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Enhancement of the sliding velocity of actin filaments in the presence of ATP analogue: AMP-PNP

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Abstract

The sliding velocity of actin filaments was found to increase in the presence of ATP analogues. At 0.5 mM ATP, the presence of 2.0 mM of AMP-PNP enhanced the filament velocity from 3.2 up to 4.5 μm/s. However, 2 mM ADP decreased the velocity down to 1.1 μm/s. The results suggest that the complex conformations of myosin cross-bridges interacting with an actin filament in the presence of ATP analogues makes the entire filament move faster.

Keywords: Actin; Myosin; Weakly bound state; ATP analogue; Sliding velocity

1. Introduction

Recent advancement of single molecular technology [1–3] has enabled us to measure the processive movement of a myosin molecule along an actin filament. In case of myosin V, for example, processive step size was approximately 36 nm [4]. However, the size for myosin II was not constant [5] against the single hydrolysis cycle of ATP [6]. The processivities are the major important aspects of motor proteins but balances between motors are another distinct feature for smooth sliding movement. The coupling between ATPase and migration step is not strictly determined presumably due to so called 'loose coupling' [7]. Why is coupling loose?

In living organelle and myofibril, many myosin heads are interacting with a single filament of actin. Muscle contraction, as well as sliding movement in an in vitro sliding assay, is actualized by cooperative interactions among many actomyosin complexes. In such a case, however, the myosin heads hydrolyze ATP molecules in a totally stochastic manner. Consequently, they might be in an arbitrary state such as power stroke, weak binding, dissociation and so on. How can they acquire the cooperativity? How do the actin filaments realize their smooth sliding? We have surmised that the key to the secret for looseness of coupling must be lurking in this point [8].

In a conventional in vitro motility assay system, actin filament could slide smoothly along myosin

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heads spread on the glass surface [9]. The velocity depends on the concentration of the ATP nucleotide [10]. Both the force generation and sliding movement were assumed to be manifested by myosin as a motor protein. However, many results have been reported about the role of actin filament on the smooth sliding movement as follows: the velocity was sensitized in the presence of both GTP and ATP [11]. Filaments were distorted during migration [12]. Elastic properties of a sliding filament were estimated quantitatively from the geometrical nature [13]. Transversal fluctuation of a filament depends on ATP concentration [14] and is propagating from the tail to the front of moving filaments [15,16]. The nature of fluctuation changes from isotropic to anisotropic with the increase of ATP concentration [17,18].

We have prepared 'Madara' actin filament [12] or speckled filament, and measured the fluctuation of sliding velocity of local points on a filament with high accuracy of 8 nm [19]. At this precision, the state of myosin heads, i.e. binding strongly, weakly to or detaching from an actin filament could be detected separately along the same actin filament. In this study, we report that the sliding velocities of an actin filament were decreased in the presence of ADP [20] but were accelerated up to approximately 150% in the presence of equal amount of AMP-PNP, which is known to stabilize the weak binding intermediates state of acto-subfragment-1 complex [21], in an in vitro motility assay. The results indicate that actin filaments coordinate the different kinds of sliding forces from both weak and strong binding state of myosin heads to effectuate the uniform velocities of the entire filament, and that the cooperativity between myosin heads could be realized by mechanical interactions through an actin filament.

2. Materials and methods

2.1. Proteins and reagents

ADP, ATP and 5′-adenylylimidodiphosphate (AMP-PNP) were purchased from Sigma Co. Ltd. Other reagents were from Wako Pure Chemicals. Actins and myosins were prepared from rabbit skeletal muscle. Actin monomers were extracted from acetone powder with a G-buffer [2 mM Tris–HCl (pH 8.0), 0.2 mM ATP, 0.1 mM $CaCl_2$] and purified according to the method of Spudich and Watt [22] followed by gel chromatographic purification removing denatured oligomers. Myosin molecules were obtained from a minced muscle with a Guba–Straub solution and were purified by the method of Perry [23]. Heavy meromyosin (HMM) was prepared by α-chymotriptic digestion of myosin according to Sekine and Okamoto [24].

2.2. Motility assay of actin filaments

Actin filaments were labeled with tetramethylrhodamine-phalloidins [25]. Their observation was performed under an optical microscope with a fluorescent illumination unit (Nikon, TMD-phot, 100× oil object lens, 10× relay lens), 100 W high pressure mercury lamp. The labeled actin filaments were incubated in standard solution (25 mM KCl, 25 mM imidazole–HCl (pH 7.4), 4 mM $MgCl_2$, 0.5% 2-mercaptoethanol) at room temperature (approx. 25 °C) for 60 min. The HMM solution (0.05 mg/ml) was perfused between cover slips (18×18 and 24×50 mm) treated with 3-methylbutyl acetate with 1% collodion (Wako and Nakarai, respectively). After 60 s of perfusion, HMMs not attaching on the glass surface were removed with a standard solution with 1.0 mg/ml bovine serum albumin (BSA) (Sigma). After another 60 s, fluorescent actin filaments were perfused followed by replacing an assay solution containing various concentrations of ATP and ATP analogues. Photobleaching was reduced enzymatically with the use of an oxygen-scavenging system [0.1 mg/ml glucose oxidase (Sigma), 3 mg/ml glucose, 0.018 mg/ml catalase (Wako)] [25].

2.3. Analysis for fluorescent images of an actin filament

The fluorescent images were recorded by using a videocassette recorder (Panasonic, S-VHS NV-SB 1000W) with a high sensitive ICCD video camera (Video Scope International, Ltd. ICCD-350F). The selected area of video images was fed into a computer (Apple Computer Inc., Power Macintosh 7600/132) with a video grabber board

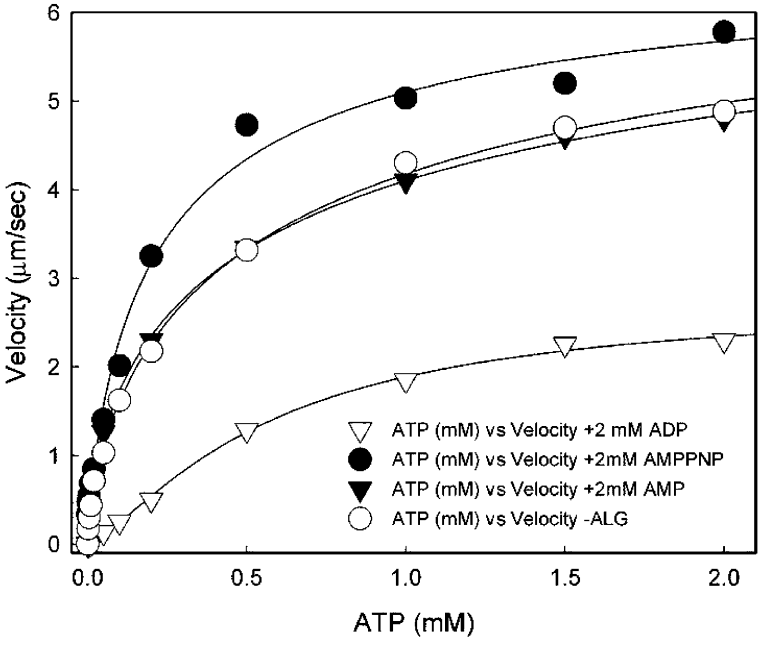

Fig. 1. ATP-dependent increase of sliding velocities of actin filament. Sliding velocities of actin filaments were calculated from the displacement of filament front during 1.0 s at normal motility assay conditions as described in text. Open circles indicate the absence of analogues. Filled circle, filled triangle and open triangles indicate the presence of 2 mM AMP-PNP, AMP and ADP, respectively. Each curve associated with the data were drawn by fitting to Hill's equation. For detailed experimental conditions, see text.

(Scion Co., LG-3 PCI) at every interval of 1/30 s. Instantaneous velocities of each filament were measured from the displacements for every 0.1 s.

'Madara' actin filaments, speckled fluorescent filaments, were prepared as described in the previous report [12]. Local fluctuations of migrating velocity was calculated as follows:

X–Y coordinate vector of speckled points $x(t_n)$,$y(t_n)$ recorded from the image analyzing program at time t_n will be denoted as:

$$\overrightarrow{X(t_n)} = (x(t_n),y(t_n))$$

Where t_n denotes a sequence point of measuring time as $t_n+1=t_n+0.1$ s.

Velocity at time t_n is defined as:

$$\overrightarrow{V(t_n)} = \overrightarrow{X(t_{n+1})} - \overrightarrow{X(t_n)}$$

Velocity components at time t_n with respect to that at time t_{n-1} is evaluated as:

$$Vt(t_n) = \left|\overrightarrow{V(t_n)}\right| \sin(\theta)$$

$$Vl(t_n) = \left|\overrightarrow{V(t_n)}\right| \cos(\theta)$$

Here, θ is the angle between $\overrightarrow{V(t_n)}$ and $\overrightarrow{V(t_{n-1})}$. Vt is the velocity component perpendicular to the direction of sliding and Vl is the component parallel to the direction. If the local points of the filament move along a straight line, Vt should constantly be zero. If the points move at a uniform velocity, Vl should be constant. In order to avoid misunderstanding the time range, we dare not to divide the value by 0.1 s giving the velocity in standard units, taking into account that the value is certain to represent the velocity of the focused point.

Table 1
Ratio of impurities of reagents

Reagents	Contaminations (%)		
	ATP	AMP-PNP	ADP
ATP	99.0	N.D.	1.0
AMP-PNP	1.0	98.0	1.0
ADP	14.0	N.D.	86.0

Analytical conditions: Shodex IEC DEAE-420N column, 20 mM Tris–$HClO_4$, $NaClO_4$ (pH 9.0) detected with absorbance at 260 nm.

3. Results

Fig. 1 indicates the ATP-dependent increase of sliding velocity of actin filaments as observed with a conventional in vitro motility assay. The sliding velocity of filaments without ATP analogues increased up to 5 μm/s with half maximal ATP concentration of approximately 200 μM. AMP at 2 mM did not affect the velocity of the filaments. However, 2 mM ADP strongly decreased the sliding velocity down as low as to 2.5 μm/s. Two millimoles of AMP-PNP exhibited a remarkable enhancement of the velocity up to approximately 7 μm/s. In order to estimate the contaminated nucleotide, those samples of AMP-PNP, ATP, ADP and AMP were analyzed with HPLC. As indicated in Table 1, contaminations of other phosphate-compounds were not significant enough to explain both the increase and the decrease of sliding velocities.

To confirm the change of velocities of filaments either in the presence of AMP-PNP or in the presence of ADP, these concentrations were varied in the presence of 2 mM ATP. Fig. 2 shows the results in addition to the control experiments in the case of AMP. Both the increase of the velocity in the presence of AMP-PNP and the decrease in the presence of ADP occurred in a cooperative manner.

We have, in this time, contrived the analytical method to quantitatively evaluate the mechanical fluctuations of a single sliding filament by measuring the local points with 8.0 nm accuracy as described in Section 2. Fig. 3 demonstrates the fluctuations of sliding velocities of three local points along an actin filament approximately 5 μm long. In this report, the fluctuation was expressed as the standard deviation (S.D.) of a distribution of instantaneous velocities sampled at 0.1-s interval. Transversal fluctuation was measured as low as 40 nm. In contrast, longitudinal fluctuation

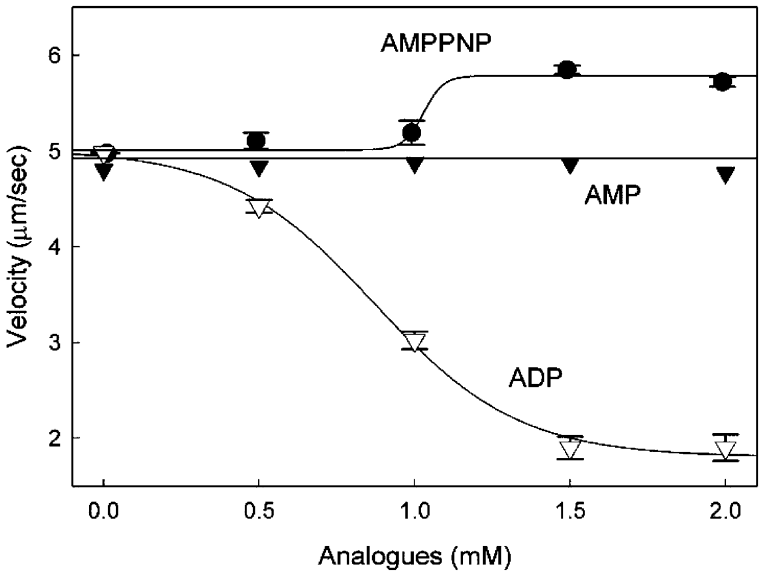

Fig. 2. Sliding velocities as a function of ATP analogues in the presence of 2 mM ATP. Sliding velocities of actin filaments were calculated by the same manner as in Fig. 1. Error bars indicate the experimental error for 15 independent experiments.

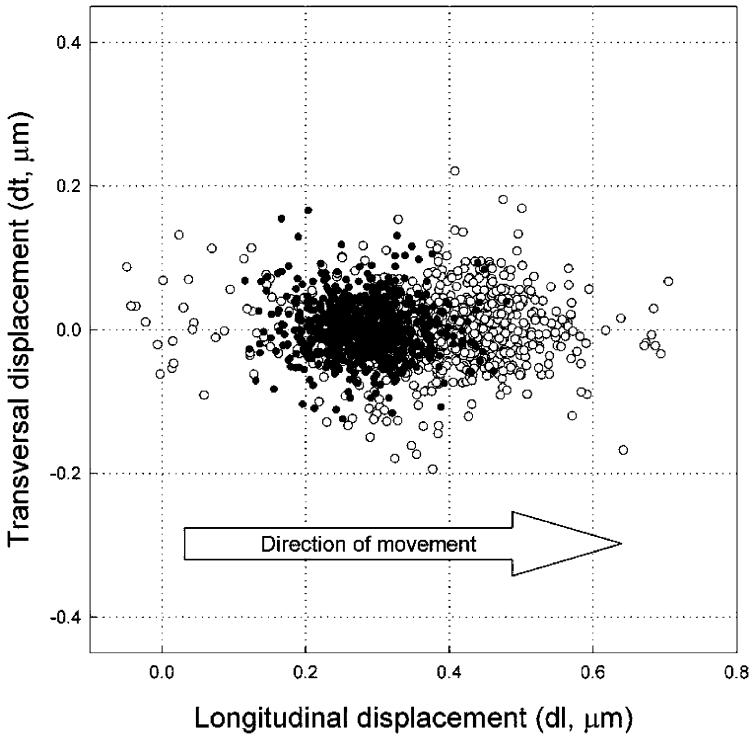

Fig. 3. Scatter diagram of velocity fluctuation of local points within sliding filaments. Fluctuations in the presence of 0.5 mM ATP were shown, calculated as in text. Filled and open symbols represent the fluctuation in the absence and presence of 2.0 mM AMP-PNP, respectively. Detail equations for calculation are shown in text.

increased up to 60 nm, where the average sliding velocity of the filament was approximately 4.0 μm/s.

Both the longitudinal and transversal fluctuations in the presence of ATP analogues were compared in the absence of ATP (Fig. 4). Considering the fact that the fluctuations must be affected by the concentration of HMM spread on the glass surface, we changed the HMM concentration from 25 to 50 μg/ml. At 25 μg/ml HMM, longitudinal fluctuations were larger than transversal ones in every case. However, no appreciable difference was detected between the ATP analogues we examined. However, the fluctuation decreased with the addition of AMP, AMP-PNP and ADP in this order at 50 μg/ml HMM.

This suppression of fluctuation occurred at 50 μg/ml HMM. The effect of ATP analogues, on the other hand, appeared in the distribution of the sliding velocities. In this context, we have examined the frequency distribution of velocities at 0.5 mM ATP both in the absence and in the presence of 2 mM AMP-PNP. Both distributions turn out uniform Gaussian as shown in Fig. 5.

4. Discussions

In Fig. 1 we have examined the effect of AMP. However, there has been no clear evidence that AMP could bind the myosin nucleotide binding site. AMP at 2 mM, whether they could bind to myosin or not, did not affect the velocity of the filaments. In contrast, a strong decrease in the sliding velocity down to as low as 2.5 μm/s under 2 mM ADP showed close conformity with the fact that ADP-bound myosin bind strongly to actin molecules and reduce their sliding velocity [20]. ADP was reported to increase shortening velocity at low calcium concentration [26] to activate the sliding velocity. Even though we could not detect

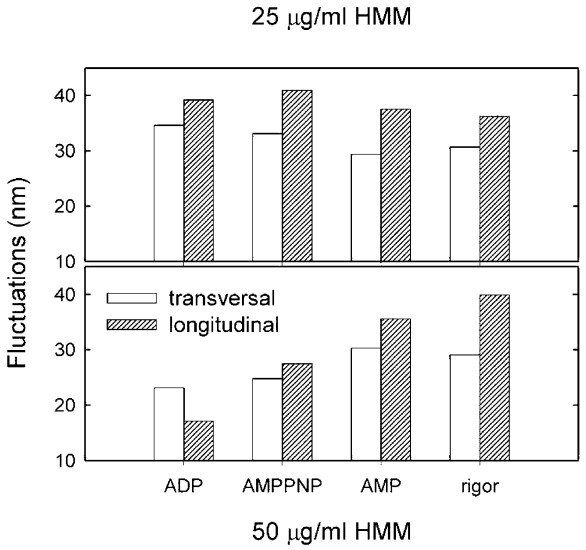

Fig. 4. Comparison of fluctuations in the absence of ATP. Both transversal and longitudinal fluctuations were shown in open and hatched boxes, respectively. In upper histograms, HMMs were spread for 1 min at 25 μg/ml, and at 50 μg/ml in lower ones. Taking into the accuracy of measurement, no significant differences were observed in the case of 25 μg/ml HMM. However, ADP remarkably lowered them even those in rigorous conditions.

such activation as far as examined AMP-PNP exhibited a remarkable enhancement of the velocity. The amount of enhancement was not supposed to be caused by ATP as contaminated within the AMP-PNP specimens used in an assay as shown in Table 1. Contaminations of other phosphate-compounds were not significant enough to explain both the increase and the decrease of sliding velocities.

At subsaturating ATP concentrations the velocity of sliding is probably a balance between the active head and actomyosin rigor bonds that cause a marked resistance to sliding. The reduction of rigor bond would be predicted to increase the sliding velocity of filaments. However, it is also predicted that AMP-PNP might reduce the forces from the active heads interacting to the same actin filament. We have examined the extent of rigor-making-heads within our experimental assays by using unlabelled actins following the method of Homsher et al. [20]. No appreciable differences were detected (data not shown). We would rather focus on the fact that AMP-PNP is a competitive inhibitor for myosin ATP catalytic site. The results in Fig. 1 indicate that the inhibitor, AMP-PNP, for

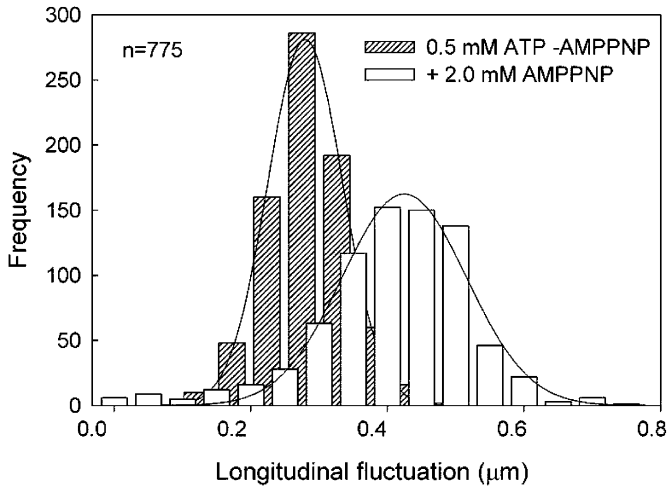

Fig. 5. Frequency distribution of the longitudinal fluctuations. Open boxes indicate the frequency of each class for every 50 nm in the presence of 2.0 mM ATP, 2.0 mM AMP-PNP, hatched boxes in the absence of AMP-PNP. Single Gaussian peak curves were drawn to fit with each distribution. The curve shows good agreement with the distributions even in the presence of both ATP and AMP-PNP.

myosin ATPase does increase the sliding activity of the actin filament.

To confirm the change of velocities of filaments either in the presence of AMP-PNP or in the presence of ADP, these concentrations were varied in the presence of 2 mM ATP as shown in Fig. 2. Both the increase of the velocity in the presence of AMP-PNP and the decrease in the presence of ADP occurred in a cooperative manner. The fact demonstrates that the interaction between ATP binding actomyosins and AMP-PNP (or ADP) binding, ones happens to be cooperative. We assume the cooperativity might be a close relation to the activation of sliding velocity both by 2 mM AMPPNP in this report and by ADP in the presence of very low ATP concentration in other reports [26]. The cooperativity emerged in sliding velocity should be supported by actins, myosin heads or both. The acceleration of the sliding velocity reported previously [11] where they demonstrate sensitization of ATP-bound myosin (fast-motor) by GTP-bound myosin (slow-motor) is of good concordance with our observation reported here.

We contrived the analytical method to quantitatively evaluate the mechanical fluctuations of a single sliding filament by measuring the local points with 8.0 nm accuracy as described in Section 2. We dare to mention about the crucial differences between the fluctuations reported here and the preceding ones subjected to single myosin molecular analysis. The fluctuations we have focused on here are not the fluctated motion of 'myosin' heads but several portions of a single 'actin' filament interacting with many myosin heads. In this sense, we did not take into account whether the portion is interacting with myosin heads or not. We are sure, however, that the fluctuation calculated here is caused from not only a single actomyosin interaction but also from the sum of the fluctuations of individual motors. Another distinct point is that the fluctuation is measured in a two-dimensional manner. As the fluctuated motions of actin filament must be anisotropic, we separated them into two components: displacements longitudinal to the moving direction; and those transversal to it.

Because the structural mobility of actin filament must be larger than that of the individual motor step size, actin filament can easily cancel the uniformity of interacting heads. Our main interest, therefore, is how the fluctuations from individual motors are transferred to smooth motion of the actin filament interacting with many independently acting heads. Comparison between transversal and longitudinal fluctuations (Fig. 5) clearly demonstrates the multitude of the direction of each local point moving along an actin filament.

Both the longitudinal and transversal fluctuations in the absence of ATP were compared in the presence of ATP analogues (Fig. 4). In spite of different concentrations of HMM, no appreciable difference was detected between the ATP analogues so far examined. However, the fluctuation decreased with the addition of AMP, AMP-PNP and ADP in this order at 50 μg/ml HMM. Observed differences might be caused from rigidity of nucleotide-binding head and/or neck region. The relation between bound nucleotide of myosins and the fluctuation of actin filament interacting to them will be discussed elsewhere.

The fact that the fluctuation of the velocities in the presence of ATP or AMP-PNP came up with a uniform and single Gaussian distribution in each case as shown in Fig. 5 strongly suggest that the observed fluctuation of sliding velocity would not be composed of the sum of the faster and the normal components, but of some coordinated integration of both. Our results indicate that both ATP-bound HMM and AMP-PNP bound HMM interacting with the same actin filament at the same time works cooperatively to enhance its sliding velocity even if their ATP hydrolysis is stochastic. In conclusion, independent energy supply from each HMM interacting with a single actin filament works cooperatively in a mechanical manner for realizing smooth movement of a single actin filament.

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