Regulation of tension development by MgADP and Pi without Ca^{2+}

Role in spontaneous tension oscillation of skeletal muscle

Hideharu Shimizu, Takashi Fujita, and Shin'ichi Ishiwata

Department of Physics, School of Science and Engineering, Waseda University, Okubo, Shinjuku-ku, Tokyo 169, Japan

ABSTRACT The length of sarcomeres in isolated myofibrils fixed at both ends spontaneously oscillates when MgADP and Pi coexist with MgATP in the absence of Ca^{2+} (Okamura, N., and S. Ishiwata, 1988. *J. Muscle Res. Cell. Motil.* 9:111–119). Here, we report that MgADP and Pi function as an activator and an inhibitor, respectively, of tension development of single skeletal muscle fibers in the absence of Ca^{2+} and the coexistence of MgADP and Pi with MgATP induces spontaneous tension oscillation. First, the isometric tension sharply increased when the concentration of MgADP became higher than ~3× that of MgATP and saturated at ~90% of the tension obtained under full Ca^{2+} activation; in parallel with this sigmoidal increase of tension, MgATPase activity appeared. The inhibition of contraction by the regulatory system seems to be desuppressed by the allosteric effect of actomyosin-ADP complex, similarly to so-called rigor complex. The ADP-induced tension was decreased along a reversed sigmoidal curve by the addition of Pi; actomyosin-ADP-Pi complex, which has no desuppression function, may be formed by exogenous Pi; accompanying the decline of tension, spontaneous oscillations of tension and sarcomere length appeared. It is suggested that the length oscillation of each (half) sarcomere would occur through the transition of cross-bridges between force-generating (on) and non-force-generating (off) states, which may be regulated by the mechanical states (strain) of cross-bridges and/or thin filaments.

INTRODUCTION

Muscle contraction occurs as a result of mutual sliding of actin (thin) and myosin (thick) filaments with the consumption of chemical energy liberated from ATP hydrolysis. So, current studies on the molecular mechanism of muscle contraction have been focused on (*a*) mechanical (structural) properties, (*b*) enzymatic (thermodynamic) properties and (*c*) mechano-chemical coupling, i.e., the connection between the molecular mechanism of force generation and the mechanism of ATP hydrolysis, in actin and myosin complexes. The molecular mechanism of regulation is still unclear, although it has been established that the first event in triggering contraction is the binding of Ca^{2+} to regulatory proteins (Ebashi and Endo, 1968; Weber and Murray, 1973).

Recently, much effort has been made to connect the mechanical changes of muscle fibers with the kinetics of ATP hydrolysis by actomyosin complex. The leading theory is that cross-bridges can be classified into two types, i.e., a weakly-binding state with ATP or ADP-Pi which hardly contributes to tension and a strongly-binding state with ADP or without nucleotides which is responsible for tension development (Eisenberg and Hill, 1985; Goldman and Brenner, 1987; Goldman, 1987; Brenner, 1990). The regulation by Ca^{2+} occurs through the change of kinetic constant(s) at some step(s) between the weakly- and strongly-binding states, and/or within the weakly- and/or strongly-binding states, but not through the association and dissociation step of actin and myosin (Chalovich et al., 1981; Millar and Homsher, 1990). Based on such results, a detailed kinetic scheme for actomyosin ATPase has been proposed and analyzed by computer simulation (e.g., Pate and Cooke, 1989). Further, the effects of ADP and Pi on contraction have been studied (Hibberd and Trentham, 1986; Schoenberg and Eisenberg, 1987); MgADP potentiates isometric tension obtained in the presence of Ca^{2+} (Cooke and Pate, 1985; Kawai and Halvorson, 1989), whereas Pi depresses the tension (Rüegg et al., 1971; Herzig et al., 1981; Altringham and Johnston, 1985; Cooke and Pate, 1985; Hibberd et al., 1985; Kawai, 1986; Nosek et al., 1987; Cooke et al., 1988; Chase and Kushmerick, 1988; Kawai and Halvorson, 1991). Those results support the above scheme, implying that actomyosin(AM)-ADP complex is in a strongly binding state, whereas AM-ADP-Pi complex is in a weakly-binding state. Also, there is a report showing that even in the absence of Ca^{2+}, tension is developed to some extent upon the addition of MgADP (Hoar et al., 1987). It should be noted, however, that in spite of these various studies, both the effects of high concentrations of MgADP and the synergistic effects of MgADP and Pi in the absence of Ca^{2+} have not yet been examined.

Several years ago, we found a new phenomenon in

Address correspondence to Dr. Ishiwata.

0006-3495/92/05/1087/12 $2.00

skeletal myofibrils, i.e., spontaneous oscillatory contraction, named SPOC, and clarified the conditions for oscillation, which seems to reflect an essential aspect of dynamical coupling between the mechanical and chemical properties of the contractile system composed of actin, myosin and regulatory proteins (Okamura and Ishiwata, 1988). That is, when high concentrations of MgADP and Pi coexist with MgATP and without Ca^{2+}, the lengths of all sarcomeres in isolated myofibrils fixed at both ends spontaneously oscillate, exhibiting a rapid lengthening phase and a slow shortening phase with a period of one to several seconds, where the oscillation of sarcomeres is usually out of phase to each other. The SPOC conditions, requiring the coexistence of MgADP and Pi with MgATP but not requiring Ca^{2+}, strongly suggest that not only the molecular mechanism of tension development but also its regulatory mechanism are involved in the oscillation mechanism.

Several reports have already been published on spontaneous oscillation of chemically or mechanically skinned fibers or fibrils of vertebrate striated muscle (Goodall, 1956; Lorand and Moos, 1956; Armstrong et al., 1966; Fabiato and Fabiato, 1978; Brenner, 1979; Iwazumi and Pollack, 1981; Stephenson and Williams, 1982; Onodera and Umazume, 1984; Onodera, 1990; Sweitzer and Moss, 1990). However, the conditions are apparently different from those of SPOC, e.g., submicromolar concentrations of free Ca^{2+} for skeletal (Iwazumi and Pollack, 1981; Stephenson and Williams, 1982) or cardiac muscle (Fabiato and Fabiato, 1978; Sweitzer and Moss, 1990) and high pH in the absence of Ca^{2+} for skeletal muscle (Onodera and Umazume, 1984; Onodera, 1990). These conditions for oscillation are apparently different from each other; besides the above two types of oscillations are limited to a narrow range of free Ca^{2+} concentrations or pH value, whereas the SPOC occurs over a wide range of concentrations of MgADP and Pi. In spite of these differences, there is one important point in common; all the conditions produce partial activation with a low level of tension, so that the state of oscillating fibers appears to be located in an intermediate state between contraction and relaxation. Therefore, the fluctuation between contraction and relaxation which occurs in each (half) sarcomere depending on the mechanical and chemical states of the contractile system may be an essential feature of all the types of spontaneous oscillations reported so far. The present work represents a first step towards examining this possibility and getting an insight into not only the mechanism of spontaneous oscillation but also the molecular mechanism of contraction and regulation of muscle.

MATERIALS AND METHODS

Solvent conditions

Solvent conditions for the measurement of developed tension of fibers and for the observation of states of myofibrils were as follows: ~120 mM K^+, 1 or 4 mM EGTA, 0.1 mM P^1,P^5-di(adenosine-5′)pentaphosphate (AP_5A; Lienhard and Secemski, 1973) and 10 or 20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS). The pH value was adjusted to 6.5–8.2 for each solvent by the addition of KOH or HCl at 25°C. Appropriate concentrations of ATP, ADP, K_2HPO_4, KH_2PO_4, $MgCl_2$, and $CaCl_2$ were added. The ionic strength (I.S.) was maintained at 0.15 M by adjusting the concentration of KCl. In the case of fibers, the concentration of free Mg^{2+} was maintained at 2 mM by adjusting the total concentration of $MgCl_2$. The concentrations of the above chemicals were determined by computer calculation using the published values for stability constants (cf Horiuti, 1986). When it was necessary to maintain the concentration of MgATP in fibers constant, 1 mg/ml creatine phosphokinase (CPK) and 20 mM creatine phosphate (CP) were added. CPK and CP were purchased from Sigma Chemical Co. (St. Louis, MO); ATP, ADP and AP_5A were from Boehringer Mannheim GmbH (Mannheim, Germany). MOPS was purchased from Dojindo (Kumamoto, Japan). Other chemicals were of reagent grade.

Preparation of muscle fibers and myofibrils

Rabbit psoas glycerinated muscle fibers were prepared as described previously (Ishiwata et al., 1985). The glycerinated fibers were used after storage at −20°C for between three weeks and three months; within this period, the mechanical properties of the fibers reported here were unchanged. Single glycerinated fibers (~5 mm long, ~40 μm thick) were carefully prepared from a bundle of fibers immersed in 50% (*v*/*v*) glycerol solution on a cold bench under a stereo microscope. Then, one end of each single fiber was fixed to a thin tungsten wire attached to a tension transducer, of which characteristic frequency was ~40 Hz (UL-2GR, Nippon Denkei Co. Ltd., Tokyo) and the other end to a thin tungsten wire attached to a micromanipulator with enamel; this work was done in air at room temperature without removal of glycerol solution from the fibers except at both ends, where the enamel was smeared. The fibers thus fixed were first stored in a rigor solution (solution A with Triton X-100) containing 60 mM KCl, 5 mM $MgCl_2$, 10 mM Tris-maleate buffer (pH 6.8), 1 mM EGTA and 1% (*v*/*v*) Triton X-100, for 20 min at 25°C. The following treatment and all the experiments were done at 25°C. The fibers were then transferred to a relaxing solution (110 mM KCl, 6 mM $MgCl_2$ (free Mg^{2+} = 2 mM), 4 mM ATP (MgATP = 3.5 mM), 10 mM MOPS (pH 7.0), 4 mM EGTA and 0.1 mM AP_5A), in which the sarcomere length of fibers was estimated by means of laser light diffraction (He-Ne laser, type GLS 5110, NEC Inc., Tokyo, Japan; cf Ishiwata et al., 1985) and adjusted to 2.3–2.5 μm with a micromanipulator. Oscillation of sarcomeres in muscle fibers was detected by monitoring the fluctuation of the fine structure of the first-order diffraction lines. Even if the tension oscillation was not observable, the fine structure fluctuated maintaining the average position of the diffraction lines, which reflects the unorganized oscillation of sarcomere lengths.

Myofibrils were prepared by gently homogenizing glycerinated fibers under a rigor condition (solution A) at 2°C and the states of myofibrils were observed under a phase-contrast microscope at 25°C as described previously (Ishiwata and Funatsu, 1985).

Measurement of tension

The isometric tension generated by single fibers was measured by using a tension transducer according to the following procedure. First, the fibers immersed in a relaxing solution were transferred to a certain standard solution and the tension (P_0) was measured. After relaxing them again, the tension (P) was measured in an assay solution. Then, the measurement of tension (P_1) was repeated under the above standard condition. An example of typical records is shown in Fig. 3, which was recorded by a chart recorder having a cut-off frequency of 0.5 Hz (VP-6533A, Matsushita Communication Industrial Co. Ltd., Yokohama, Japan). Thus, the relative tension was estimated from the ratio of P to the average of P_0 and P_1. A single data point was obtained from each fiber. The volume of solution was ~0.3 ml; the solution was stirred with a miniature magnetic stirrer.

Measurement of ATPase activity

ATPase activity of fibers was estimated as follows. The concentration of Pi released from four single fibers into 0.3 ml of solution during 10 min after the addition of ADP was measured by a modified malachite green method (Lanzetta et al., 1979; Kodama et al., 1986; Ohno and Kodama, 1991). The ATPase activity was expressed in a unit of (number of released Pi)/(number of myosin heads)/s, with the assumption that myosin accounts for 50% (w/w) of total protein content in fibers. The amount of proteins of fibers was determined by a modified Lowry method (Ohnishi and Barr, 1978).

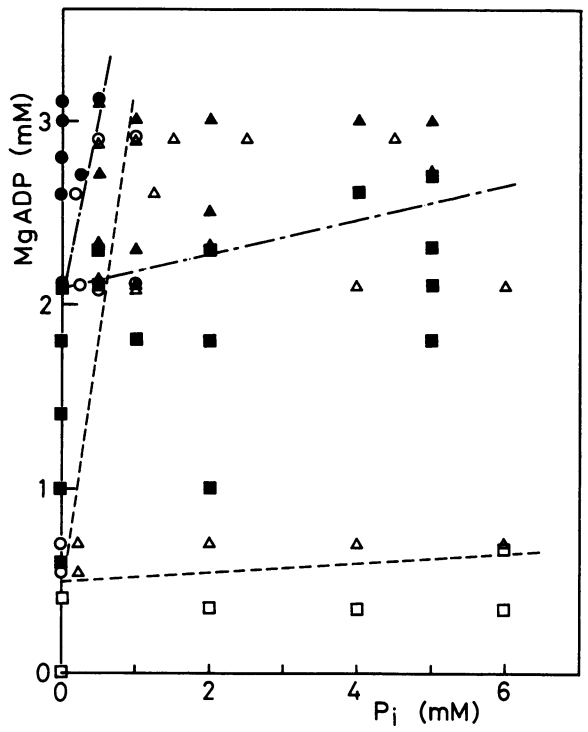

FIGURE 1 State diagram showing states of myofibrils, i.e., contraction (○, ●; myofibrils shorten without oscillation), relaxation (□, ■) and SPOC (△, ▲) at different concentrations of MgATP (0.2 mM, *open symbols;* 1 mM, *closed symbols*). The states of myofibrils were observed under a phase-contrast microscope. Ordinate and abscissa, the concentrations of MgADP (calculated) and Pi (added), respectively. Other solvent conditions, 4 mM $MgCl_2$, 10 mM MOPS (pH 7.0), 4 mM EGTA and 0.1 mM AP_5A. Ionic strength (I.S.) was adjusted with KCl to be 0.15 M. Temperature, 25°C.

RESULTS

Spontaneous oscillatory contraction (SPOC) characterized by microscopic observation of myofibrils

We have previously obtained a state diagram showing three states of myofibrils, i.e., spontaneous oscillation (SPOC), relaxation and contraction without oscillation, which are induced by adding various concentrations of ADP and Pi in the presence of 0.2 mM ATP and the absence of Ca^{2+} (Okamura and Ishiwata, 1988; Ishiwata et al., 1991). Here, we have refined the state diagram by controlling the concentrations of MgATP (0.2 or 1.0 mM) and MgADP, i.e., true substrates of actomyosin ATPase, and the ionic strength. The states of myofibrils, of which both ends were attached to a glass surface, were observed under a phase-contrast microscope as described in detail previously. The characteristics of the state diagram thus obtained were essentially the same as those found previously (Fig. 1; cf Okamura and Ishiwata, 1988; Ishiwata et al., 1991). That is, the SPOC region appeared between the contraction and relaxation regions; myofibrils which are relaxed in the absence of MgADP and Pi (corresponding to the origin of the diagram) were transformed into the contraction state by adding MgADP (along the ordinate) and then started to oscillate spontaneously on further addition of Pi (in parallel with the abscissa). Across the boundaries shown by dashed or once-broken lines, the state of myofibrils changed gradually and reversibly. There appears to be a triple point on the ordinate around which the three states of myofibrils merge. Across the triple point along the ordinate, the state of myofibrils gradually changed from relaxation to contraction; between the triple point and the origin on the ordinate, myofibrils may be partially activated and generate tension, though it seems that the tension was so small that myofibrils attached to a glass surface could not shorten appreciably (cf the text dealing with Fig. 4). So, we regard the concentration of MgADP at the triple point as a good measure to indicate a "critical MgADP concentration" above which ADP-induced contraction occurs. As described in the previous paper (Okamura and Ishiwata, 1988), the shortening velocity of myofibrils under the condition of ADP-induced contraction was low, about one tenth of the standard velocity of no-load shortening.

It should also be noted in Fig. 1 that on increasing the concentration of MgATP the SPOC region shifted

upward to the region of high concentrations of MgADP, suggesting that MgADP competes with MgATP for binding to actomyosin complex. To further clarify this point, we examined the relationship between the critical MgADP concentration and the concentration of MgATP. Fig. 2 shows that the critical MgADP concentration increases nearly in proportion to the concentration of added MgATP. The slope of the straight line was ~3, suggesting that the ratio of the apparent association constant of MgATP to that of MgADP with actomyosin complex was ~3. Fig. 2 is a state diagram in the sense that the straight line showing the relationship between the critical MgADP concentration and the concentration of MgATP corresponds to a boundary across which the state of myofibrils changes reversibly. When the concentration of MgATP is very low, lower than ~0.1 mM, myofibrils contracted without the addition of ADP, probably due to the activation by rigor complex (cf Bremmel and Weber, 1972; Weber and Murray, 1973).

Effects of MgADP on isometric tension of single fibers

We have measured the isometric tension generated by the ADP-induced contraction by using single glycerinated fibers. Fig. 3 is an example of record showing the reversibility and reproducibility of tension development with the change of concentration of MgADP. This isometric tension induced by MgADP in the absence of Ca^{2+} reached nearly 90% of that induced normally by the addition of Ca^{2+} instead of MgADP (see below). Fig. 4 summarizes these results, showing that the tension is generated with the addition of MgADP in the absence of Ca^{2+}; in the presence of 1 mM MgATP, the tension was abruptly increased at ~3 mM MgADP and almost saturated at ~10 mM MgADP. This concentration of 3 mM MgADP exactly corresponds to the critical MgADP concentration defined above. Such a sigmoidal curve of tension development and the presence of critical MgADP concentration indicate that MgADP functions as an allosteric effector and induces cooperative interaction between thin and thick filaments.

Next, we examined the effects of MgADP on the isometric tension under a contracting condition in the presence of Ca^{2+}. As summarized in Fig. 5, in the presence of 1 mM MgATP, the tension increased by ~10%, reached a plateau at ~3 mM MgADP and decreased on further increase in the concentration of MgADP. On the other hand, in the presence of 2 mM MgATP, the increase of tension was much larger, to nearly the same extent as reported before (cf Cooke and Pate, 1985; Kawai and Halvorson, 1989) and reached a

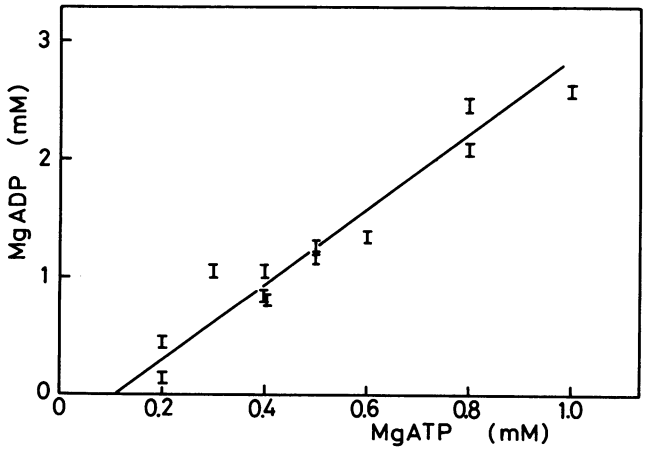

FIGURE 2 Critical concentrations of MgADP at various concentrations of MgATP without Pi. The critical concentration of MgADP, above which contraction of myofibrils started to occur, was determined under a phase-contrast microscope by stepwise increasing MgADP concentration along the ordinate in Fig. 1 at each concentration of MgATP (calculated). Other conditions, the same as in Fig. 1 except that Pi was absent.

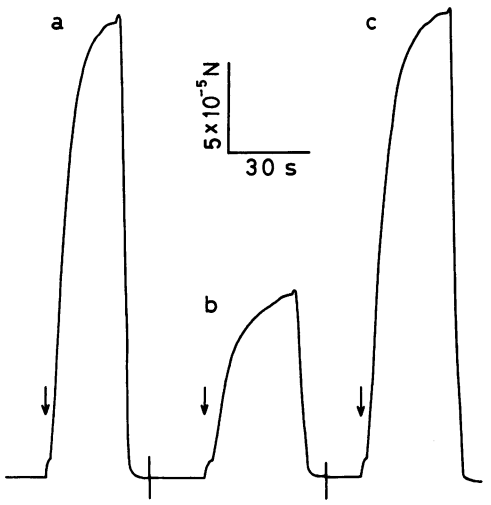

FIGURE 3 Recordings of tension development of a single fiber. Solvent conditions: 1 mM MgATP, 15 mM MgADP (*a* and *c*) or 5 mM MgADP (*b*), 2 mM Mg^{2+} (free), 4 mM EGTA, 10 mM MOPS (pH 7.0), 0.1 mM AP_5A, concentration of free Mg^{2+} maintained at 2 mM with $MgCl_2$ and I.S. maintained at 0.15 M with KCl. Temperature, 25°C. Tension was measured in sequence from *a* to *c*. Throughout the present work, the tension under a certain standard condition was measured before (*a*) and after (*c*) the measurement under the assay condition (*b* in this example). At each arrow, relaxing solution (1 mM MgATP, 2 mM Mg^{2+} (free), 4 mM EGTA, 10 mM MOPS (pH 7.0), 0.1 mM AP_5A and I.S. = 0.15 M) was exchanged with the above solution containing MgADP. Vertical and horizontal bars indicate 5×10^{-5} N and 30 s, respectively.

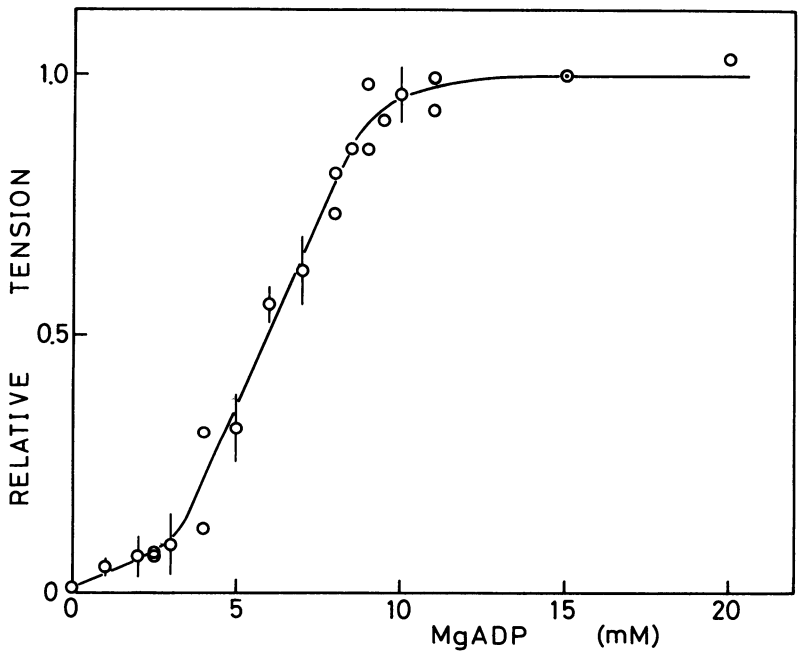

FIGURE 4 Effect of MgADP on tension development of single fibers in the presence of ATP and the absence of Ca^{2+}. Solvent conditions: the same as in Fig. 3 except that the concentration of MgADP was changed. Temperature, 25°C. Tension was normalized with respect to that at 15 mM MgADP (◉). Vertical bars, SD when more than three data points were obtained at each MgADP concentration.

plateau at ~7 mM MgADP. Thus, MgADP functions as a potentiator for the tension development irrespective of the presence or absence of Ca^{2+}; however, MgADP of much higher concentrations functions as a suppressor, probably due to competitive binding to an actomyosin complex with respect to MgATP, as already suggested above.

Data in Fig. 5 were normalized for the tension generated under the condition where MgADP was removed by adding an ATP-regenerating system. The slight increase of tension upon removal of the ATP-regenerating system (see the tension on the ordinate in Fig. 5) may be ascribed to the accumulation of MgADP in lattice spaces of the fiber.

Finally, we compared the tension generated in the presence (Fig. 5) and the absence (Fig. 4) of Ca^{2+} with 1 mM MgATP and 15 mM MgADP using the same fibers. The tension induced by MgADP in the absence of Ca^{2+} was 87 ± 2% (SD calculated from five experiments) of that in the presence of Ca^{2+}.

Activation of MgATPase of fibers by the addition of MgADP in the absence of Ca^{2+}

Although we have confirmed that the tension induced by MgADP in the absence of Ca^{2+} attained nearly 90% of

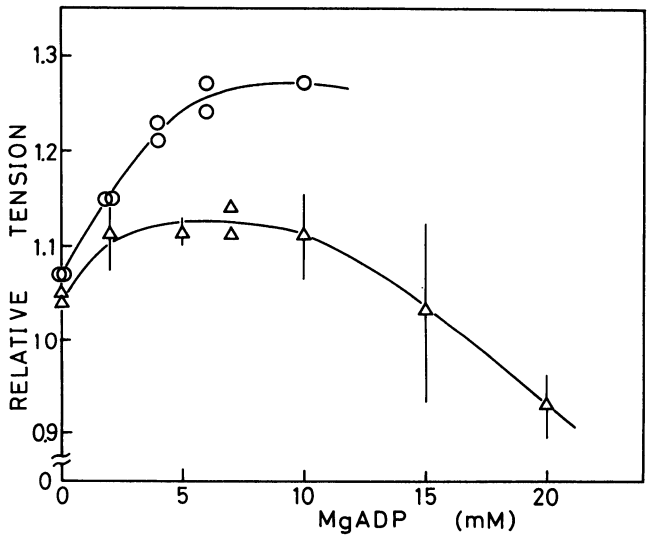

FIGURE 5 Effect of MgADP on tension development of single fibers in the presence of ATP and Ca^{2+}. Solvent condition: 1 mM (△) or 2 mM (○) MgATP, 2 mM Mg^{2+} (free), 1 mM EGTA (pCa adjusted to be 5.0 with $CaCl_2$), 20 mM MOPS (pH 7.0), 0.1 mM AP_5A and I.S. = 0.15 M. Temperature, 25°C. Tension was normalized with respect to that obtained in the presence of 1 mM MgATP and with the addition of 1 mg/ml of creatine phosphokinase and 20 mM creatine phosphate to suppress ADP contamination. Vertical bars, SD of more than three data points.

that in the presence of Ca^{2+}, there was a possibility that the tension is similar to rigor tension attained without ATP hydrolysis. So, we examined the ATPase activity of fibers by measuring the concentration of released Pi. Fig. 6 shows that accompanying the addition of MgADP, ATPase activity appeared and finally reached about 0.85 (Pi/myosin head/s). Thus, we conclude that the ADP-induced contraction generates active tension which is accompanied by ATP hydrolysis. It should be noted that the ATPase activity appears at slightly lower MgADP concentration than that required for tension development (cf Figs. 4 and 6).

Effects of Pi on isometric tension in the presence or absence of MgADP

Referring to the results of Figs. 1 and 4, we examined the effects of Pi on the tension induced by MgADP, i.e., the tension under SPOC conditions. Fig. 7 shows that the tension was decreased on increasing the concentration of Pi; first, in the presence of 15 mM MgADP and 1 mM MgATP where the ADP-induced tension was saturated (Fig. 4), the drop of tension occurred after some lag phase, along a reversed sigmoidal curve (~2 mM Pi at the point of inflection of the curve) and the extent of the tension drop was about 70% at 5 mM Pi. On the other hand, in the presence of 8 mM MgADP and 1 mM MgATP, where the ADP-induced tension was just before the saturation point (Fig. 4), the tension decreased monotonously without a lag phase (~1 mM Pi at the midpoint of the tension drop) and reached ~20% of the original value. In this respect, the result in the presence of 2 mM MgATP with 10 mM MgADP at pH 7.0 (cf *insert,* Fig. 10 *a*) was similar to that in the presence of 1 mM MgATP with 8 mM MgADP but not to that with 15 mM MgADP, although the extent of the tension drop was large.

We have noticed that the tension of single fibers start to oscillate spontaneously when the concentration of Pi is >1–2 mM, i.e., on the right-hand side region of the descending phase of the tension drop in Fig. 7. A typical record is shown in Fig. 8. The wave form of the tension

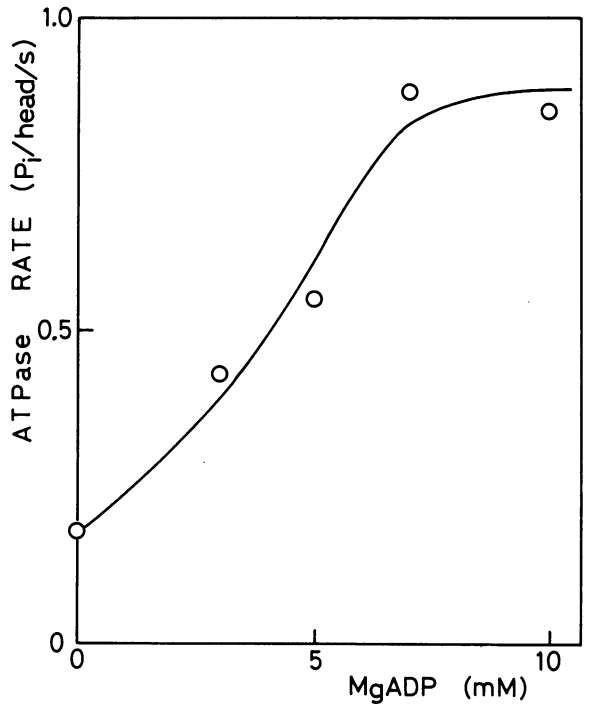

FIGURE 6 ATPase activity of single fibers on increasing MgADP concentration in the presence of ATP and absence of Ca^{2+}. Solvent conditions: 1 mM MgATP, 2 mM Mg^{2+} (free), 4 mM EGTA, 10 mM MOPS (pH 7.0), 0.1 mM AP_5A and I.S. = 0.15 M. ATPase rate is represented in a unit of (number of released Pi)/(number of myosin heads)/s. The concentration of myosin heads in fibers was assumed to be 0.2 mM. Temperature, 25°C.

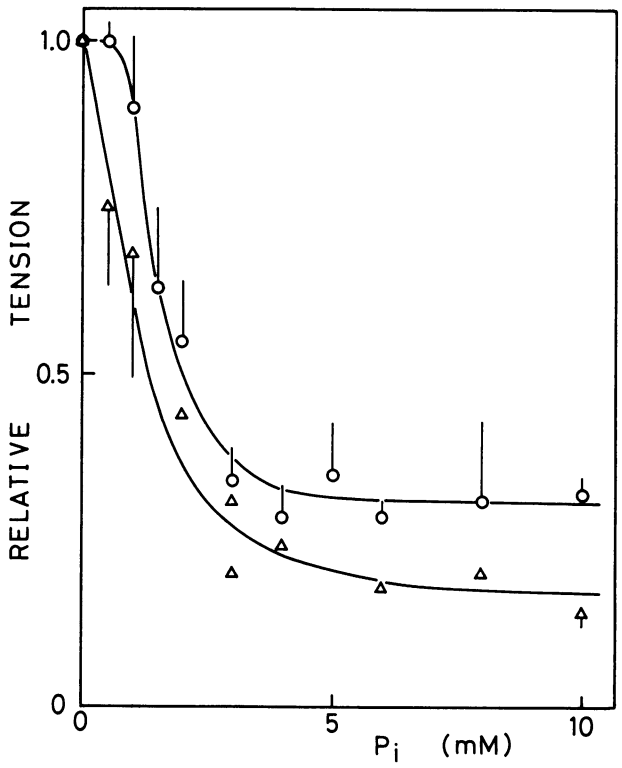

FIGURE 7 Effect of Pi on tension of single fibers induced by MgADP in the presence of ATP and absence of Ca^{2+}. Solvent conditions: (○, △), 1 mM MgATP and either 8 (△) or 15 (○) mM MgADP. Other conditions, 2 mM Mg^{2+} (free), 4 mM EGTA, 10 mM MOPS (pH 7.0), 0.1 mM AP_5A and I.S. = 0.15 M. Temperature, 25°C. Tension was normalized with respect to that obtained without Pi at each condition. When the tension oscillated as shown in Fig. 8, the initial peak tension was plotted. Vertical bars, SD of more than three data points.

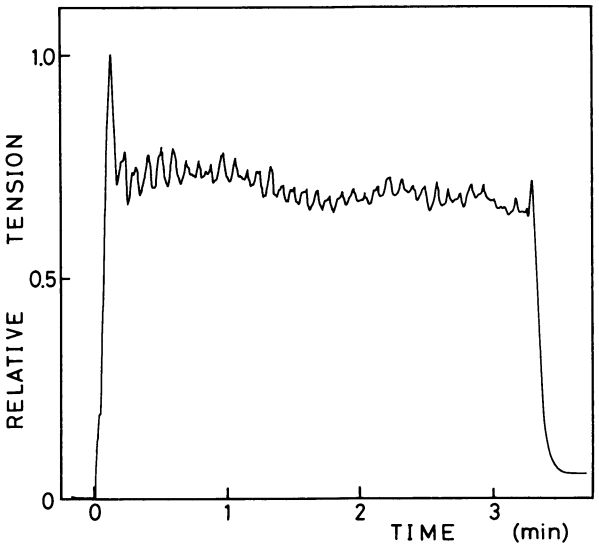

FIGURE 8 Example of tension oscillation of a single fiber observed under the SPOC condition. Solvent conditions: 1 mM MgATP, 15 mM MgADP, 1 mM Pi, 2 mM Mg^{2+} (free), 4 mM EGTA, 10 mM MOPS (pH 7.0), 0.1 mM AP_5A and I.S. = 0.15 M. Temperature, 25°C.

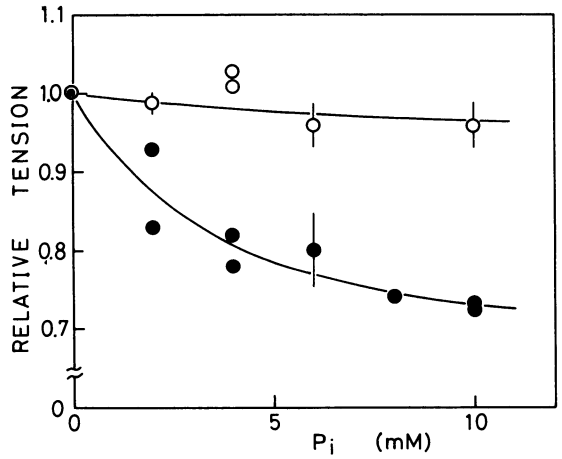

FIGURE 9 Effect of Pi on tension of single fibers developed in the presence of ATP and Ca^{2+} with or without ADP. Solvent conditions: 3.5 mM MgATP (●) or 1 mM MgATP and 15 mM MgADP (○) with 2 mM Mg^{2+} (free), 1 mM EGTA (pCa adjusted to be 5.0 with $CaCl_2$), 20 mM MOPS (pH 7.0), 0.1 mM AP_5A and I.S. = 0.15 M. Temperature, 25°C. Tension was normalized with respect to that obtained without Pi under each condition. Vertical bars, SD of three data points.

oscillation was not simple, but there appears to be some period of oscillation, ~11 s in this example. Accompanying this tension oscillation, propagation of the SPOC wave, i.e., the propagation of the rapid lengthening phase of sarcomeres (cf Okamura and Ishiwata, 1988; Ishiwata et al., 1991), was observed here and there along the long axis of fibers under a phase-contrast microscope. Even when the tension became negligibly small with the addition of Pi (cf *insert*, Fig. 10 *a*), the intensity fluctuation of the fine structure of laser light diffraction lines indicated that the sarcomere lengths spontaneously oscillated.

Next, we examined the effects of Pi on the tension generated under contraction conditions with Ca^{2+} and with or without MgADP (Fig. 9). The results under a standard contracting condition were similar to those reported previously (Cooke and Pate, 1985; Kawai, 1986; Kawai et al., 1987); the extent of the tension drop was as much as 30% at 10 mM Pi. On the other hand, the tension hardly decreased in the presence of 15 mM MgADP, which is in contrast to the results in the absence of Ca^{2+} (Fig. 7).

Effects of pH on isometric tension in the presence of MgADP and Pi

Finally, we examined the effects of pH on the ADP-induced tension in the presence of Pi and the absence of Ca^{2+}. In the pH range of 6.5–8.2, the higher the pH value, the larger the tension. The midpoint of pH was ~7.6 (Fig. 10 *a*); in other words, on increasing the pH, the midpoint of tension drop with the addition of Pi was shifted to higher concentrations of Pi (*insert*, Fig. 10 *a*). Next, we replotted the relative tension against the estimated concentrations of diprotonated Pi (Fig. 10 *b*) or protonated Pi (*insert*, Fig. 10 *b*). Fig. 10 *b* clearly shows that the tension decreases in proportion to the concentration of diprotonated Pi, irrespective of whether it is changed by the pH value or the total concentration of added Pi. On the other hand, there was no correlation between the tension drop and the concentration of protonated Pi (*insert*, Fig. 10 *b*).

DISCUSSION

Concentrations of ATP, ADP and Pi inside fibers

In the present study on muscle fibers, the concentration of MgATP was kept low, mostly 1 mM (2 mM in some cases), to examine the competitive action of MgADP. Therefore, when the ATPase activity was high, the MgATP concentration in the center of fibers may have become quite low. The MgATP concentration under a steady state was calculated based on the diffusion equation (cf Cooke and Pate, 1985) by assuming that the fibers are cylinders of radius 20 μm and the ATPase activity inside the fibers is uniform and 0.85/myosin head/s), which is the maximum value obtained under the conditions without Ca^{2+} in the present work. When

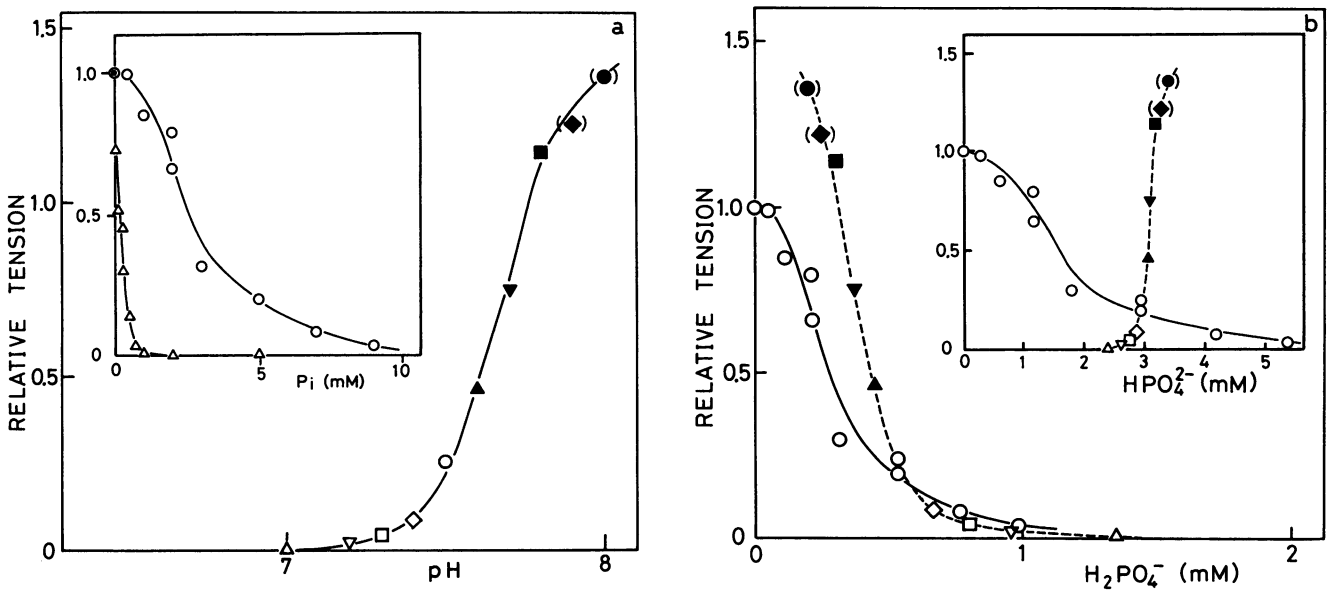

FIGURE 10 Effect of Pi at different pH values on tension of single fibers induced by MgADP in the presence of ATP and absence of Ca^{2+}. Solvent conditions: 2 mM MgATP (different from 1 mM used in Fig. 7), 10 mM MgADP, 2 mM Mg^{2+} (free), 5 mM Pi, 4 mM EGTA, 10 mM MOPS, 0.1 mM AP_5A and I.S. = 0.15 M (*a*); note that the concentrations of MgATP and MgADP used in Fig. 7 are 1 mM and either 8 or 15 mM, respectively. The pH value was adjusted for each solution by the addition of HCl or KOH. The effect of Pi concentration was examined at pH 7.0 (△) and 7.5 (○) (*insert* in *a*). Temperature, 25°C. The tension was normalized with respect to that (standard tension) obtained without Pi at pH 7.5 (◎, *insert* in *a*) according to the same procedure as described in Fig. 3. Two parentheses indicate that the standard tension obtained after the measurement of tension under the assay condition became <80% of the initial standard tension; this tended to occur when large tension was developed at high pH. Relative tension was replotted against the concentration of diprotonated Pi, $H_2PO_4^-$ (*b*) or protonated Pi, HPO_4^{2-} (*insert* in *b*). The concentration of each Pi species was calculated by using published values for stability constants (cf Materials and Methods). Symbols correspond to those in *a*.

the concentration of MgATP outside the fibers was 1 (or 2) mM, the concentration of MgATP at the center of the fibers was estimated to be 0.15 (or 1.15) mM or 0.86 (or 1.86) mM for a value of the diffusion coefficient of ATP of either 2×10^{-7} (MgATP; Mannherz, 1968) or 1.2×10^{-6} cm^2/s (ATP^{3-}; Kushmerick and Podolsky, 1969), respectively. Thus, the concentration of MgATP inside the fibers may have become a half of that outside the fibers when the ATPase activity was high. It should be noted, however, that this is the case only for the core region of the fibers; in the broader outer region, the concentration of MgATP would have been much closer to that outside the fibers.

The reported values of ATPase activity in muscle fibers in the presence of Ca^{2+} are scattered in the range of 0.9 to 3.2/myosin head/s (Chaen et al., 1981; Cooke and Pate, 1985; Sleep, 1984; Webb et al., 1986). When the MgATP concentration outside the fibers is as low as 1 (or 2) mM, the threshold of ATPase activity above which the MgATP concentration at the core of muscle is exhausted may be ~1 (or 2)/myosin head/s. The ATPase activity, however, should be lowered with the addition of MgADP in the presence of Ca^{2+}, so that only a part of the conditions within the range examined in the present work may have met the above threshold of ATPase activity.

Conversely, the concentrations of MgADP and Pi inside the fibers will increase in proportion to the ATPase activity. Thus, the effective concentrations of such substances will be modified as considered above.

In summary, the data presented here appear to be quantitatively correct under conditions where the tension level (ATPase activity) is low, but we should keep in mind that the data obtained at high tension are less quantitative.

Activation of fibers with ADP

Both isometric tension and ATPase activity of muscle fibers sigmoidally increased with the addition of MgADP under the relaxing condition (Figs. 4 and 6). Fig. 2 suggests that the added MgADP competes with MgATP for the binding site and that relaxed muscle is activated when some fraction of myosin heads is occupied by MgADP. Thus, the SPOC region is determined by the

ratio of the concentrations of MgADP and MgATP but not by the MgADP concentration.

If MgADP is a simple competitor for MgATP, ATPase activity is considered to be suppressed. In fact, the ATPase activity of muscle fibers was decreased by the addition of MgADP under a contracting condition (Hoar et al., 1987). In the present work, behavior of MgADP as a simple competitor was observed in the decreasing phase of tension obtained with increasing concentration of MgADP under a contracting condition (Fig. 5).

On the other hand, under a relaxing condition, MgADP functioned as a competitive activator (Fig. 4). The most probable interpretation for this is that AM-ADP complexes (AM, actomyosin complex), which become predominant with the addition of MgADP, function as a desuppressor, i.e., a suppressor of the inhibitory function of regulatory proteins, similarly to so-called rigor complex (Bremmel and Weber, 1972; Weber and Murray, 1973; Greene and Eisenberg, 1980); probably, the step of Pi release and/or some step(s) of isomerization of AM-ADP-Pi complex, which are suppressed in the absence of Ca^{2+} (Chalovich et al., 1981), may be released allosterically and cooperatively through the conformational change of thin filaments by the formation of AM-ADP complex, so that the ATPase activity appears. Judging from the large tension (Fig. 4) and high ATPase activity (Fig. 6) attained, the suppression seems to be nearly fully released.

Even under a contracting condition with Ca^{2+}, the addition of ADP slightly elevated the isometric tension (Fig. 5; Cooke and Pate, 1985; Kawai, 1986). This fact suggests that although the suppression imposed by the regulatory system is released by Ca^{2+}, MgADP may have an extra function as an activator; this does not necessarily mean, however, that the step of ATPase reaction that is regulated by an AM-ADP complex is different from that regulated by Ca^{2+}. The decrease in tension observed with further addition of MgADP (Fig. 5) may be ascribed to the competitive binding of MgADP to the binding site of MgATP as mentioned above; this phenomenon is probably similar to that observed when the concentration of MgATP is lowered to $< \sim 20$ μM, where a rigor complex instead of the AM-ADP complex may play a key role as an allosteric regulator (Cooke and Bialek, 1979).

Deactivation of fibers with Pi

Several reports have shown that muscle fibers under a contracting condition are deactivated by the addition of Pi (Rüegg et al., 1971; Herzig et al., 1981; Altringham and Johnston, 1985; Cooke and Pate, 1985; Kawai, 1986; Nosek et al., 1987; Cooke et al., 1988; Chase and Kushmerick, 1988; Kawai and Halvorson, 1991). We confirmed that the addition of 10 mM Pi monotonously decreased the isometric tension under a contracting condition by ~30% (Fig. 9), whereas we found that the decrease in tension was not observed if a high concentration of MgADP coexisted in the presence of Ca^{2+} (Fig. 9). The effect of Pi on the ADP-induced tension in the absence of Ca^{2+} was peculiar; with the increase of Pi concentration the tension decreased along a reversed sigmoidal curve (Fig. 7; *insert*, Fig. 10 *a*); besides, the tension drop was as much as 70% or more in the presence of 1 mM MgATP (Fig. 7), and nearly 100% in the presence of 2 mM MgATP (*insert*, Fig. 10 *a*). As discussed above, the data obtained in the presence of 2 mM MgATP are considered to be more quantitative, so that we can conclude that the ability of Pi to suppress the tension is extraordinarily strong for the ADP-induced activation in the absence of Ca^{2+}.

Such features can be simply interpreted according to the kinetic scheme of actomyosin ATPase (cf the following review articles; Hibberd and Trentham, 1986; Goldman, 1987; Goldman and Brenner, 1987; Brenner, 1990; Homsher and Millar, 1990; Geeves, 1991) as follows. Under the condition of ADP-induced contraction in the absence of Ca^{2+}, myosin heads will be mostly (>50%) in an AM-ADP complex, which is in a strong binding state and functions as an allosteric desuppressor of the inhibition, as pointed out repeatedly above. If Pi binds to this key complex and transforms it to an AM-ADP-Pi complex that is in a weak binding state having no desuppression function, the fibers will be easily deactivated again. If this is the case, as the Pi concentration is increased, the tension will decrease along a sigmoidal curve of tension vs MgADP concentration (Fig. 4) in an opposite direction, so that the decrease of tension occurs along either a reversed sigmoidal curve or a monotonous curve depending on the concentration of coexisting MgADP, or to be more precise, on the ratio of the concentrations of MgADP and MgATP (Figs. 7 and 10 *a*).

Now, Pi is considered to be a mixture of diprotonated form ($H_2PO_4^-$) and monoprotonated form without (HPO_4^{2-}) and with potassium ($KHPO_4^-$), and so on. There is a debate as to whether the diprotonated form (Dawson et al., 1986; Nosek et al., 1987) or all the species of Pi (Chase and Kushmerick, 1988) are responsible for the force reduction under a usual contracting condition with Ca^{2+}. Our results summarized in Fig. 10 *b* suggest that, at least under a SPOC condition, the diprotonated form is responsible. Although the relative tension curve obtained by changing the pH value at 5 mM Pi was slightly different from that obtained by

changing the Pi concentration at pH 7.0 (Fig. 10 *b*), this can be ascribed to the direct effect of pH on tension.

Synergistic effects of ADP and Pi

When both MgADP and Pi over critical concentrations, which depend on the concentration of MgATP, were added to the relaxing solution, spontaneous tension oscillation (SPOC) of muscle fibers was observed (Fig. 8). As far as we examined, the tension oscillation was not developed by the addition of either MgADP or Pi alone in the presence of MgATP and irrespective of the concentration of Ca^{2+}. Although the tension oscillation was not observed on a chart recorder, the intensity fluctuation of the fine structure of laser light diffraction lines, which originates from unorganized SPOC of sarcomeres, was clearly seen when MgADP alone was added (this phenomenon was not observed upon addition of Pi alone); this does not mean, however, that Pi is not needed for SPOC because submillimolar concentrations of Pi will be easily accumulated inside the fibers. So, until complete removal of Pi becomes possible, we can not reach a conclusion as to whether SPOC is induced by the addition of MgADP alone under the relaxing condition. It is true, in any case, that the coexistence of MgADP and Pi with MgATP is most suitable to induce stable SPOC.

The effects of MgADP and Pi on tension with Ca^{2+} (Figs. 5 and 9) are apparently similar to those without Ca^{2+} (Figs. 4 and 7). However, it is considered that the effects are qualitatively different from each other; in the presence of Ca^{2+}, MgADP and Pi may change the fraction of strong binding and weak binding states through direct interaction with myosin, whereas in the absence of Ca^{2+}, they may modulate the state of thin filaments, either the on-state or the off-state, in an allosteric and cooperative fashion through an AM-ADP (-Pi) complex similarly to rigor complex (Bremmel and Weber, 1972). We believe that this difference is essential to SPOC. Why is SPOC observed only in the latter condition but not in the former one, even under a similarly low level of tension? The key to this question must lie in the above difference.

Role of elastic components in SPOC

Here, we have neglected the contribution of the parallel elastic components composed of connectin (titin) (Maruyama, 1986; Wang, 1985), which are responsible for the resting tension (Funatsu et al., 1990). The reason is that SPOC can occur even at very short sarcomere lengths of ~ 2.0 μm, at which the extension of the parallel elastic components will be negligibly small; also, the dynamic feature of SPOC is unchanged by treatment with trypsin, to which connectin (titin) is very sensitive, until the destruction of the ordered structure of myofibrils becomes clear under an optical microscope (data not shown).

Mechanochemical coupling essential to the mechanism of SPOC

We infer that during SPOC each (half-)sarcomere cycles through turned-on and turned-off states, which are induced by stretch activation and/or release deactivation, i.e., activation during yielding and deactivation due to release caused by yielding of an adjacent (half-) sarcomere. Here, we suggest the following two types of regulatory mechanisms, where the key point is that the enzymic activity of actomyosin is modulated by the mechanical strain. First, the myosin(cross-bridge)-linked regulation: this is probable because the kinetic constant of some step(s), not only the step of Pi release but also the isomerization step of AM-ADP-Pi and/or AM-ADP complexes, may depend on the strain imposed on cross-bridges (cf Goldman, 1987; Danzig and Goldman, 1989). Second, the actin-linked regulation: the flexibility of reconstituted thin filaments is regulated by the concentration of free Ca^{2+} (Ishiwata and Fujime, 1972) and the flexibility change with Ca^{2+} is amplified by the interaction with myosin (Ishiwata and Fujime, 1971; Oosawa et al., 1973); conversely, the binding of myosin to reconstituted thin filaments increases the binding affinity of Ca^{2+} (Weber and Murray, 1973). From these properties, we infer that the strain of thin filaments induced by imposed stress may modify the functional state of thin filaments, so that the binding affinity and the state of cross-bridges are regulated. Other aspects of the molecular mechanism of SPOC will be discussed in the subsequent paper (Anazawa et al., 1992).

Physiological meaning of SPOC

It should be noted that the mechanochemical coupling essential to the mechanism of SPOC discussed above may play some role even under normal conditions. But, SPOC itself may not take place in vivo, because such high concentrations of MgADP as are required for SPOC will not be accumulated in living skeletal muscle, however severe muscle fatigue is.

On the other hand, some kinds of muscle may have the properties more suitable for SPOC. For example, cardiac myofibrils have more than ten times higher binding affinity to MgADP compared with skeletal muscle (Johnston and Adams, 1984). In practice, we found that SPOC of glycerinated cardiac muscle was observed in a lower concentration range of MgADP (Fukuda et al., 1991, and manuscript in preparation).

Cardiac muscle is not tetanizable while SPOC is observed under steady state. But, it may be important for the function of cardiac muscle that the contractile system has intrinsically such oscillatory properties.

Flight muscle, another example of which the physiological function is oscillation, needs activation by stretch in addition to Ca^{2+} to generate large tension; but the stretch is not needed if a low concentration of ADP is present (Pringle, 1967, 1978). It is inferred that the suppression, which may not be fully released by Ca^{2+}, is released by stretching of the muscle or the attachment of ADP to cross-bridges, which activates the muscle probably through the feed-back and allosteric regulation of transition between strong and weak-binding states of cross-bridges. Also, flight muscle is deactivated by quick release. In flight muscle, tension spontaneously oscillates without oscillation of membrane potential, i.e., the oscillation of free Ca^{2+} concentration, that is, the contractile system itself has oscillatory properties. In such muscle, feed-back regulation of tension through the tension-dependent (probably strain-dependent) change of state of cross-bridges as suggested in the above section may play a key role in its functioning. Thus, the molecular mechanism of oscillation of flight muscle may be essentially the same as that of SPOC.

This work was supported in part by Grants-in-Aid for Scientific Research (No. 61580226) and for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan.

Received for publication 23 August 1991 and in final form 16 December 1991.

REFERENCES

Altringham, J. D., and I. A. Johnston. 1985. Effects of phosphate on the contractile properties of fast and slow muscle fibers from an antarctic fish. *J. Physiol. (Lond.).* 368:491–500.

Anazawa, T., K. Yasuda, and S. Ishiwata. 1992. Spontaneous oscillation of tension and sarcomere length in skeletal myofibrils. Microscopic measurement and analysis. *Biophys. J.* 61:1099–1108.

Armstrong, C. F., A. F. Huxley, and F. J. Julian. 1966. Oscillatory responses in frog skeletal muscle fibers. *J. Physiol. (Lond.).* 186:26–27.

Bremmel, R. D., and A. Weber. 1972. Cooperative behavior within the functional unit of the actin filament in vertebrate skeletal muscle. *Nature (Lond.).* 238:97–101.

Brenner, B. 1979. An indirect proof of stretch-induced Ca^{++} release from the sarcoplasmic reticulum in glycerinated skeletal and heart muscle preparations. *Basic Res. Cardiol.* 74:177–202.

Brenner, B. 1990. Muscle mechanics and biochemical kinetics. *In* Molecular mechanisms in muscular contraction. J. M. Squire, editor. The Macmillan Press Ltd. London. 77–149.

Chaen, S., K. Kometani, T. Yamada, and H. Shimizu. 1981. Substrate concentration dependences of tension, shortening velocity and ATPase activity of glycerinated fibers. *J. Biochem. (Tokyo).* 90:1611–1621.

Chalovich, J. M., P. B. Chock, and E. Eisenberg. 1981. Mechanism of action of troponin-tropomyosin. *J. Biol. Chem.* 256:575–578.

Chase, P. B., and M. J. Kushmerick. 1988. Effects of pH on contraction of rabbit fast and slow skeletal muscle fibers. *Biophys. J.* 53:935–946.

Cooke, R., and W. Bialek. 1979. Contraction of glycerinated muscle fibers as a function of the ATP concentration. *Biophys. J.* 28:241–258.

Cooke, R., and E. Pate. 1985. The effects of ADP and phosphate on the contraction of muscle fibers. *Biophys. J.* 48:789–798.

Cooke, R., K. Franks, G. B. Luciani, and E. Pate. 1988. The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J. Physiol. (Lond.).* 395:77–97.

Danzig, J. A., and Y. E. Goldman. 1989. Photolysis of caged ATP in muscle fibers with negatively strained cross-bridges indicates that relaxation in the presence of MgADP is highly strain dependent. *Biophys. J.* 55:260*a*. (Abstr.)

Dawson, M. J., S. Smith, and D. R. Wilkie. 1986. The $[H_2PO_4^{-1}]$ may determine cross-bridge cycling rate and force reduction in living fatiguing muscle. *Biophys. J.* 49:268*a*. (Abstr.)

Ebashi, S., and M. Endo. 1968. Calcium ions and muscle contraction. *Prog. Biophys. Mol. Biol.* 18:123–183.

Eisenberg, E., and T. L. Hill. 1985. Muscle contraction and free energy transduction in biological systems. *Science (Wash. DC).* 227:999–1006.

Fabiato, A., and F. Fabiato. 1978. Myofilament-generated tension oscillations during partial calcium activation and activation dependence of the sarcomere length-tension relation of skinned cardiac cells. *J. Gen. Physiol.* 72:667–699.

Fukuda, N., T. Fujita, and S. Ishiwata. 1991. Effects of ADP, inorganic phosphate and calcium on spontaneous tension oscillation of glycerinated cardiac muscle. *J. Muscle Res. Cell Motil.* 12:304*a*. (Abstr.)

Funatsu, T., H. Higuchi, and S. Ishiwata. 1990. Elastic filaments in skeletal muscle revealed by selective removal of thin filaments with plasma gelsolin. *J. Cell Biol.* 110:53–62.

Geeves, M. A. 1991. The dynamics of actin and myosin association and the cross-bridge model of muscle contraction. *Biochem. J.* 274:1–14.

Goldman, Y. E. 1987. Kinetics of the actomyosin ATPase in muscle fibers. *Annu. Rev. Physiol.* 49:637–654.

Goldman, Y. E., and B. Brenner. 1987. Special topic: molecular mechanism of muscle contraction. *Annu. Rev. Physiol.* 49:629–636.

Goodall, M. C. 1956. Auto-oscillations in extracted muscle fibre systems. *Nature (Lond.).* 177:1238–1239.

Greene, L. E., and E. Eisenberg. 1980. Cooperative binding of myosin subfragment-1 to the actin-troponin-tropomyosin complex. *Proc. Natl. Acad. Sci. USA.* 77:2616–2620.

Herzig, J. W., J. W. Peterson, J. C. Rüegg, and R. J. Solaro. 1981. Vanadate and phosphate ions reduce tension and increase cross-bridge kinetics in chemically skinned heart muscle. *Biochim. Biophys. Acta.* 672:191–196.

Hibberd, M. G., J. A. Dantzig, D. R. Trentham, and Y. E. Goldman. 1985. Phosphate release and force generation in skeletal muscle fibers. *Science (Wash. DC).* 228:1317–1319.

Hibberd, M. G., and D. R. Trentham. 1986. Relationships between chemical and mechanical events during muscular contraction. *Annu. Rev. Biophys. Biophys. Chem.* 15:119–161.

Hoar, P. E., C. W. Mahoney, and W. G. L. Kerrick. 1987. $MgADP^-$ increases maximum tension and Ca^{2+} sensitivity in skinned rabbit soleus fibers. *Pfluegers Arch. Eur. J. Physiol.* 410:30–36.

Homsher, E., and N. C. Millar. 1990. Caged compounds and striated muscle contraction. *Annu. Rev. Physiol.* 52:875–896.

Horiuti, K. 1986. Some properties of the contractile system and sarcoplasmic reticulum of skinned low fibres from *Xenopus* muscle. *J. Physiol. (Lond.).* 373:1–23.

Ishiwata, S., and S. Fujime. 1971. Effect of Ca^{2+} on dynamic properties of muscle proteins studied by quasielastic light scattering. *J. Phys. Soc. Japan.* 31:1601.

Ishiwata, S., and S. Fujime. 1972. Effect of calcium ions on the flexibility of reconstituted thin filament of muscle studied by quasielastic scattering of laser light. *J. Mol. Biol.* 68:511–522.

Ishiwata, S., and T. Funatsu. 1985. Does actin bind to the ends of thin filaments in skeletal muscle? *J. Cell Biol.* 100:282–291.

Ishiwata, S., K. Muramatsu, and H. Higuchi. 1985. Disassembly from both ends of thick filaments in rabbit skeletal muscle fibers. An optical diffraction study. *Biophys. J.* 47:257–266.

Ishiwata, S., N. Okamura, H. Shimizu, T. Anazawa, and K. Yasuda. 1991. Spontaneous oscillatory contraction (SPOC) of sarcomeres in skeletal muscle. *Adv. Biophys.* 27:227–235.

Iwazumi, T., and G. H. Pollack. 1981. The effect of sarcomere nonuniformity on the sarcomere length-tension relationship of skinned fibers. *J. Cell. Physiol.* 106:321–337.

Johnston, R. E., and P. E. Adams. 1984. ADP binds similarly to rigor muscle myofibrils and to actomyosin-subfragment one. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 174:11–14.

Kawai, M. 1986. The role of orthophosphate in cross-bridge kinetics in chemically skinned rabbit psoas fibres as detected with sinusoidal and step length alterations. *J. Muscle Res. Cell Motil.* 7:421–434.

Kawai, M., K. Guth, K. Winnikes, C. Haist, and J. C. Rüegg. 1987. The effect of inorganic phosphate on ATP hydrolysis rate and the tension transients in chemically skinned rabbit psoas fibers. *Pfluegers Arch. Eur. J. Physiol.* 408:1–9.

Kawai, M., and H. R. Halvorson. 1989. Role of MgATP and MgADP in the cross-bridge kinetics in chemically skinned rabbit psoas fibers. *Biophys. J.* 55:595–603.

Kawai, M., and H. R. Halvorson. 1991. Two step mechanism of phosphate release and the mechanism of force generation in chemically skinned fibers of rabbit psoas muscle. *Biophys. J.* 59:329–342.

Kodama, T., K. Fukui, and K. Kometani. 1986. The initial phosphate burst in ATP hydrolysis by myosin and subfragment-1 as studied by a modified malachite green method for determination of inorganic phosphate. *J. Biochem. (Tokyo).* 99:1465–1472.

Kushmerick, M. J., and R. J. Podolsky. 1969. Ionic mobility in muscle cells. *Science (Wash. DC).* 166:1297–1298.

Lanzetta, P. A., L. J. Alvarez, P. S. Reinach, and O. A. Candia. 1979. An improved assay for nanomole amounts of inorganic phosphate. *Anal. Biochem.* 100:95–97.

Lienhard, G. E., and I. I. Secemski. 1973. P^1,P^5-Di(adenosine-5')pentaphosphate, a potent multisubstrate inhibitor of adenylate kinase. *J. Biol. Chem.* 248:1121–1123.

Lorand, L., and C. Moos. 1956. Auto-oscillations in extracted muscle fibre systems. *Nature (Lond.).* 177:1239.

Mannherz, G. M. 1968. ATP-spaltung und ATP-diffusion in oscillierenden extrahierten muskelfasern. *Pfluegers Arch. Eur. J. Physiol.* 303:230–248.

Maruyama, K. 1986. Connectin, an elastic filamentous protein of striated muscle. *Int. Rev. Cytol.* 104:81–114.

Millar, N. C., and E. Homsher. 1990. The effect of phosphate and calcium on force generation in glycerinated rabbit skeletal muscle fibers. A steady-state and transient kinetic study. *J. Biol. Chem.* 265:20234–20240.

Nosek, T. M., K. Y. Fender, and R. E. Godt. 1987. It is diprotonated inorganic phosphate that depresses force in skinned skeletal muscle fibers. *Science (Wash. DC).* 236:191–193.

Ohnishi, S. T., and Barr, J. K. 1978. A simplified method of quantitating protein using the biuret and phenol reagents. *Anal. Biochem.* 86:193–200.

Ohno, T., and T. Kodama. 1991. Kinetics of adenosine triphosphate hydrolysis by shortening myofibrils from rabbit psoas muscle. *J. Physiol. (Lond.).* 441:685–702.

Okamura, N., and S. Ishiwata. 1988. Spontaneous oscillatory contraction of sarcomeres in skeletal myofibrils. *J. Muscle Res. Cell Motil.* 9:111–119.

Onodera, S., and Y. Umazume. 1984. Periodic contraction of skinned muscle fiber under high pH. *Biophys., Suppl. (Jpn.).* 24:S84*a*. (Abstr.)

Onodera, S. 1990. Oscillatory contraction waves in skinned skeletal muscle at high-pH without Ca^{2+}. *Jikeikai Med. J.* 37:447–455.

Oosawa, F., S. Fujime, S. Ishiwata, and K. Mihashi. 1973. Dynamic property of F-actin and thin filament. *Cold Spring Harbor Symp. Quant. Biol.* 37:277–286.

Pate, E., and R. Cooke. 1989. A model of cross-bridge action: the effects of ATP, ADP and Pi. *J. Muscle Res. Cell Motil.* 10:181–196.

Pringle, J. W. S. 1967. The contractile mechanism of insect fibrillar muscle. *Prog. Biophys. Mol. Biol.* 17:1–60.

Pringle, J. W. S. 1978. Stretch activation of muscle: function and mechanism. *Proc. R. Soc. Lond. B.* 201:107–130.

Rüegg, J. C., M. Schadler, G. J. Steiger, and G. Muller. 1971. Effects of inorganic phosphate on the contractile mechanism. *Pfluegers Arch. Eur. J. Physiol.* 325:359–364.

Schoenberg, M., and E. Eisenberg. 1987. ADP binding to myosin cross-bridges and its effect on the cross-bridge detachment rate constants. *J. Gen. Physiol.* 89:905–920.

Sleep, J. 1984. The adenosine-triphosphatase activity of muscle-fibers. *J. Physiol. (Lond.).* 326:5*a*. (Abstr.)

Stephenson, D. G., and D. A. Williams. 1982. Effects of sarcomere length on the force-pCa relation in fast- and slow-twitch skinned muscle fibres from the rat. *J. Physiol. (Lond.).* 333:637–653.

Sweitzer, N. K., and R. L. Moss. 1990. The effect of altered temperature on Ca^{2+}-sensitive force in permeabilized myocardium and skeletal muscle. *J. Gen. Physiol.* 96:1221–1245.

Wang, K. 1985. Sarcomere-associated cytoskeletal lattices in striated muscle: reviews and hypothesis. *In* Cell and Muscle Motility. Vol. 6. J. W. Shay, editor. Plenum Publishing Corp., New York. 315–369.

Webb, M. R., M. G. Hibberd, Y. E. Goldman, and D. R. Trentham. 1986. Oxygen exchange between Pi in the medium and water during ATP hydrolysis mediated by skinned fibers from rabbit skeletal muscle: Evidence for Pi binding to a force-generating state. *J. Biol. Chem.* 261:15557–15564.

Weber, A., and J. M. Murray. 1973. Molecular control mechanisms in muscle contraction. *Physiol. Rev.* 53:612–673.