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Effect of inorganic phosphate and ADP on the myofilament sliding induced by laser flash photolysis of caged ATP

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Abstract

Using the technique of laser flash photolysis of caged ATP, we have suggested that, under nearly isometric conditions, the unitary distance of myofilament sliding per ATP molecule (myosin head powerstroke) is about 10 nm. To give further information about the mechanism of myofilament sliding, we studied the effect of inorganic phosphate (P_i) and ADP on the photoreleased ATP-induced shortening of single glycerinated muscle fibers under very small external loads. Both the velocity and the distance of the myofilament sliding induced by 150 μM ATP increased by P_i (20 mM), and decreased by ADP (0.4 mM). On the other hand, P_i and ADP showed no significant effect on the myofilament sliding induced by 100 and 75 μM ATP. The potentiating effect of P_i on the myofilament sliding with 150 μM ATP can be explained as being due to the increase in population of AM · ADP · P_i with corresponding decrease in population of AM · ADP, and also the increase in population of M · ADP · P_i and M · ATP. Meanwhile, the inhibitory effect of ADP can be simply accounted for to be due to an accumulation of AM · ADP that already finished their force generating process. The ineffectiveness of P_i and ADP on the myofilament sliding with 100 and 75 μM ATP is consistent with the view that it is caused by almost synchronized single myosin head powerstrokes.

Keywords: Myofilament sliding; ATP hydrolysis; Caged ATP; Laser flash photolysis; Inorganic phosphate; ADP

1. Introduction

Muscle contraction results from relative sliding between the thick filament consisting mainly of myosin and the thin filament consisting mainly of actin. The myofilament sliding is caused by cyclic interaction between the myosin heads extending from the thick filaments (cross-bridges) and the sites on the thin filaments coupled with ATP hydrolysis; a myosin head first attaches to actin, and then changes its angle of attachment (powerstroke) to produce myofilament sliding until it is detached from actin [1,2]. The technique of rapid laser flash photolysis of caged ATP (P^3-1-(2-nitro) phenylethyladenosine 5′-triphosphate), a biologically inert and photolabile precursor of ATP [3], has been a powerful method to obtain direct correlation between actomyosin ATPase reaction and muscle contractile response [4–6]. Using this technique, we have recently found that 75 μM photoreleased ATP produces the minimum (unitary) myofilament sliding taking place along the entire length of a single glycerinated muscle fiber, and that the distance of the unitary myofilament sliding is around 10 nm under nearly isometric conditions [7]. Since the ATP concentration producing the unitary myofilament sliding is a half of the myosin head concentration within the fiber [8], these results suggest that the distance of myosin head powerstroke, coupled with hydrolysis of one ATP molecule, is about 10 nm in nearly isometric conditions. A similar distance of myosin head powerstroke has also been suggested by Higuchi and Goldman [9].

To obtain further information about the mechanism of myofilament sliding, the present experiments were undertaken to study the effect of inorganic phosphate (P_i) and ADP on the myofilament sliding induced by laser flash photolysis of caged ATP. It will be shown that the myofilament sliding induced by 150 μM ATP is potentiated by

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P_i and inhibited by ADP, while the unitary myofilament sliding induced by 75 μM ATP is not influenced by P_i and ADP.

2. Materials and methods

2.1. Muscle fiber preparation

Glycerol-extracted muscle strips were prepared from rabbit psoas by the method of Yamada et al. [7], and further dissected into single fibers (diameter, 50–60 μm; length, 250–300 μm). The single fiber preparation was mounted horizontally in a glass experimental chamber ($3 \times 15 \times 0.8$ mm deep) filled with glycerol solution containing 50 mM KCl, 4 mM $MgCl_2$, 4 mM EGTA and 20 mM Tris-maleate (pH 7.0); one end of the fiber was glued to a rigid stainless-steel rod, which the other end was made free to shorten without any external load. Temperature of solutions in the chamber was kept at 20–22°C with a thermoelectric device. The sarcomere length of the fiber was adjusted to 2.4 μm by light diffraction with He-Ne laser light.

2.2. Solutions

The solutions used in the present experiments are listed in Table 1. Reagent grade $MgCl_2$ and EGTA were obtained from Wako Pure Chemical Co. (Osaka, Japan). ATP, ADP, P_i and glutathione (GSH, reduced form) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Caged ATP of highly purified grade was purchased from Dojindo Laboratories (Kumamoto, Japan).

2.3. Laser flash photolysis of caged ATP

After the fiber was mounted in the experimental chamber, glycerol solution was replaced by Ca^{2+} rigor solution, and further by photolysis solution containing caged ATP. For uniform diffusion of caged ATP into myofilament lattice, the fiber was kept in photolysis solution for 30–60 s. Immediately before laser flash irradiation, caged ATP solution in the chamber was replaced by silicone oil to prevent diffusion of photoreleased ATP out of the fiber. Then the fiber was made to shorten by a light flash (duration, 10 ns; wavelength, 355 nm; intensity, up to 200 mJ) from a Nd:YAG laser system (Spectra Physics, Mountain View, CA, USA; DCR-3). Details of the laser flash irradiation technique have been described previously, together with the method to estimate the amount of photoreleased ATP with a reverse-phase high-pressure liquid chromatography [7]. The amount of photoreleased ATP was altered either by changing the caged ATP concentration or by changing the laser flash intensity.

2.4. Recording of muscle fiber shortening

The shortening of the whole fiber following photorelease of ATP was recorded with a high-speed video system (200 frames/s, NAC, Inc. HSV-200) mounted on a binocular microscope (Wild, M-7, Heerbrugg, Switzerland). The videotape records were analyzed with a video microscaler (For A, Co., Tokyo, Japan, IV-550) to obtain the time course of fiber shortening. Early experiments were performed in the presence of 40 μM AP_5A (diadenosine pentaphosphate), inhibiting myokinase activity, but AP_5A had no appreciable effect on the fiber shortening following photorelease of ATP.

2.5. Electron microscopy

To ascertain uniformity of sarcomere lengths within the fiber both before and after the fiber shortening, the fibers were fixed in a 2.5% glutaraldehyde solution containing 0.2% tannic acid and then in a 1% OsO_4 solution either before or after the flash-induced shortening, and conventional longitudinal sections of the fibers were examined under low magnifications with a JEOL JEM 100CX transmission electron microscope [7].

3. Results

3.1. General features of the ATP-induced muscle fiber shortening

A typical video record of a single glycerinated muscle fiber during the course of shortening induced by 150 μM

Table 1
Composition of solutions

	$MgCl_2$	$CaCl_2$	KCl	EGTA	Caged ATP	Tes	GSH	P_i	ADP
Ca^{2+} rigor	5	2.1	120	2	0	100	0	0	0
Photolysis	5	2.1	35	2	2–4	100	30	0	0
Photolysis (+P_i)	5	2.1	0	2	2–4	100	30	20	0
Photolysis (+ADP)	5	2.1	35	2	2–4	100	30	0	0.4

All concentrations in millimol per liter. Overall ionic strength of the solutions was 200 mM with pCa 4.0. pH was adjusted to 7.0 with HCl or KOH. EGTA, ethyleneglycol-bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid; Tes, N-Tris (hydroxymethyl)-methyl-2-aminoethanesulphonic acid; GSH, glutathione (reduced form).

ATP is shown in Fig. 1. Following laser flash irradiation, each elementary fiber segment between adjacent natural markers on the fiber surface shortened uniformly along the entire fiber length. As shown in Fig. 2, all the fiber segments shortened with almost the same time course; the segments first shortened linearly with time, and then the shortening was slowed down to eventually come to a complete stop, as the photoreleased ATP was completely exhausted to zero [7]. The uniform fiber shortening was induced only with photoreleased ATP above 75 μM; 50 μM photoreleased ATP produced only a just perceptible change in fiber shape without any appreciable shortening of the whole fiber [7]. The uniform fiber shortening induced by ATP above 75 μM is therefore considered to be associated with uniform myofilament sliding taking place in every half sarcomere. In accordance with the previous report [7], electron microscopic observation of the fiber longitudinal sections indicated that the sarcomere length was uniform everywhere within the fiber in all the experimental conditions used. The disappearance of the uniform fiber shortening with the decrease of photoreleased ATP from 75 to 50 μM may result from that the uniform distribution of photoreleased ATP in each sarcomere, necessary to produce the uniform fiber shortening, is no

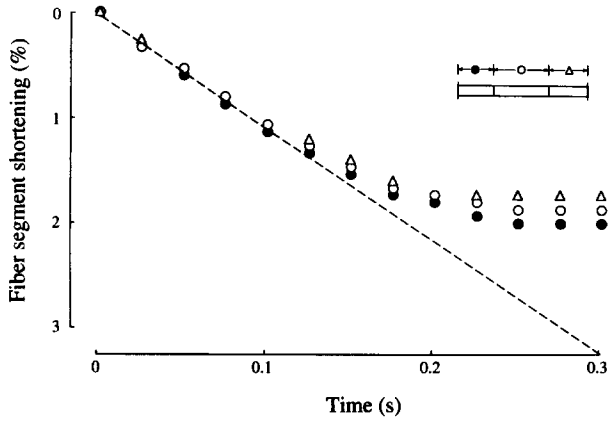


Fig. 2. Time course of shortening of three elementary segments divided by natural surface markers (inset). Note that each segment initially shortens linearly with time. The data points were obtained from the video record shown in Fig. 1.

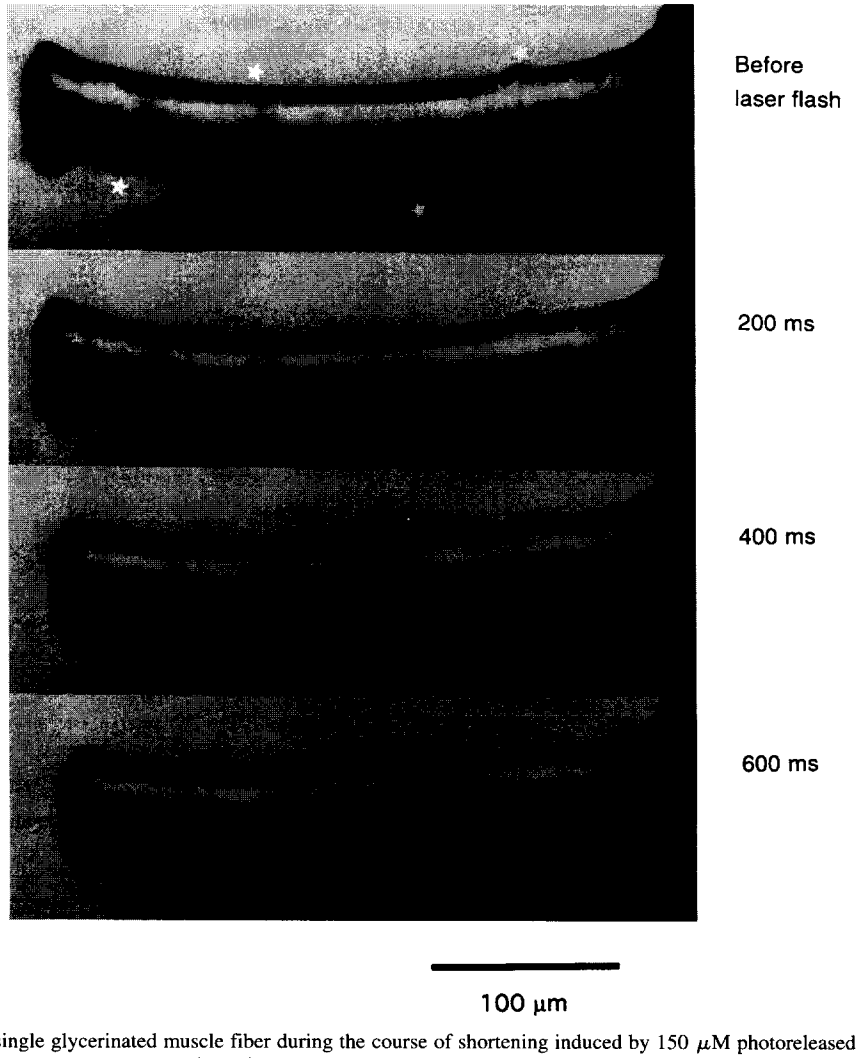


Fig. 1. A video record of a single glycerinated muscle fiber during the course of shortening induced by 150 μM photoreleased ATP. The time after a laser flash irradiation is indicated alongside each frame (in ms). Location of some natural markers on the fiber surface is indicated by asterisks in the top frame.

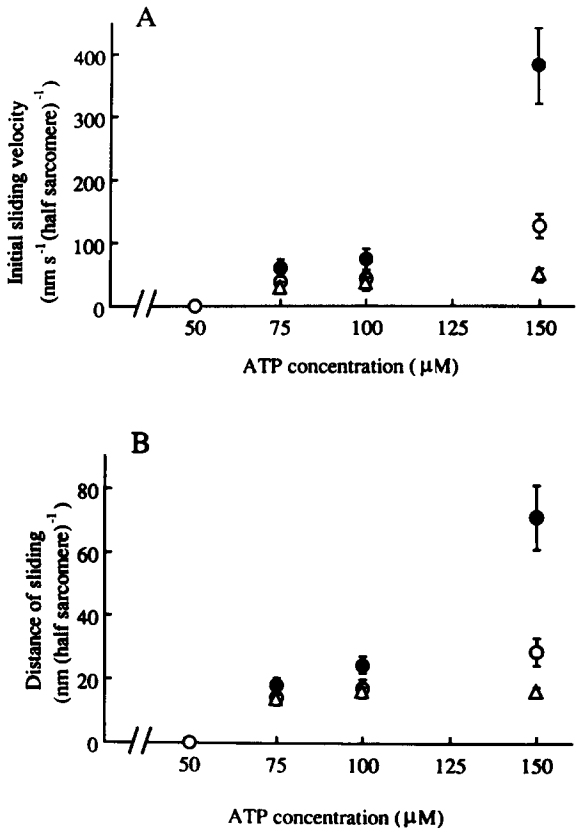


Fig. 3. Effect of P_i (20 mM) and ADP (0.4 mM) on the initial velocity (A) and the distance (B) of myofilament sliding induced by 76, 100 and 150 μM ATP. (○, control:, ●, in the presence of 20 mM P_i: △, in the presence of 0.4 mM ADP. Vertical bars denote S.E. of the mean.

longer maintained if its concentration is reduced below a critical level.

3.2. Effect of P_i and ADP on the initial velocity and the distance of myofilament sliding

The results obtained are summarized in Fig. 3. The initial velocity of myofilament sliding induced by 150 μM ATP was 127 ± 19 nm s^{-1} (half sarcomere)$^{-1}$ (mean ± S.E., $n = 13$) in the control condition. It increased to 380 ± 61 nm s^{-1} (half sarcomere)$^{-1}$ ($n = 9$) in the presence of P_i (20 mM) (t-test, $P < 0.01$), while it decreased to 49 ± 10 nm s^{-1} (half sarcomere)$^{-1}$ ($n = 6$) by 0.4 mM ADP ($P < 0.05$). The distance of myofilament sliding induced by 150 mM ATP was 28.2 ± 4.2 nm ($n = 13$) (half sarcomere)$^{-1}$ in the control condition, increased to 70.9 ± 9.9 nm (half sarcomere)$^{-1}$ ($n = 9$) in the presence of P_i ($P < 0.01$), and decreased to 16.5 ± 1.0 nm (half sarcomere)$^{-1}$ ($n = 6$) in the presence of ADP ($P < 0.01$).

In contrast with the distinct P_i-induced potentiation and the ADP-induced inhibition on the myofilament sliding with 150 μM ATP, both P_i and ADP showed no significant effect on the myofilament sliding with 100 and 75 μM ATP. The initial myofilament sliding velocity with 100 μM ATP was 44 ± 8, 73 ± 17 and 35 ± 10 nm s^{-1} (half sarcomere)$^{-1}$ ($n = 9$, 11 and 6) in the control, in the presence of P_i, and in the presence of ADP, respectively. The myofilament sliding distance was 17.2 ± 3.0, 24.5 ± 2.7 and 16.5 ± 1.0 nm (half sarcomere)$^{-1}$ ($n = 9$, 11 and 6) in the control, in the presence of P_i, and in the presence of ADP, respectively. The differences between the control and the test values were all statistically insignificant.

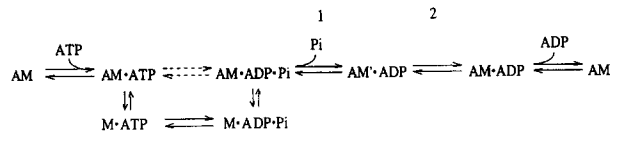
The effect of P_i and ADP was also statistically insignificant concerning the myofilament sliding with 75 μM ATP. The initial myofilament sliding velocity was 38 ± 7, 60 ± 13, 28 ± 10 nm s^{-1} (half sarcomere)$^{-1}$ ($n = 9$, 11 and 6) in the control, in the presence of P_i, and in the presence of ADP, respectively. The myofilament sliding distance was 14.6 ± 2.8, 18.3 ± 2.3 and 14.0 ± 1.1 nm (half sarcomere)$^{-1}$ ($n = 8$, 6 and 6) in the control, in the presence of P_i and in the presence of ADP.

4. Discussion

4.1. Mechanism of the potentiating effect of P_i and inhibitory effect of ADP on the myofilament sliding with 150 μM ATP

In the present experiments, the concentration of ATP within a muscle fiber was limited to be equal to, or smaller than, the myosin head concentration in the fiber (150 μM) [8], so that the average number of ATP molecule available for a single myosin head was one with 150 μM ATP and less than one with 100 and 75 μM ATP. Therefore, a considerable fraction of myosin heads remained attached to actin to form rigor linkages, providing a considerable internal resistance against the ATP-induced myofilament sliding. In fact, Yamada et al. [7] estimated the internal resistance against the ATP-induced myofilament sliding to be nearly equal to the maximum isometric force P_o, by comparing the initial myofilament sliding velocity with the force-velocity relation of fully Ca^{2+}-activated fibers in the presence of 2 mM ATP. Therefore, the ATP-induced myofilament sliding observed in the present study took place against a large internal resistance, though no external load was applied to the fiber.

The reaction scheme for the myosin heads (M) interacting with actin (A) by utilizing ATP is [10–12]:



where generation of force for myofilament sliding is likely to be linked to reaction 2. According to the above scheme, the addition of P_i will affect reaction 1 to increase the

population of AM · ADP · P_i with corresponding decrease in the population of AM′ · ADP. In the steady state, this decreases the population of force-generating myosin heads to result in a decrease of steady isometric force in fully Ca^{2+}-activated muscle [13,14].

The distance of myofilament sliding with 150 μM ATP was about 70 nm (half sarcomere)$^{-1}$ in the presence of 20 mM P_i, a distance several times the myofilament sliding distance with 75 μM ATP, indicating that many myosin heads would repeat their cyclic reaction with actin several times during the course of myofilament sliding. In such a non-steady condition, the P_i-induced increase of AM · ADP · P_i population slows down reaction 1, and will prolong the period in which the myosin heads are allowed to repeat their powerstrokes associated with reaction 2. This would result in a less synchronized myosin head powerstrokes to build up a larger distance of myofilament sliding until ATP is totally exhausted. As the above explanation assumes that the number of AM′ · ADP generating sliding force at a given moment is smaller than that in the control condition, it is necessary to explain why P_i increases the initial velocity of myofilament sliding despite the decreased population of AM′ · ADP. Since P_i is reported to have no effect on the unloaded shortening velocity of muscle fibers [13], it is unlikely that P_i facilitates myosin head detachment from actin after the completion of reaction 2.

A possible explanation for the potentiating effect of P_i on the initial velocity of myofilament sliding may be that P_i reduces the internal resistance against myofilament sliding to an extent that would more than compensate the decreased population of AM′ · ADP responsible for myofilament sliding. The initial velocity of myofilament sliding is reported to never saturate even with the photoreleased ATP above 1 mM [9], suggesting that some fraction of myosin heads forming rigor A · M links may not readily bind with the released ATP. If the binding of the released ATP with rigor myosin heads is assumed to be facilitated by the P_i-induced increase of AM · ADP · P_i population, which would in turn initiate recycling of reactions before reaction 1, then the overall effect of P_i would be to reduce the internal resistance against myofilament sliding to result in an increase in the initial velocity of myofilament sliding.

On the other hand, the addition of ADP will affect reaction 2 to increase the population of AM · ADP, and consequently slows down reaction 2 and the rate of myosin head detachment from actin. The resulting increase of the internal resistance can, at least qualitatively, account for the inhibitory effect of ADP on both the velocity and the distance of myofilament sliding with 150 μM ATP. In fact, ADP is reported to reduce the shortening velocity and to increase steady isometric tension in glycerinated fibers [13].

4.2. Ineffectiveness of P_i and ADP on the unitary myofilament sliding

The effect of P_i and ADP on the myofilament sliding induced by 100 and 75 μM ATP was statistically insignificant, though P_i showed a tendency to increase the initial myofilament sliding velocity. On the basis of the reaction scheme, the ineffectiveness of P_i and ADP seems to indicate that when the concentration of ATP within the fiber is smaller than the concentration of myosin heads, the myosin heads utilizing ATP would perform their single powerstroke almost synchronously. This implies that most myosin heads pass through reactions 1 and 2 almost synchronously and only once, so that P_i and ADP cannot effectively affect the resulting myofilament sliding. The above idea is consistent with the fact that 75 μM ATP produces the unitary myofilament sliding that reflects the distance of a single myosin head powerstroke [7]. Much more experimental work with different techniques are of course needed to further clarify the molecular mechanism of myofilament sliding coupled with ATP hydrolysis.

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References

[1] Huxley, A.F. (1957) Prog. Biophys. Biophys. Chem. 7, 255–318.
[2] Huxley, H.E. (1969) Science 164, 1356–1366.
[3] Kaplan, J.H., Forbush, B. and Hoffman, J.F. (1978) Biochemistry 17, 1929–1935.
[4] Goldman, Y.E., Hibberd, M.G., McCray, J.A. and Trentham, D.R. (1982) Nature 300, 701–705.
[5] Goldman, Y.E., Hibberd, M.G. and Trentham, D.R. (1984) J. Physiol. 354, 605–624.
[6] Dazing, J.A., Hibberd, M.G., Trentham, D.R. and Goldman, Y.E. (1991) J. Physiol. 432, 639–680.
[7] Yamada, T., Abe, O., Kobayashi, T. and Sugi, H. (1993) J. Physiol. 466, 229–243.
[8] Ferenczi, M.A., Homsher, E. and Trentham, D.R. (1984) J. Physiol. 352, 575–599.
[9] Higuchi, H. and Goldman, Y.E. (1991) Nature 352, 352–354.
[10] Lymn, R.W. and Taylor, E.W. (1971) Biochemistry 10, 4617–4624.
[11] Eisenberg, I. and Greene, L.E. (1980) Ann. Rev. Physiol. 42, 293–309.
[12] Sleep, J.A. and Hutton, R.L. (1980) Biochemistry 19, 1276–1283.
[13] Cook, R. and Pate, E. (1985) Biophys. J. 48, 789–798.
[14] Kentish, J.C. (1986) J. Physiol. 370, 585–604.