lengths below about 2.6 μm, by contrast, usually contracted homogeneously (Fig. 7). All sarcomeres along the specimen shortened slightly (usually 20–100 nm per sarcomere). Specimens that contracted homogeneously may have generated higher levels of tension than their more disordered counterparts, 0.64 versus 0.57 N mm^{-2} but the increase is not statistically significant. Even myofibrils

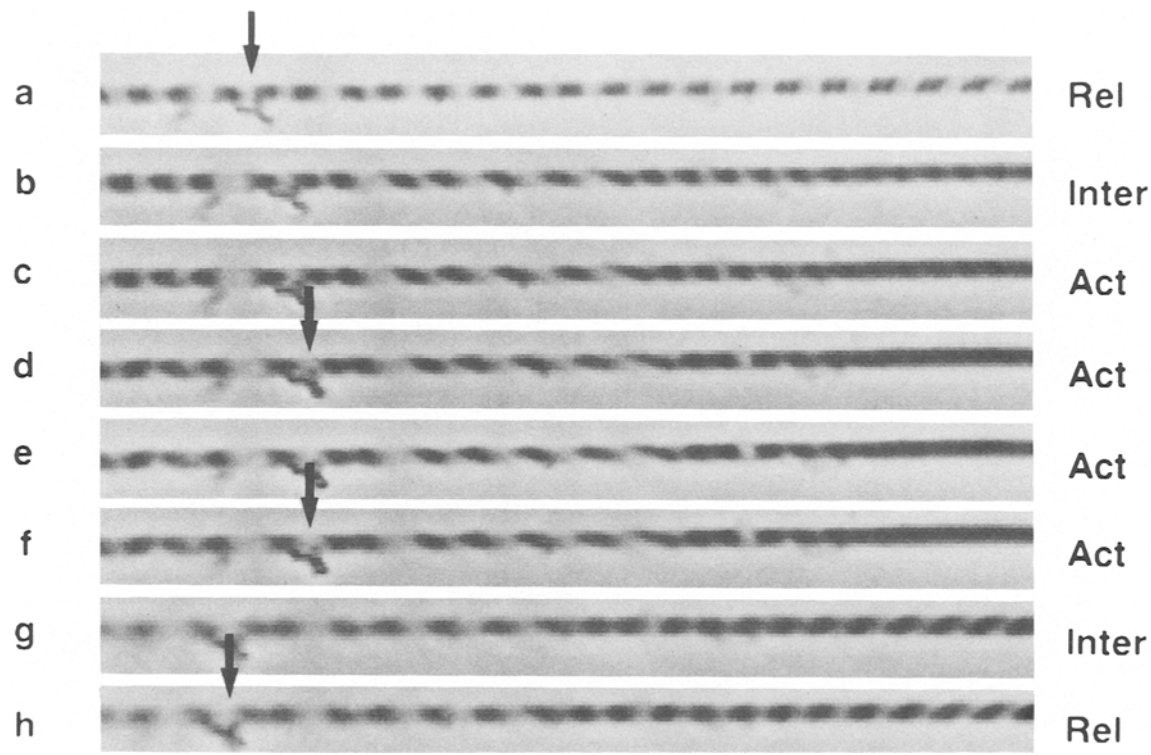

Fig. 6. Series of video images taken during activation of myofibril doublet. Rel = relaxed myofibrils; Inter = partially activated; Act = activated with pCa 5.5. Arrow indicates position of natural marker that translated during activation, remained stationary during the plateau of active tension and returned to a slightly different position when the myofibrils were again relaxed.

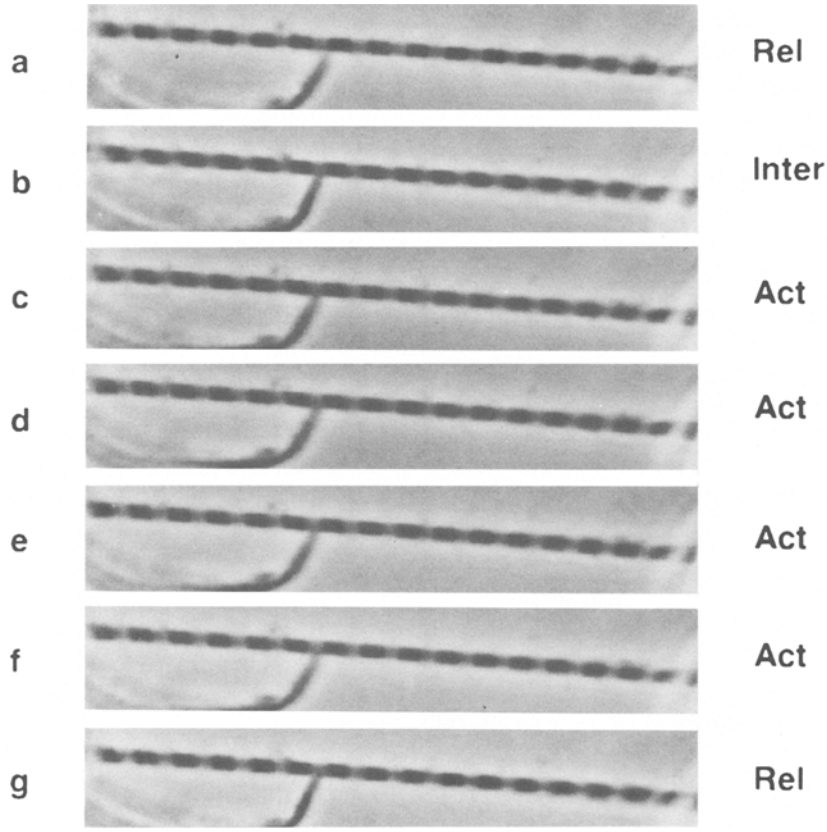

Fig. 7. Series of video images taken during homogeneous activation of single myofibril. Rel = relaxed; Inter = partially activated; Act = activated with pCa 5.5. Initial sarcomere length of 3.00 μm (a), while sarcomeres shortened slightly to 2.95 μm during the active plateau (c–e). The slight skewing of the A-bands during activation (and increase in measured tension) was the only visible sign of contraction. The force transducer needle (right side), shifted < 1 μm during contraction. Homogeneous contractions at this long sarcomere length are rare and the next contraction from this specimen was inhomogeneous while active tension remained nearly identical.

that contract homogeneously at short sarcomere lengths usually became inhomogeneous during contractions at longer sarcomere lengths. As with specimens that initially exhibited a range of sarcomere lengths during contraction, the distribution of lengths is not bimodal. Along with a population of sarcomeres that shortened to lengths of 1.8–2.4 μm, other sarcomeres were stretched and remained isometric at intermediate lengths of 2.5–3.6 μm during many seconds of activation.

Specimens varied in the number of reproducible activation cycles they could endure. Rarely, only two or three measurements could be obtained from a specimen. This was often due to the myofibril pulling free of the glue on subsequent activations, or even breaking in half during activation. However, the bulk of specimens maintained a healthy striation pattern for four to eight activation cycles, while one specimen generated high force for 14 sequential cycles before force significantly deteriorated.

Six specimens (44 activations) were used to measure an approximate force-pCa relation in myofibrils, as shown in Fig. 8. pCa 4.5–5.5 fully activated the myofibril, while pCa 6.0 consistently gave about one-third of maximal force. The importance of this plot is not its accuracy in measuring the Hill coefficient (about 3.3) or pCa_{50} (about 5.9) but simply the fact that myofibrils exhibit calcium sensitivity that is consistent with expectations from larger preparations. Previous studies (Brandt *et al.*, 1984; de Beer *et al.*, 1988; Nosek *et al.*, 1990) have reported similar force-pCa curves in skinned psoas fibres. No

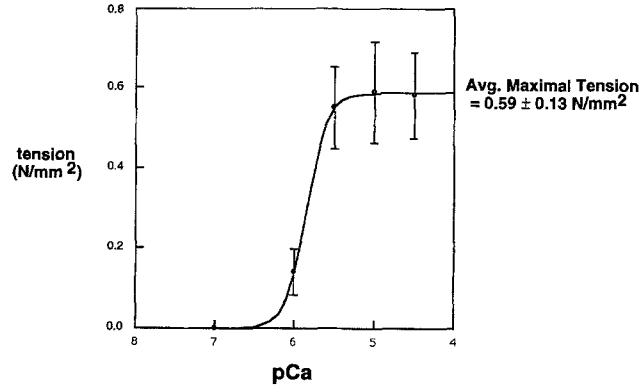

Fig. 8. Force-pCa curve for isolated myofibrils. Line was fit using Hill equation.

significant difference was found in developed tension between pCa 5.5 and 4.5; thus, our maximal tension measurements were made over this range of calcium concentration.

Representative tension traces are shown in Fig. 9 for four sequential activations of one specimen. To minimize temperature-dependent artefacts during an activation sequence, baseline tension was first measured (leftmost arrow) after exchange of relaxing for fresh relaxing solution. All new solutions were injected from syringes that had equilibrated to room temperature. In Fig. 9, arrows denote the beginning of the stable plateau just following each solution exchange. The first plateau of relaxation was followed by addition of activating solution (downward deflections after first plateau) and then a plateau of active tension generation (centre arrow). When the activating solution was exchanged for relaxing solution again (second group of downward deflections), tension returned to its previous baseline value (rightmost arrow followed by postactivation plateau). The large fluctuations in tension were caused by solution flow during solution pCa changes. Because solution exchanges were performed manually, the time course of the four traces is different and does not reflect any changes in contractile behaviour. It is clear that levels of active tension were nearly identical in the four traces.

During active tension generation, force did not decrease for tens of seconds. Specimens could remain steadily activated for minutes at a time but possible drift in force-transducer baseline precluded the use of such long duration protocols. In Fig. 9, baseline tension was at the same level before and after activation in each of the

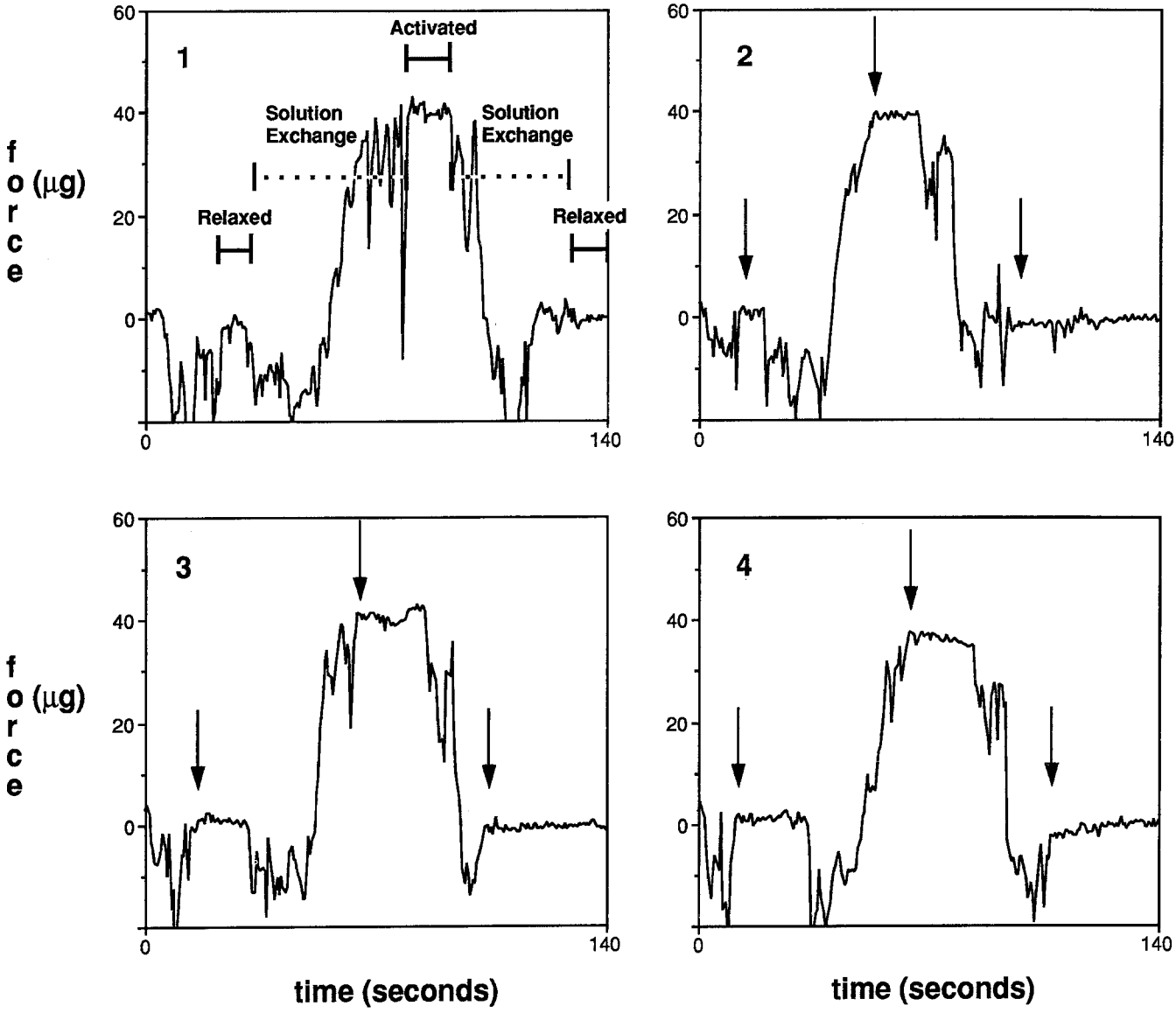

Fig. 9. Force traces of developed tension from four sequential activations of a single myofibril. Arrows indicate onset of pause in solution flow (large downward deflections) during which baseline and active tension levels were determined. Myofibril diameter was 0.97 μm.

force traces. Changes in relaxed tension before and after activation were usually $<10\%$ of maximal tension in these 140 s protocols. However, we sometimes found slightly lower passive tension after activation than before activation; this was probably caused by residual temperature differences between the newly added solution and the temperature of solution in the specimen chamber. In these cases, active force was calculated as the average of the difference between the active plateau and the two relaxed plateaus.

Discussion

Reliability of measurements

Clearly, force per cross-sectional area in all of these reports is highly sensitive to the accuracy of specimen diameter measurements. This is particularly critical with thin myofibrils. It was found that measurements using the phase image led to an overestimate of specimen width by 28% when compared with measurements made by electron microscopy and with reported values of interfilament spacing by X-ray diffraction (see Materials and Methods). We scaled our optical measurements by this factor to more accurately represent cross-sectional area. A second source of error, which would tend to increase our relative tension measurements, was left uncorrected. This second source of error was due to the fact that myofibrils are rarely perfectly circular in cross-section. When mounted, myofibrils often were twisted slightly, appearing as a gentle narrowing and widening along their length. In all cases, we measured the largest diameter observed along the entire myofibril and calculated cross-sectional area with the assumption that the myofibril was a simple cylinder. No effort was made to calculate the true, elliptical cross-section.

Finally, in comparing tension generation in myofibrils with that in larger specimens, an estimate must be made of the nonmyofibrillar area of the fibre cross-section. Eisenberg and colleagues (1974, 1976; also Mobley & Eisenberg, 1975) have measured the apparent myofibrillar space in intact specimens (frog and guinea pig) and found it to range from 83 to 92% of the cross-sectional area. If the fibre were composed of myofibrils alone, its effective tension level would obviously increase. Using the generous assumption of 20% nonmyofibrillar area, tension generation in intact and skinned fibres should be scaled up by 25% to compare with tension generation in myofibrils. All literature values for active tension cited in this paper have been scaled upward using this correction. Additional overestimates in cross-sectional area are likely to be present in data from skinned fibres that have not been osmotically compressed to their original volume. However, such errors in skinned fibre measurements cannot be reliably estimated here.

Tension measurements are also sensitive to the accuracy of force calibrations. The force transducer was calibrated in two ways with consistent results (for details see Fearn *et al.*, 1993). Its simplicity of design leaves little room for unsuspected artefact. In addition, measurements of passive force per unit area (discussed in next section) fell between values reported for skinned whole fibres. The similarity of our passive tension measurements with literature values argues against a systematic error in our force measurements.

Passive tension

When divided by cross-sectional area, passive force in myofibrils was found to lie between two curves reported for skinned psoas fibres. The exponential shape of the curve (Fig. 3) was similar to that measured in both skinned and intact fibres. Measurements of stress relaxation were also consistent with observations from whole fibres. Following quick stretch, passive tension declined rapidly with little change in sarcomere length. This observation supports the hypothesis that stress relaxation is due to an internal rearrangement of sarcomeric proteins, possibly connecting filaments.

Passive tension was reproducible at any given sarcomere length, regardless of whether sarcomere length was achieved by a slow stretch or slow release before the measurement (data not shown). However, if the sarcomeres within the preparation had been stretched beyond overlap of thick and thin filaments at any time, passive tension measurements no longer fell on the same curve. This observation is consistent with the report of Trombitás and colleagues (1991; also Maruyama *et al.*, 1989) that beyond about 3.8 μm sarcomere length, antibodies to epitopes on the connecting filament (e.g. Sigma T-11) are pulled irreversibly away from the A-band, while at shorter sarcomere lengths the antibodies remain at a fixed distance from the ends of the A-band. Such an irreversible change in epitope position is indicative of a change in connecting-filament conformation; this would be reflected in changes in passive tension if the connecting filament is the major source of such tension.

Active tension

Levels of active tension in isolated myofibrils were twice the typical values of 0.20 N mm^{-2} (scaled up by assuming 20% nonmyofibrillar cross-sectional area) found in skinned psoas fibres (e.g. Martyn & Gordon, 1988). Skinned frog and rabbit soleus fibres have also been reported to generate (scaled up) tension in the range of 0.18–0.28 N mm^{-2} (Hellam & Podolsky, 1969; Julian & Moss, 1981, 1984; Ferenczi *et al.*, 1984; Krasner & Maughan, 1984). Two exceptionally high tension values have been reported by Iwazumi and Pollack (1981) (0.36 N mm^{-2} for mechanically skinned rabbit soleus fibres at long sarcomere length) and by Nosek and colleagues (1990) (0.41 N mm^{-2} for rabbit psoas at unspecified length and elevated pH). But even these exceptional values are still lower than the mean value we found in single myofibrils.

Maximal tension developed by intact fibres, while generally higher than that generated by skinned fibres, is significantly lower than in isolated myofibrils. Typical tension levels reported for intact frog fibres, even when corrected for a nonmyofibrillar space of 20%, are 0.28–0.38 N mm^{-2} (Gordon *et al.*, 1966; Edman, 1971; ter Keurs *et al.*, 1978; Moss, 1979); the tension of 0.59 N mm^{-2} found in myofibrils is higher. Measurements by Iwazumi (1987b) support our findings of high tension generation in myofibrils: the bullfrog atrial myofibrils used in Iwazumi's study were approximately 1 μm diameter (Iwazumi, personal communication), and the reported force values lead to tension of 0.40–0.50 N mm^{-2}

If, indeed, our tension measurements offer an accurate assessment of the maximal force-generating ability of the contractile proteins, why do these diminutive specimens out-perform both skinned and intact fibres? In comparison with skinned fibres, one advantage of isolated myofibrils may be their small size. A single or doublet myofibril, with its 1–2 μm diameter, is unlikely to be diffusion limited either for entrance of ATP, or egress of inorganic phosphate. It has been widely reported that inorganic phosphate can suppress active tension levels in skinned fibres (Nosek *et al.*, 1987; Chase *et al.*, 1992; Martyn & Gordon, 1992). Within the isolated myofibril, any phosphate compounds generated should be in rapid equilibrium with the external bathing medium, and no force suppression should occur. A second cause of decreased tension in skinned fibres could arise from a calcium gradient from the periphery to the core (least activated) of the specimen. Similar gradients may be hypothesized for ATP, even in the presence of a regenerating system. In support of diffusionally generated gradients, Elzinga and colleagues (1989; also Lin *et al.*, 1988) have measured force per cross-sectional area in skinned fibres and found evidence of decreasing tension with increasing fibre diameter. Elzinga and colleagues concluded that the accumulation of orthophosphate ion within contracting segments could account for a significant part of the decline in relative force in thicker segments.

More of a puzzle is the ability of isolated myofibrils to generate higher tension than intact fibres. Intact fibres are assumed to be fully activated when the tetanus is 'fused', and no further fluctuations in tension are visible. However, there is evidence that tetanically stimulated intact fibres may exhibit partial activation. Sharnoff and colleagues (1984) reported that, as contraction began, electrically-stimulated intact fibres showed discrete regions of activation. Their holographic images, which detected minute changes in light scattering in the plane parallel to fibre length, showed long, narrow regions of localized contractile activity. These thin, separated streaks of motion were interpreted to mean that myofibrils were activated discretely, each along most of its length. Localized activation was also found by Rozycka and Gonzalez-Serratos (1993). They measured fluorescent calcium-indicator images within intact, electrically stimulated fibres. In cross-section, they found distinct regions of high free calcium side-by-side with regions of submicromolar calcium. Furthermore, it has been widely observed that higher tension can be produced in fused tetani by increasing the rate and amplitude of stimulation. If tetanically stimulated fibres are not fully activated, this may explain why force per myofibril is lower than in fully activated, single myofibrils.

Thus, there is evidence that measurements of tension generation in muscle fibres do not reflect the full force-generating potential of the contractile proteins. Skinned fibres may be incompletely activated, or they may have their force generating ability compromised by buildup of hydrogen-ion or phosphate. In intact fibres, active tension may be generated by only a subset of myofibrils. Isolated myofibrils, on the other hand, are forced into a fully activated state when the surrounding calcium concentration increases; their small diameter precludes significant gradients of ATP hydrolysis products which might otherwise limit their maximal force-generating ability. Isolated myofibrils, though fragile, may be ideally suited specimens for the study of exposed and accessible contractile machinery, with the advantage of maintaining the constituent proteins in their natural structural organization.

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