Spontaneous oscillation of tension and sarcomere length in skeletal myofibrils

Microscopic measurement and analysis

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ABSTRACT We have devised a simple method for measuring tension development of single myofibrils by micromanipulation with a pair of glass micro-needles. The tension was estimated from the deflection of a flexible needle under an inverted phase-contrast microscope equipped with an image processor, so that the tension development is always accompanied by the shortening of the myofibril (auxotonic condition) in the present setup. The advantage of this method is that the measurement of tension (1/30 s for time resolution and about 0.05 μg for accuracy of tension measurement; 0.05 μm as a spatial resolution for displacement of the micro-needle) and the observation of sarcomere structure are possible at the same time, and the technique to hold myofibrils, even single myofibrils, is very simple. This method has been applied to study the tension development of glycerinated skeletal myofibrils under the condition where spontaneous oscillation of sarcomeres is induced, i.e., the coexistence of MgATP, MgADP and inorganic phosphate without free Ca^{2+}. Under this condition, we found that the tension of myofibrils spontaneously oscillates accompanied by the oscillation of sarcomere length with a main period of a few seconds; the period was lengthened and shortened with stretch and release of myofibrils. A possible mechanism of the oscillation is discussed.

INTRODUCTION

The molecular mechanism of muscle contraction and tension generation has been studied in detail for several decades by using intact, or mechanically or chemically skinned single fibers (a few tens of μm in diameter) (cf Cooke, 1986; Goldman and Brenner, 1987; Pollack, 1990). However, such an approach has several disadvantages for the study of structure and function of muscle; first, it is difficult to change quickly or even to maintain the internal chemical environment because of slow diffusion of chemical substances. Second, it is difficult to analyze the fine structure of sarcomeres, e.g., the optical properties of constituent proteins, with high spatial resolution under an optical microscope. Although the former problem has been partly overcome by the recent introduction of caged compounds (Goldman and Brenner, 1987; Homsher and Miller, 1990), it will be difficult to resolve the latter one.

Recently, Kishino and Yanagida (1988) succeeded in measuring the tension (of the order of one ng) imposed on a single actin filament by using a pair of glass micro-needles and an optical system which detects a displacement of the needle with a spatial resolution of 0.1 nm under an optical microscope (Kamimura, 1987; Iwazumi, 1987*a*). This system and such an in vitro reconstituted system (e.g., Chaen et al., 1989) are undoubtedly powerful techniques to elucidate the function and the mechanism of a minimum motile unit consisting of actin and myosin molecules.

On the other hand, from a physiological point of view, a single myofibril is considered to be the minimum structural and functional unit of muscle. This implies that the physiological function of myofibrils may not be a simple summation of the function of each motile unit; to elucidate the molecular mechanism of contraction and force generation, we will finally need to investigate the structure and function of motile units in an organized contractile system of muscle, i.e., a myofibril.

Here, we have devised a simple method appropriate for investigating both the mechanical properties, i.e., active or resting tension or stiffness, of myofibrils and their fine structure at the same time under an inverted optical microscope equipped with a micromanipulator. It seems that our method is simpler than the previous ones (Borejdo and Schweitzer, 1977; Iwazumi, 1987*a* and *b*; Bartoo et al., 1988).

We applied this technique and setup to measure the generated tension of single myofibrils and small bundles of myofibrils having a width of 1–10 μm, especially under conditions where the length of every sarcomere spontaneously oscillates (so-called SPOC condition, in which high concentrations of MgADP and Pi coexist with MgATP; Okamura and Ishiwata, 1988; Ishiwata et al., 1991). It was recently found that the tension spontaneously oscillates in single fibers under the SPOC condition (see the preceding paper; Shimizu et al., 1992). However, in the fiber system, not only is the wave form

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of tension oscillation complicated, but also it is difficult to measure the oscillation of sarcomere lengths under an optical microscope. The present study has demonstrated that those difficulties can be overcome. A preliminary report of this work has been presented (Anazawa et al., 1990).

MATERIALS AND METHODS

Solutions

Rigor solution, 60 mM KCl, 5 mM $MgCl_2$, 10 mM Tris-maleate (pH 6.8) and 1 mM EGTA; relaxing solution, 0.12 M KCl, 4 mM $MgCl_2$, 4 mM ATP, 20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS, pH 7.0) and 4 mM EGTA; contracting solution, 0.12 M KCl, 4 mM $MgCl_2$, 4 mM ATP, 20 mM MOPS (pH 7.0), 2 mM EGTA, and 1.9 mM $CaCl_2$; SPOC solution, 0.12 M KCl, 4 mM $MgCl_2$, 0.2 mM ATP, 4 mM ADP, 4 mM K-Pi, 20 mM MOPS (pH 7.0), and 4 mM EGTA. The pH value was adjusted for each preparation of solution. ATP and ADP were purchased from Boehringer Mannheim GmbH (Mannheim, Germany); MOPS was from Dojindo (Kumamoto, Japan). Other chemicals were of reagent grade.

Preparation of myofibrils

Single myofibrils and small bundles of myofibrils were prepared by gently homogenizing rabbit psoas glycerinated muscle fibers as described previously (Ishiwata and Funatsu, 1985), except that 1 mM leupeptin was added to 50% (*v*/*v*) glycerol, 0.5 mM $NaHCO_3$, and 5 mM EGTA to suppress the proteolysis of parallel elastic components during glycerination (Funatsu et al., 1990). Glycerol and undissolved large aggregates in the suspension of myofibrils were removed by low-speed centrifugation in the rigor solution at 2°C. The protein concentration of the suspension of myofibrils was estimated by means of the biuret reaction.

Perfusion chamber and manipulation of myofibrils

The main part of the perfusion chamber with a size of 24 × 60 × 1 mm was made of Lucite (Fig. 1). The open base of the chamber was sealed with a cover slip (24 × 60 mm), of which the outer part was fixed to the Lucite with Vaseline. A suspension of myofibrils was allowed to settle in the chamber, then a pair of glass micro-needles were put down and the upper surface of the solution was covered with a cover slip (22 × 22 mm), which is indispensable for obtaining a clear image by phase-contrast microscopy. The solution, of which the effective volume in the chamber was 50 μl, was exchanged by continuously sucking it up from an outlet through a peristaltic pump and adding fresh solution from an inlet with a micropipette; because of the small space between the upper cover slip and the outlet (or inlet), the volume of solution (~50 μl) in the space under the cover slip remained nearly constant even if the solution was strongly sucked up; because only the solution that overflowed from the space was sucked up, steady exchange of solution was made possible. Because the micro-needles moved slightly during flow of the solution, the exchange of solution was not performed during experiments.

A pair of glass micro-needles were fixed with epoxy resin to glass rods of which the thickness at the tip was ~0.3 mm. The size of the micro-needles was 5–15 μm thick and 0.3–1.5 mm long, depending on the required stiffness. The glass rods were each connected to a micromanipulator, i.e., the one with a flexible micro-needle was coupled to a water-pressure-controlled 3-D-micromanipulator (WR-90, Narishige, Tokyo; its drift with time is much smaller than that of an oil-pressure-controlled one, within one pixel on a TV monitor [~50 nm] in 1 min irrespective of imposed load) and the other with a stiffer micro-needle to an oil-pressure-controlled 3-D-micromanipulator (MO-188, Narishige). Myofibrils which are sticky to glass surface were attached to a pair of glass micro-needles, perpendicularly to the micro-needles, as shown in the enlarged figure in Fig. 1 and as described in the Results section. The stiffness (Hooke's elastic constant) of glass micro-needles used in the present work was determined under an optical microscope to be ~300 μg/μm for a stiffer needle by direct measurement and 1.06 μg/μm for a flexible needle by cross-calibration (Kishino and Yanagida, 1988; Ishijima et al., 1991). We confirmed that the deflection of the flexible micro-needle disappeared without oscillation (over-damping) within 1/30 s, indicating the characteristic frequency of the needle was larger than 30 Hz, which is the limit of time resolution in our system.

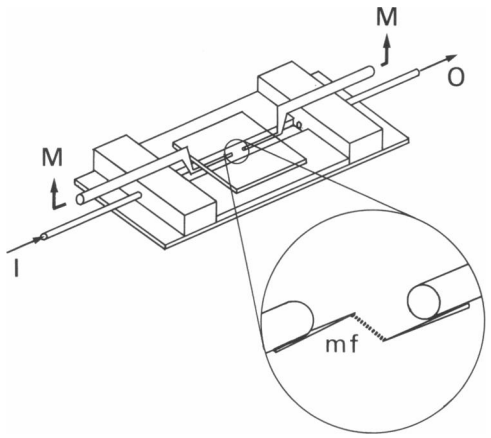

FIGURE 1 Schematic view of a perfusion chamber and a pair of glass micro-needles holding a myofibril (*mf*). Solution is put in through an inlet (*I*) and sucked up through an outlet (*O*). Both micro-needles are attached to micromanipulators (*M*). For details, see Materials and Methods.

The perfusion chamber was set on the stage of an inverted microscope (DIAPHOT-TMD, Nikon Co., Ltd., Tokyo), on which the micromanipulators were mounted. For holding myofibrils, a dry type of phase-contrast objective lens (Plan 40 × DM, Nikon) was used and for measuring tension, an oil immersion type of phase-contrast objective lens (Plan Apo 60 × oil DM, NA = 1.40, Nikon) was used to obtain a higher spatial resolution.

Measurement of tension and sarcomere length

A schematic diagram showing the system for microscopic analysis of myofibrils is shown in Fig. 2. A single myofibril or a small bundle of myofibrils was held by a pair of glass micro-needles as described in Results and the image was monitored by a CCD camera (C3077, Hamamatsu Photonics K.K., Hamamatsu, Japan) equipped with a TV zoom lens (0.9 – 2.25×, Nikon, Tokyo); a stiffer needle was used for the manipulation, i.e., stretching and imposing a load on myofibrils, and a flexible one was used for tension measurement. The displacements of both needles were measured by use of a double-channel position detector (Width analyzer C3161, Hamamatsu Photonics K.K.); to increase the accuracy of the measurement, the positions of

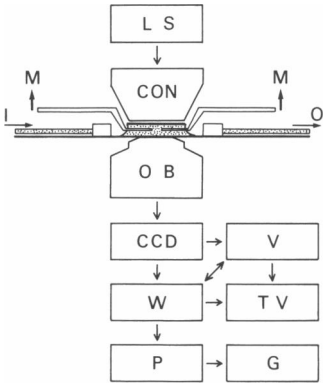

FIGURE 2 Schematic diagram showing a system for microscopic analysis of myofibrils. A cross-section of a perfusion chamber is illustrated. (*LS*) light source; (*CON*) condenser lens; (*OB*) objective lens; (*CCD*) CCD camera; (*V*) videotape recorder; (*W*) width analyzer; (*TV*) TV monitor; (*P*) personal computer; (*G*) graphic display; (*M*, *I*, and *O*) the same as in Fig. 1. For details, see Materials and Methods.

the edge of the needle were averaged along the needle within the window (cf Fig. 4 *b*). When the objective lens of 60× was used, the spatial resolution was ~50 nm (distance between adjacent pixels). The time resolution was 1/30 s. Data were analyzed in real time with a personal computer (PC-9801, NEC Inc., Tokyo) to obtain the developed tension (= [displacement of the flexible needle from an equilibrium position] × [Hooke's elastic constant of the needle]) and the average sarcomere length (= [total length of myofibril, i.e., the distance between the two micro-needles]/[number of sarcomeres]). In the present work, the accuracy of tension measurement was ~0.5 μg (= 0.05 μm [distance between adjacent pixels] × 1.06 μg/μm (Hooke's elastic constant of the flexible needle)). Images stored on a video tape (S-VHS; recorder HV-V1000, Mitsubishi Electric Co., Ltd., Tokyo) can also be analyzed afterwards through the width analyzer and computer (cf Fig. 2), though the spatial resolution is halved (~0.1 μm).

Analysis of oscillation wave of tension

The wave form of tension oscillation with time was analyzed by FFT and the power spectrum was obtained. Data were accumulated every 30 ms for ~31 s (total of 1,024 data points) under each experimental condition. The major peak at the lowest frequency region was considered to correspond to the fundamental period of oscillation. Practically, we determined the fundamental period (T) as the inverse of the weighted average of frequencies in the peak zone located at the lowest frequency region of power spectra, i.e., $1/T = (\Sigma_i \text{ amplitude} \times \text{frequency})/(\Sigma_i \text{ amplitude})$, where the summation was taken over the frequencies of which the amplitudes were > 30% of the largest amplitude in the peak.

RESULTS

How to hold both ends of myofibrils

A suspension of myofibrils (~0.1 mg/ml) in rigor solution was put in the chamber and washed with the rigor solution; this washing did not shift myofibrils attached to the glass surface, but floating myofibrils which would disturb the manipulation of the micro-needles were removed. Then, a pair of glass micro-needles were slowly inserted into the solution vertically to the stage and placed at ~100 μm above the glass surface. The upper surface of the solution was covered with a cover slip (24 × 24 mm).

Myofibrils suitable for experiments, i.e., those in which striated structure looks regular with sharp *Z*-lines but neither distorted nor twisted and only one end was attached to a glass surface whereas the other end was detached, were selected by moving the stage of the optical microscope. We can use any size of myofibrils for experiments, even a single myofibril thinner than 1 μm if it is observable.

A single myofibril or a small bundle of myofibrils thus selected was held by a pair of glass micro-needles according to the procedure schematically shown in Fig. 3. First, one of the micro-needles (L) was inserted beneath the floating end of the myofibril and slightly drawn up; because myofibrils adhere to glass, the end of the myofibril was loosely attached to the needle by this procedure. Another micro-needle (R) was touched onto the other end of the myofibril from above (Fig. 3 *a*). Then, the needle L was carefully moved along the arrow without slackening but without over-stretching the myofibril; it has been pointed out that there is a possibility

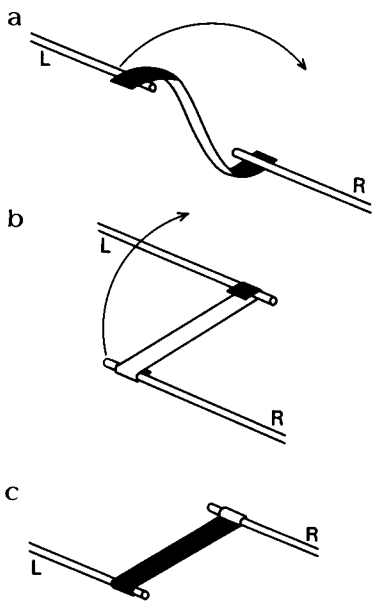

FIGURE 3 Schematic illustration showing the procedure (*a* to *c*) of holding both ends of myofibrils with a pair of micro-needles (L and R) under an inverted phase-contrast microscope. For details, see the text.

that myofibrils are damaged by over-stretching (Iwazumi, 1987*b*). The same procedure was repeated once more for the needle *R* (Fig. 3 *b*). The myofibril was thus twined around a pair of micro-needles (Fig. 3 *c*); this procedure is similar to that used to hold the ends of smooth muscle (cf Warshaw and Fay, 1983). Finally, both needles were placed at >10 μm above the bottom glass surface and set so as to make the myofibril parallel to the optical stage and perpendicular to both needles. A phase-contrast micrograph of a single myofibril held by needles is shown in Fig. 4. The ordered structure of sarcomeres appears to be intact, extending over the whole myofibril (Fig. 4 *a*).

Measurement of tension development

The tension generated by myofibrils was measured by detecting the displacement of the flexible micro-needle by means of image processing (see Fig. 4 *b*). Fig. 5 is an

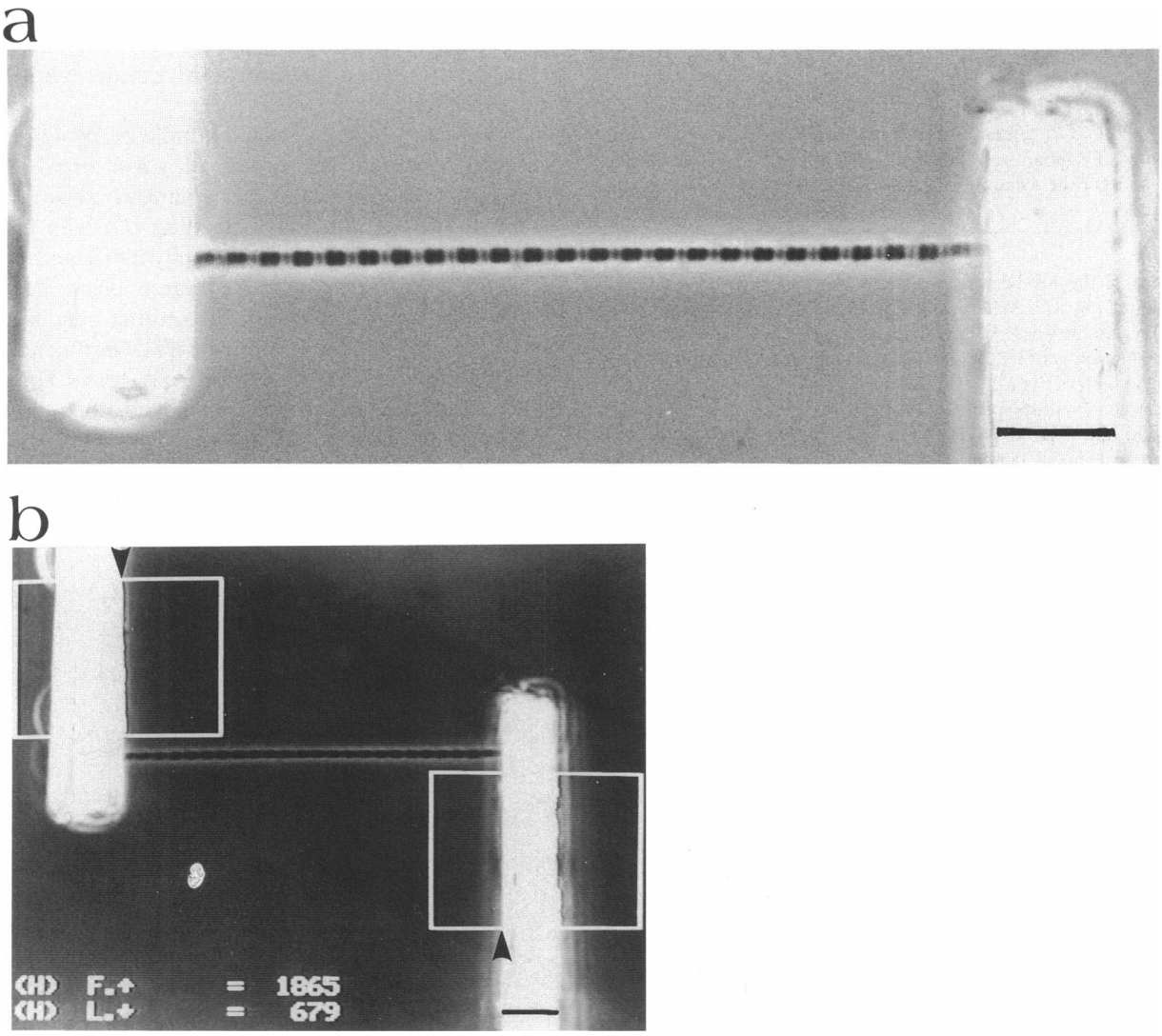

FIGURE 4 Optical micrograph of a single myofibril mounted in the perfusion chamber in rigor solution. (*a*) Phase-contrast micrograph of a single myofibril of which both ends were fixed to a pair of glass micro-needles, taken on Kodak Plus-X 35 mm photographic film. Scale bar, 10 μm. (*b*) The image of *a* taken by a CCD camera and processed by the width analyzer was displayed on a TV monitor (PVM-1442Q, Sony, Tokyo). The positions of the two micro-needles, i.e., the right-hand edge (*L*, *downward arrowhead*) of the left needle and the left-hand edge (*F*, *upward arrowhead*) of the right needle, were detected by setting a threshold for brightness in two windows. In the windows, black-and-white pictures are superimposed on a phase-contrast image. The positions of *F* and *L* are also indicated by the use of 2560 pixels (1280 pixels for a video tape image) on the monitor after averaging the position of the edge for each needle. Scale bar, 10 μm. For more details, see Materials and Methods.

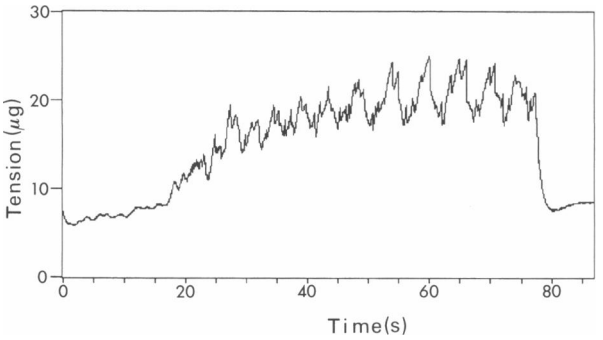

FIGURE 5 Time course of tension development under a SPOC condition. At time zero, injection of SPOC solution was started into the perfusion chamber in place of relaxing solution; after the steady SPOC was attained, relaxing solution was injected again. There was a lag time of ~3 s for exchanging solutions. A small bundle of myofibrils, ~3 μm thick and 36 sarcomeres long, was used. The average sarcomere length changed between 2.4 μm and 2.0 μm. For conditions, see Materials and Methods.

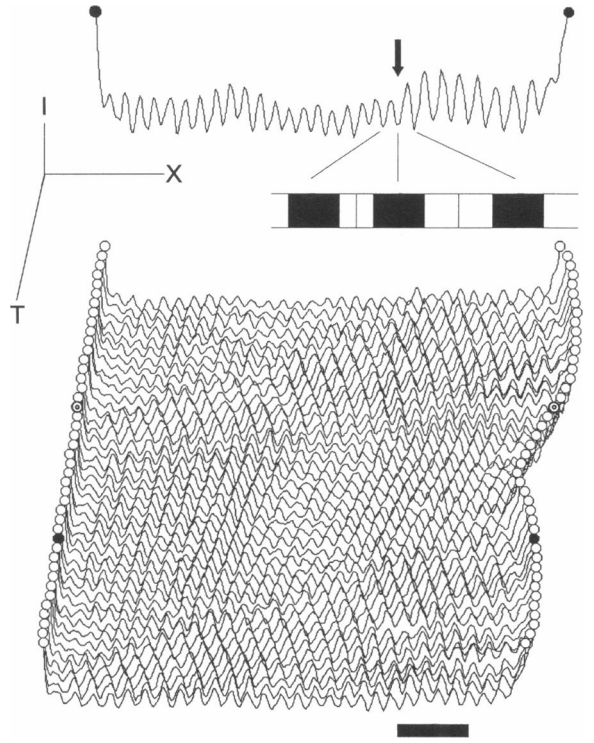

FIGURE 6 Time course of image profile of myofibrils showing propagation of a SPOC wave. *T*, *X*, and *I* axes, respectively, represent time course of SPOC, position along a myofibril and brightness of the phase-contrast image. Two circles at both sides correspond to the edges of the micro-needles. Serial images (every 1/15 s from top to bottom) show that the lengthening phase of (half) sarcomeres propagates with time from right to left along the long axis of the myofibril (see from the side). It can be seen that all the sarcomeres entered a shortening phase at a moment indicated by two double circles. In this example, a small bundle of myofibrils (~5 μm thick, 31 sarcomeres long) composed of several single myofibrils was used to obtain a typical organized state of SPOC. The needle at the left-hand side is stiff, so that the developed tension is represented by movement of the flexible needle on the right-hand side. An image profile of a myofibril corresponding to that with two filled circles is shown at the top of the figure as a typical example where the lengthening phase and shortening phase coexist, the boundary between them being indicated by an arrow; a peak and a valley of the image profile, respectively, correspond to the *I*-band and the *A*-band of the sarcomere as schematically shown, where the degree of lengthening and shortening of sarcomeres is exaggerated. Scale bar, 10 μm.

example of the time course of tension development under a SPOC condition. Myofibrils developed oscillatory tension, and the sarcomere lengths also oscillated; even at the very early stage before the tension was appreciably developed, the length of sarcomeres had already started to oscillate (see the small tension fluctuation at the initial lag phase of Fig. 5). It should also be noted that sarcomere lengths spontaneously oscillate even under an isometric condition, as previously reported (cf Okamura and Ishiwata, 1988). The lengthening phase of a (half) sarcomere propagated to adjacent sarcomere successively (SPOC wave). Usually, this SPOC wave propagated over a few to several sarcomeres along myofibrils but not over the whole length of myofibrils; SPOC waves appeared here and there, not at definite places but at random, and disappeared when two waves collided with each other; the travelling distance of a SPOC wave was usually restricted. Correspondingly, the tension oscillated with small amplitudes containing high-frequency components as shown in Fig. 5.

Organized state of SPOC

In some cases, a SPOC wave steadily and repeatedly propagated from one end to the other of myofibrils consisting of more than 10 sarcomeres (we call this an "organized" state of SPOC hereafter). A typical example is shown in Fig. 6 (corresponding tension records are shown in Fig. 7); a quick lengthening phase of the (half) sarcomere appeared from the right end of the myofibril and propagated to the left end with a nearly constant velocity (~70 μm/s in this example); after a lengthening phase of the order of 0.1 s, the sarcomeres started to shorten again slowly. The first sarcomere at the right end continued to shorten whereas the SPOC wave propagated through the myofibril, during which time the developed tension of the myofibril gradually decreased. Even after the last sarcomere at the left end had started to shorten (see the profile indicated by two double circles in Fig. 6), the first sarcomere kept on shortening for a while, very slowly; during this process, the devel-

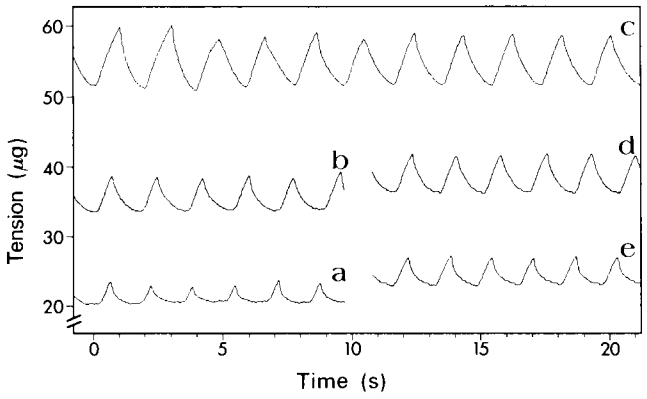

FIGURE 7 Reversible change with load (average tension) of the wave form of tension oscillation of myofibrils under an organized state of SPOC. A load imposed on the myofibril was stepwise increased from *a* to *c* and reversibly decreased from *c* to *e*. A sample is the same as in Fig. 6. For detailed analysis, see Figs. 8 and 9.

oped tension of the myofibril continued to increase (see the middle part of Fig. 6). And, when the tension reached the maximum, the first sarcomere started to lengthen again. Note that every sarcomere length oscillated with nearly the same saw-tooth wave form, irrespective of the developed tension (sustained load) of the myofibril.

It is empirically considered that the following factors are favorable to realize the organized state of SPOC: relatively thick myofibrils (the organized state is difficult to generate in a thin single myofibril, but easy in a thick bundle of myofibrils) with an appropriate total length and after staying in the SPOC state for a while. A small amount of stretch frequently triggers the organized state. Once the organized state was attained, it was stable against perturbation such as forced stretch or release and continued for more than 10 min without changing the essential features of its wave form. In the above example, the SPOC wave started from the side of the flexible needle, but this was not always the case; after the return to relaxation, the side from which the SPOC wave starts could not be controlled.

Analysis of tension oscillation of SPOC

After the organized state of SPOC was attained (cf Fig. 6), the stiffer micro-needle was moved slowly (~ 1 μm/s) so as to increase and reversibly decrease the load imposed on the myofibril. The tension response observed under a steady state for each load is shown in Fig. 7. Through this process, the pattern of propagation of the SPOC wave was unchanged. With increase of th load, both tension level and amplitude of oscillatio increased, and average sarcomere length slightly ir creased. Also, Fig. 7 suggests that the period of oscill tion increased and the wave form became nearly symme rical with the increase of load.

Next, the wave form of oscillatory tension was ana lyzed by FFT. Fig. 8 shows the power spectra obtaine from the data in Fig. 7. The spectra contained the mai peak at 0.5–0.6 Hz and its higher harmonics. It becam clear from the power spectra that with the increase c load, the main peak shifted to the lower frequenc region, indicating that the fundamental period of SPO increased, and besides, the amplitude of higher harmor ics decreased, suggesting that the wave form of SPO approached a sinusoidal form. The results of analysis ar summarized in Fig. 9, showing that the period of SPO (also the average sarcomere length) is nearly linear t the applied load and the change of the period i reversible.

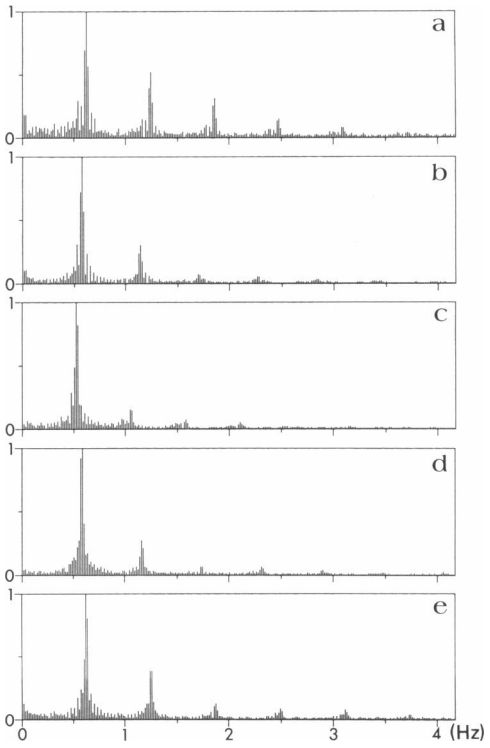

FIGURE 8 Power spectra of the wave form of tension oscillation myofibrils. The power spectra, *a*, *b*, *c*, *d*, and *e*, (correponding to *a*–*e* Fig. 7) were obtained by FFT of tension oscillation data for ~31 (1,024 data points, with sampling every 30 ms). Abscissa, frequenci (Hz); ordinate, amplitude of each frequency component (arbitra unit). For more details, see Materials and Methods.

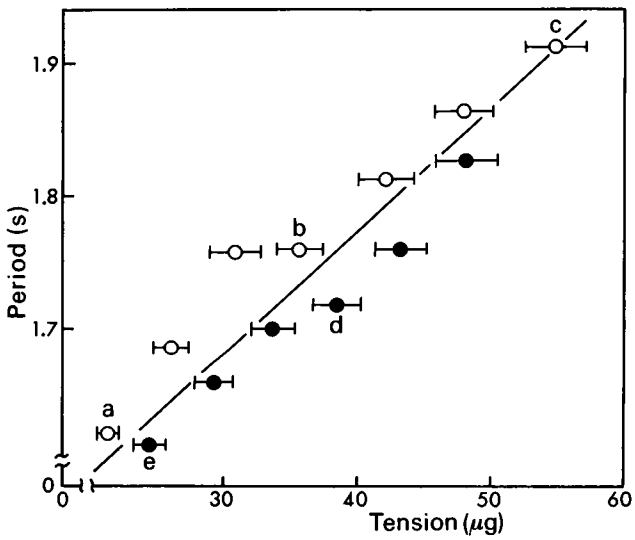

FIGURE 9 Dependence on load (average tension) of the fundamental period of tension oscillation. Abscissa, oscillated tension averaged (±SD, horizontal bars) with time; ordinate, a fundamental period obtained by FFT analysis of tension oscillation as shown in Fig. 8. Data were obtained in the order of *a*, *b*, *c*, *d*, and *e* by stepwise stretching (○) of the myofibril from the lower left to the upper right and then reversely shortening (●) it. Corresponding average sarcomere length (±SD; in μm unit) (oscillated total length of myofibril was averaged with time and divided by the number of sarcomeres) was 2.06 (0.02) for *a*, 2.22 (0.05) for *b*, 2.35 (0.07) for *c*, 2.19 (0.05 for *d*, and 1.97 (0.04) for *e*.

DISCUSSION

Advantages and disadvantages of our method

Up to the present, there have been only a few reports on the measurement of tension generated by a single skeletal myofibril, in spite of its importance (Borejdo and Schweitzer, 1977; Iwazumi, 1987 *a* and *b*; Bartoo et al., 1988; Pollack, 1990). The main reason is the difficulty of gently holding both ends of a myofibril while maintaining the internal structure.

The advantages of our method are that the means to hold myofibrils is simple, and the technique to measure the developed tension is also simple; besides, it is possible to observe the internal structure of myofibrils at the same time. The main disadvantage may be that the spatial (50 nm) and time (1/30 s) resolutions are limited by those intrinsic to the television camera-video system. Although the time resolution would be improved by using a high-speed camera, it will be more difficult to improve the spatial resolution as long as the system of image analysis with a television camera is used. If the movement of an object such as a glass micro-needle is suppressed to quite a small amount, an ultrasensitive detection system having a spatial resolution of the order of 0.1 nm can be used (Kamimura, 1987). Thus, this disadvantage of our system should be overcome in the future.

Our technique seems to be appropriate to examine the static properties of myofibrils such as resting tension rather than the dynamic properties, especially of fast phenomena; in fact, the present method has been successfully applied to investigate, for example, the contribution of the elasticity of connectin (titin) filaments (Wang, 1985; Maruyama, 1986) to the resting tension (concerning muscle fibers, see Funatsu et al., 1990). These results will be published elsewhere.

In the present work, we first applied our technique for measuring the oscillatory tension of myofibrils under a SPOC condition. Although the tension oscillation under SPOC conditions has been measured by using single fibers with a conventional method (Shimizu et al., 1992), it was impossible to detect the oscillation of sarcomere lengths at the same time. It has become possible, probably for the first time in the case of any auto-oscillation of skeletal muscle reported so far, to examine quantitatively the correlation between the oscillations of tension and sarcomere lengths by use of the method and system reported here to manipulate myofibrils and analyze the internal structure.

Phenomenological aspects of SPOC of myofibrils

We selected, as a typical example, an organized state of SPOC in which the SPOC wave steadily and repeatedly propagates from one end of myofibrils to the other. As reported previously (Okamura and Ishiwata, 1988), under SPOC conditions the length of each sarcomere (strictly speaking, half-sarcomere; cf Ishiwata et al., 1991) spontaneously oscillates with a saw-tooth wave form composed of a relatively rapid lengthening phase and a slow shortening phase. Here, the SPOC wave is defined as such a wave that propagates the lengthening phase of a (half)sarcomere to the adjacent one, the next one to that and so on. We found that in the organized state of SPOC, myofibrils generated oscillatory tension; the tension gradually decreased during the propagation of a SPOC wave and started to increase again after the SPOC wave had reached the end of myofibrils (cf Fig. 6). Thus, in the organized SPOC, the fundamental period of tension oscillation (Fig. 8) is equal to the average period of oscillation of sarcomere length, whereas this is not the case for the unorganized SPOC.

Because there is the restriction that the length of myofibrils plus the displacement of the flexible glass micro-needle, i.e., (the developed tension)/(the Hooke's elastic constant of the micro-needle), is maintained nearly constant in our experimental setup (auxotonic condition), the oscillations of tension and length of myofibrils are related as a mirror-image to each other. Thus, in the present experiments, we could not examine separately the oscillation of developed tension and the oscillation of total length of myofibrils. In future, we should examine them independently by keeping either the total length (isometric condition) or the developed tension (isotonic condition) constant, for example, by combining a piezo-electric element and a feed-back electronic system.

Effects of stretching of myofibrils on SPOC

What is the effect of stretching (imposing a load on) myofibrils on SPOC? Apparent features of the effects seem to be very similar to those in flight muscle (cf Pringle, 1967, 1978). In both cases, the activation of myofibrils is induced (stretch activation); one aspect of stretch activation in SPOC, the increase of the level of tension, is seen in Fig. 7. Concerning the mechanism of stretch activation, two types of mechanisms have been discussed in the preceding paper (Shimizu et al., 1992), where it is assumed that the enzymic activity is enhanced by the strain imposed on cross-bridges and/or thin filaments. We consider that such a large activation is possible because myofibrils are in the partially activated state, being intermediate between contraction and relaxation. Here, note that the stretch-induced increase of tension by partial Ca^{2+} activation (Endo, 1972, 1973) may belong to the same category.

The main reason why the period of SPOC was increased by the stretching of myofibrils (Figs. 8 and 9) is that the shortening phase was slightly prolonged. Whether or not the shortening velocity of each sarcomere obeys Hill's tension-velocity (P-V) relation is to be examined in future.

Relationship between oscillations of tension and sarcomere length

How is the oscillation of each sarcomere length related to the oscillation of tension of myofibrils? This is clearly seen in an "organized" state of SPOC. Fig. 6 suggests that the steady oscillation of each sarcomere length may not be directly related to the level of tension, and accordingly not related to the slow oscillation of tension generated by myofibrils, although the period of oscillation is affected by the level of tension. First, note that all (half)sarcomeres will bear nearly the same load at every moment, judging from the fact that the velocity of propagation of tension (mechanical impulse) along a myofibril is very fast, for example, 170 m/s under a contracting condition and only three to four times slower under a relaxing condition (Schoenberg et al., 1974), so that irrespective of conditions, the tension will be transmitted within a microsecond over the whole length of a myofibril. Thus, the oscillation of sarcomere length seems to be independent of the level of tension, because, as can be seen in Fig. 6, whereas the sarcomere at the right end starts to yield when the imposed load becomes maximum, the yielding of the sarcomere at the left end starts when the load becomes nearly minimum; this feature is not fixed but changeable, as is especially clear in an unorganized state of SPOC. Note that in spite of this fact, the wave forms of length oscillation of sarcomeres are similar to each other. From such considerations, we infer that the oscillation of sarcomeres may be controlled not by the level of tension but by the change of tension, i.e., mechanical impulse.

The integrated work performed by myofibrils during SPOC is effectively zero in an auxotonic condition, because the work performed during the contracting phase just cancels that performed during the lengthening phase. However, because of the feature described above, in the example shown in Fig. 6 the sarcomeres at the right-hand half received work from the outside but those at the left-hand half performed work to the outside. What is the difference among these sarcomeres? Here, the following questions arise: first, how is the timing of oscillation of each sarcomere controlled and second, how is the ATPase activity coupled to the oscillation of sarcomeres, which may be different in every sarcomere? In other words, is the feature of mechano-chemical coupling, i.e., the correspondence between chemical reaction (response) and mechanical response (event), different in every sarcomere? The answers to these questions may give us a key to resolve the mechanism of SPOC and, moreover, some insight into the mechanism of contraction and its regulation coupled to the mechanism of the ATPase reaction.

Molecular mechanism of SPOC

What is the mechanism of SPOC of each sarcomere and the propagation of the SPOC wave along a myofibril? Although we cannot present a definite answer, the key to resolve such questions may be present in the fact that, as pointed out above, each sarcomere bears the same load (produces the same tension) regardless of the difference of the internal state, that is, either lengthening or

shortening sarcomeres, and even stopped sarcomeres bear the same load.

Such a situation that half-sarcomeres in different states can maintain the same external force is probably possible because they are in an intermediate state between contraction and relaxation, in which they may self-control their state in response to the external force. The intermediate state can be induced not only by the SPOC condition as in the present work but also by low concentrations of Ca^{2+} (Fabiato and Fabiato, 1978; Iwazumi and Pollack, 1981; Stephenson and Williams, 1981) or high pH (Onodera and Umazume, 1984; Onodera, 1990).

Also note the inference that the oscillation of sarcomere length may be triggered by mechanical impulse but not by the level of tension. The mechanical impulse will be produced by quick yielding and reshortening of sarcomeres and propagate along a myofibril regardless of the level of tension. Based on these considerations, we present the following idea as a key to the mechanism of spontaneous oscillation of myofibrils. First, even if the internal states of sarcomeres happen to be different from each other, each sarcomere can bear the same load by self-control through changing the population of several chemical states of cross-bridges such as AM-ADP-Pi complex (AM, actomyosin), and AM-ADP complex. However, the response to the mechanical impulse may be different for each chemical state of cross-bridges (cf Huxley and Simmons, 1971; Goldman, 1987; Brenner, 1990); we assume that a particular chemical state which is weak against the mechanical impulse may be dominant in nearly stopped half-sarcomeres, so that they seem to yield easily. This yielding will produce a new mechanical impulse.

In summary, we suggest that the principal role of MgADP and Pi in SPOC is to maintain the intermediate state of (half)sarcomeres, that is, probably to make AM-ADP-Pi complex and AM-ADP complex dominant so that the state can be self-controlled in response to the mechanical impulse to keep the myofibril a dynamic state, SPOC.

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