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ATP-dependent fluctuations of single actin filaments in vitro

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Abstract

Single filaments of actin were observed to fluctuate in the direction perpendicular to their longitudinal axes when they hydrolyzed ATP in the presence of myosin. The transversal fluctuations of actin filaments were identified by reading the transversal displacements of the filaments under a fluorescence microscope. The transversal fluctuations in the absence of ATP decreased their intensity as the number of myosin molecules contacting directly with actin filaments increased. In the presence of ATP, on the other hand, the amplitude of the transversal fluctuations increased in proportion to the ATP concentration up to a certain level, while the sliding velocity of the filaments did not increase significantly over the same range of ATP concentration. The present observation suggests that the chemical energy released from ATP along actin filament binding to myosin molecules is first and primarily converted into kinetic energy of fluctuations in the form of the displacement movements of the filaments in the direction perpendicular to their longitudinal axes.

Keywords: Actin; ATP; Fluctuation; Myosin; Sliding

1. Introduction

Sliding movement between actin and myosin filaments that occurs during muscle contraction is a process transducing the chemical binding energy initially stored in ATP. There has already been identified a rich catalogue of chemical reactions involved in the transduction process [1–9]. Nonetheless, we note that the physical characterization of the process has relatively been scarce. Direct observation of actin filaments modified with a fluorescent dye in motility assay systems [10–12] reveals a rather uniform sliding movement of the filaments, but the movement itself has been pretty flexible [13–15]. Sometimes, actin filaments exhibit rotational or reptational movement depending upon the environmental conditions [16–18]. The present contrast between longitudinal and non-longitudinal movements of actin filaments urges us to examine the contribution of non-longitudinal movements more thoroughly in order to clarify the physical processes involved in the energy transduction.

In this article, we measure and characterize the displacement of an actin filament in the direction perpendicular to its longitudinal axis when the filament is placed in an in vitro motility assay system in the presence or absence of ATP.

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2. Materials and methods

2.1. Purification of proteins

Actins and myosins were prepared from rabbit skeletal muscle. Actins were extracted from acetone powder and were purified according to the method of Spudich and Watt [19]. Myosins were obtained from a minced muscle with a Straub solution and were prepared by the method of Petty [20]. Further purification was carried out by an ion-exchanging chromatography (DEAE-cellulose (Pharmacia)) [21]. Actins were stored on ice and were used within a month after their preparation. Myosins were frozen in liquid nitrogen and then stored at −80°C.

2.2. Observation of actin filaments

Actin filaments were labelled with tetramethylrhodamine-phalloidins (Sigma) [14]. Their observation was performed under an optical microscope with a fluorescent illumination unit (Nikon, TMD-phot, ×100 oil object lens, ×10 relay lens). The labelled actin filaments were placed in 25 mM KCl, 20 mM HEPES (pH 7.8), 4 mM $MgCl_2$, and 1 mM DTT solution at room temperature (25°C). Myosins dissolved in a solution of high salt concentration (0.6 M KCl, 10 mM HEPES (pH 7.0), 1 mM DTT) were perfused between a cover slip (18 × 18 mm) and a cover glass (24 × 50 mm) coated with silicon (Sigma, Sigmacote) [22]. Roughly 60 s after the perfusion,

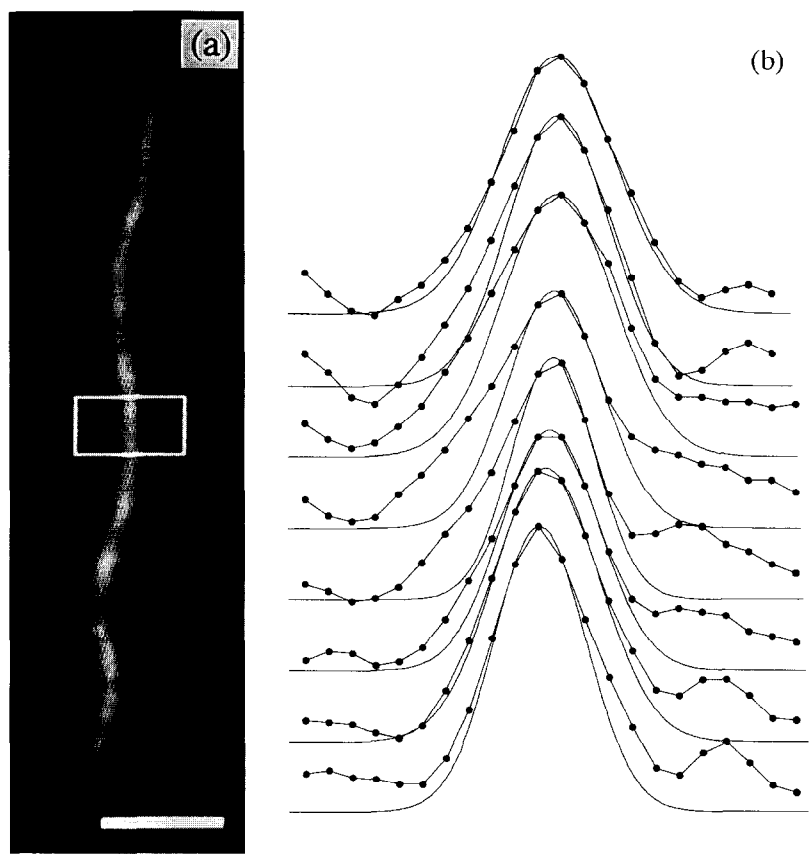

Fig. 1. A schematic drawing of an intensity profile and a video image of a single actin filament observed under a fluorescence microscope. The filament was placed on a plane parallel to the screen. Its image (a) was taken at every frame of an even TV field (bar denotes 5 μm). Intensity profile transversal to the longitudinal axis of the filament (i.e. along the scanning line of a TV screen) was supposed to represent a Gaussian distribution so that the center of the fluorescent intensity (b) could be estimated. The center was equated to a point on the skeleton line of the actin filament. The resolution was 8 nm.

myosin molecules that did not attach on the glass surface were removed with a solution of high salt concentration and then various solutions with 1 μg/ml of the labelled actin filaments were filled. In order to control the concentration of ATP at a constant level, 1 mM phosphocreatine and 0.1 mg/ml creatine phosphokinase were added. Photobleaching was prevented enzymatically with the use of an oxygen-scavenging system (0.1 mg/ml glucose oxidase, 3 mg/ml glucose, 0.018 mg/ml catalase) [12].

Movements of the actin filaments were in all cases planar [23].

2.3. Analysis for fluorescent images of an actin filament

The fluorescent images were recorded by using a video cassette recorder (Sony, S-VHS SLV-R5) with a high sensitive video camera (Hamamatsu, C2400-8). The selected area of video images was fed into a computer (Epson, PC-286UX) through digitization by using a video board (Cybertech Co., CT-9800B) at intervals of 1/30 s. Only one field of the TV-frame was used in order to avoid the time delay due to the interlaced raster scanning. Video images were continuously retrieved without compression. The noises residing in the obtained digital data were reduced by a simple smoothing filter with 3×3 pixels. In order to obtain a high resolution power under the given pixel size, we determined the position of each point on the center-skeleton line of an actin filament as referring to a Gaussian interpolation of every three adjacent data points by following the procedure reported in [24] (see Fig. 1). Reading of the directional angle of the longitudinal axis of the filament on the two-dimensional planar plane was accomplished within the accuracy of less than five degrees.

2.4. Standard deviation of displacements

In order to estimate the fluctuations of the displacement of an actin filament, we figured out the standard deviation of the displacements in the direction perpendicular to the longitudinal axis averaged over all the points measured along the filament. The number of measured points on the skeleton-line of the actin filament was 40, and each of them was spaced equally (93 nm).

3. Results

3.1. Fluctuations of an actin filament

We observed the transversal fluctuations of an actin filament contacting rigidly with myosin heads under rigor conditions. Fig. 2 shows the transversal displacements of one part of an actin filament in rigor conditions over 8 s, in which the myosin concentration was 100 μg/ml. The amplitude of the displacements did not exceed 50 nm in this case. The movement was random to the extent it was seen there. Fig. 3 shows the distribution of the displacements over 20 independent samples, each of which has 256 data points. The standard deviation was calculated to be 13.6 nm. This value was certainly greater than the resolution power of our measurement apparatus (8 nm).

3.2. Effect of the concentration of myosins on fluctuations

The effect of the concentration of myosins placed on a glass surface upon the fluctuations of an actin filament was first examined in the absence of ATP. The standard deviation of the transversal displacements was found to decrease with the increase of the concentration of myosins as shown in Fig. 4. When the concentration was decreased and reached 10 μg/ml in the absence of ATP, the standard devia-

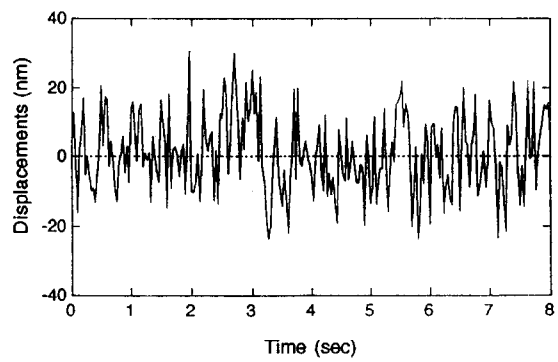

Fig. 2. Time development of the transversal displacement of an arbitrary point on the skeleton line along an actin filament in the presence of myosin molecules on a glass surface. An actin filament was fixed on myosin molecules in the absence of ATP. The position of a point on the skeleton line was determined at every 1/30 s. Transversal displacements of the point were measured as deviations from the average value evaluated over 8 s.

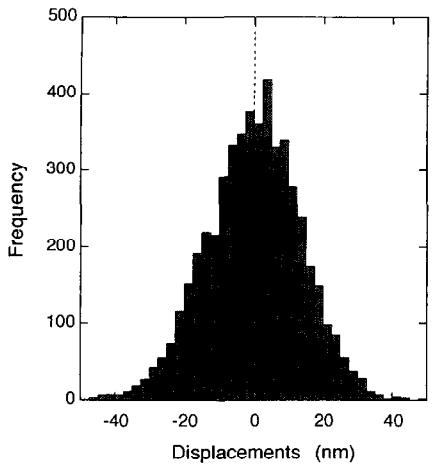

Fig. 3. Frequency-distribution of transversal displacements measured at every 1/30 s. The displacements were measured of arbitrary points on the skeleton line along an actin filament at every 1/30 s in the absence of ATP. The concentration of myosin molecules applied was 100 μg/ml. The standard deviation of the displacements was about 14 nm, and this value was estimated from 5000 samples.

tion of the displacements increased up to about 20 nm. Under such conditions, most parts of the filament were detached from the glass surface. On the other hand, when the concentration of myosins increased above 50 μg/ml the fluctuations of the filament were significantly tamed down. The standard deviation remained at a constant level of about 14 nm at a myosin concentration above 100 μg/ml.

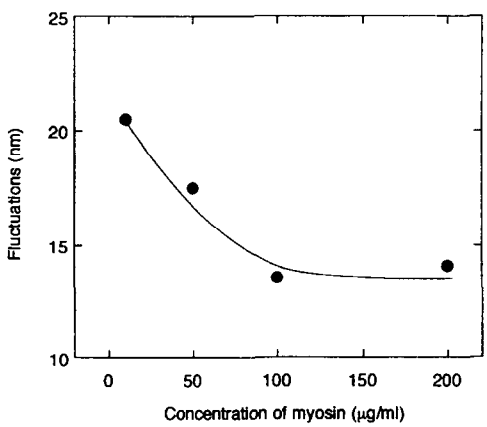

Fig. 4. Fluctuation intensity of transversal displacements measured in its standard deviation vs. the concentration of myosin molecules dispersed on the glass surface.

3.3. ATP-dependent fluctuations and the sliding velocity of an actin filament

Both the sliding velocity of an actin filament and its transversal fluctuations were measured when ATP was hydrolyzed in the presence of myosins. The sliding movement of the filaments was measured at a myosin concentration of 100 μg/ml. Actin filaments exhibited a smooth sliding movement under these conditions.

As the concentration of ATP increased, both the fluctuation intensity and the sliding velocity increased in an ATP-dependent manner (see Fig. 5). The dashed curve for the fluctuation intensity was a consequence of applying a curve-fitting of a Michaelis–Menten formula of hyperbolic function to the observed data points. The intensity of the transversal fluctuations rapidly grew with ATP concentration over the range of 1–10 μM, and reached a plateau of roughly 65 nm at 2 mM ATP. Although the actin filaments could not exhibit explicitly observable sliding movement at an ATP concentration below 2 μM, the sliding velocity slowly increased as ATP concentration increased above 2 μM [12]. The value of the ATP concentration that realized sliding velocity 0.7 μm/s, that equals one half of

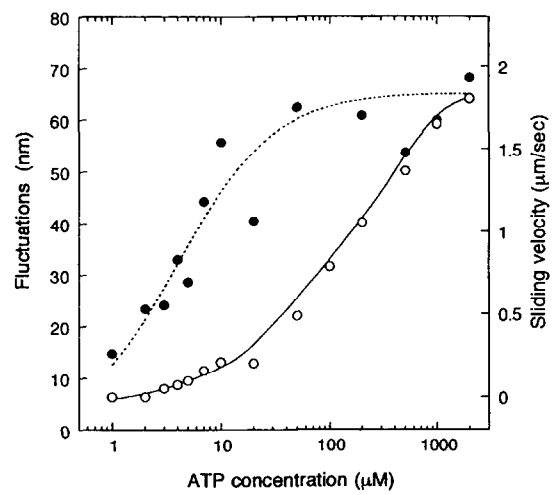

Fig. 5. ATP-dependent transversal fluctuations and the sliding velocity of an actin filament. The fluctuation intensity of transversal displacements measured in its standard deviation (filled circle) is on the left and the sliding velocity (open circle) is on the right. The data were collected from three independent sample filaments of the similar nature.

the maximally attainable velocity, was about 100 μM, whereas the ATP concentration realizing the transversal fluctuations, whose intensity is roughly half of the maximal attainable, was about 4 μM.

The fact that the transversal fluctuations of an actin filament can significantly be enhanced compared to its longitudinal sliding movement at low ATP concentrations suggests that the energy released from ATP is first converted into transversal movements of the filament. In fact, the relative number of those myosin molecules binding actively to actins could be much smaller than that of those binding rigidly at an ATP concentration of 1–10 μM. Sliding movement could become actually effective only when the ATP concentration increases up to such a level that the relative number of myosin molecules binding actively to actins becomes significant.

4. Discussion

We observed that the transversal fluctuations of an actin filament in the direction perpendicular to its longitudinal axis decrease as the number of myosin molecules contacting with the filament increases in the absence of ATP. In particular, the intensity of the fluctuations measured in units of the standard deviation of the displacement decreased roughly as being inversely proportional to the concentration of myosin molecules applied below 100 μg/ml (Fig. 4). The standard deviation of the displacement measured at the myosin concentration of 100 μg/ml was 13.6 nm, and this value was consistent with the one estimated from measurements of the flexibility of myosin heads under similar conditions [25,26].

Furthermore, the distribution of transversal fluctuations of an actin filament in the absence of ATP (Fig. 3) agreed quite well with a Gaussian distribution. The present Gaussian characteristic suggests that transversal fluctuations of an actin filament in the absence of ATP originate in thermal fluctuations from the ambient. In contrast, the distribution of transversal fluctuations in the presence of 2 mM ATP markedly differed from a Gaussian, indicating that transversal fluctuations could also be activated by sources other than random thermal fluctuations.

The intensity of the transversal fluctuations of an actin filament measured in units of the standard deviation of displacements increased with the ATP concentration and was saturated at the level of 65 nm at about 0.1 mM ATP. The value of the ATP concentration that realized half the maximal transversal fluctuations was read to be 4.1 μM according to the curve-fitting by a Michaelis–Menten formula. This characteristic coincides with the ATPase activity in that the value of ATP concentration realizing half the maximum rate of ATP hydrolysis in an actomyosin

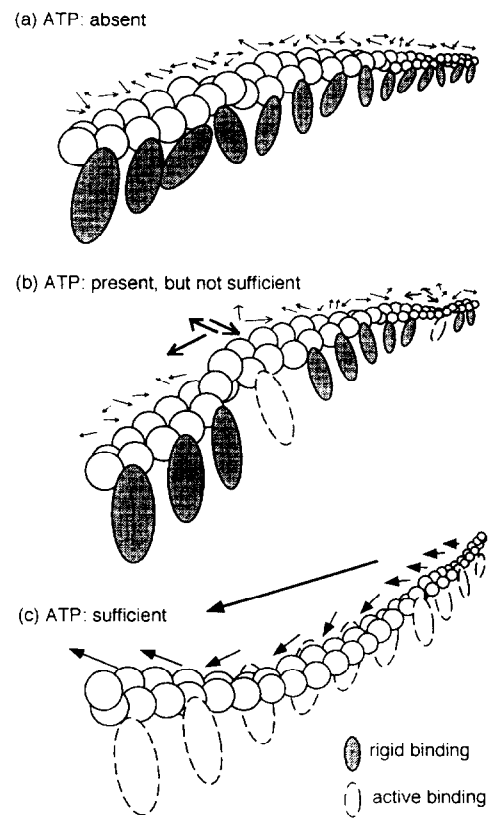

Fig. 6. A schematic representation of fluctuations along an actin filament binding to myosin molecules. (a) ATP is absent. Actin filaments binding rigidly to myosin molecules fluctuate as responding to thermal agitations coming from the ambient. (b) ATP is present, but not sufficient. A limited number of myosin molecules binds actively to actin filaments. Energy released from ATP could transform into that of fluctuations both transversally and longitudinally along the filaments. (c) ATP is sufficiently present. All myosin molecules binding to actin filaments are active. Fluctuations induced by ATP hydrolysis at each binding site may correlate longitudinally as being aided by, for instance, the polarity of actin filaments, and accordingly could yield a longitudinal sliding movement.

complex is roughly 4 μM [12]. The present coincidence points to the possibility of a direct connection between the ATPase activity of an actomyosin and the transversal fluctuations of an actin filament.

One of the physical mechanisms for making an actin filament move toward its transversal direction may be a rotational movement. In fact, an actin filament sliding on myosin molecules can rotate [16,18]. If the rotational movement of an actin filament is induced from its interaction with myosins, the resulting transversal movement of the filament could serve as a bridge connecting the ATPase activity and the sliding movement of the filament. The suggested energy transduction is two-staged. The chemical binding energy stored in an ATP could first be transferred into the kinetic energy inducing the transversal movement of an actin filament, followed by the mechanical energy transduction from transversal to longitudinal movements. The mechanical energy transduction may be due to a correlated motion of those constituent monomers in the filament.

Above all, transversal movement of an actin filament pumped up by ATP hydrolysis at the sites contacting with myosin molecules raises an intriguing question of mechanics asking how the contacting regions distributed along the filament could act upon and be acted upon [27,28] in a coordinated manner in the whole body. The transformation from ATP-activated transversal fluctuations of an actin filament to the longitudinal sliding movement may be one possibility for coordinating the whole actomyosin complexes mechanically.

In summary, we suggest in Fig. 6 a simple picture of the energy transduction underlying the ATPase activity in actomyosin complexes. When ATP is absent, actin filaments bound rigidly to myosin molecules fluctuate only as responding to thermal agitations coming from the ambient. No explicit correlation of fluctuations would be expected longitudinally along the filaments. In contrast, as the ATP concentration is slightly increased above zero, the number of those myosin molecules exhibiting active binding to actins would increase. However, the ATP-induced fluctuations could not establish their longitudinal correlation because of the interferences coming from those myosins binding to actins rigidly. As the ATP concentration further increases, all of the myosins contacting with actins would become active. Once the phase correlation of fluctuations is established longitudinally, a macroscopic sliding movement could be expected to set in.

References

[1] E. Eisenberg and C. Moos, Biochemistry, 7 (1968) 1486.
[2] R.W. Lymn and E.W. Taylor, Biochemistry, 10 (1971) 4617.
[3] L.A. Stein, R.P. Schwarz, Jr., P.B. Chock and E. Eisenberg, Biochemistry, 18 (1979) 3895–3909.
[4] J.A. Sleep and R.L. Hutton, Biochemistry, 19 (1980) 1276–1283.
[5] E. Eisenberg and L. Greene, Annu. Rev. Physiol., 42 (1980) 293.
[6] M.A. Geeves, R.S. Goody and H. Gutfreund, J. Muscle Res. Cell Motility, 5 (1984) 351.
[7] L.A. Stein, P.B. Chock and E. Eisenberg, Biochemistry, 23 (1984) 1555–1563.
[8] E. Eisenberg and T.L. Hill, Science, 227 (1985) 999.
[9] E.W. Taylor, J. Mol. Chem., 266 (1991) 294–302.
[10] S.J. Kron and J.A. Spudich, Proc. Natl. Acad. Sci. USA, 83 (1986) 6272–6276.
[11] H. Honda, H. Nagashima and S. Asakura, J. Mol. Biol., 191 (1986) 131–133.
[12] Y. Harada, A. Noguchi, A. Kishino and T. Yanagida, Nature, 236 (1987) 805–808.
[13] H. Nagasima and S. Asakura, J. Mol. Biol., 136 (1980) 169.
[14] T. Yanagida, M. Nakase, K. Nishiyama and F. Oosawa, Nature, 307 (1984) 58–60.
[15] S. Fujime, S. Ishiwata and T. Maeda, Biophys. Chem., 20 (1984) 1.
[16] T. Nishizaka, T. Yagi, Y. Tanaka and S. Ishiwata, Nature, 361 (1993) 269–271.
[17] Choi-man Ng and R.D. Ludescher, Biochemistry, 33 (1994) 9098–9104.
[18] J. Kas, H. Strey and E. Sackmann, Nature, 368 (1994) 226–229.
[19] J.A. Spudich and S. Watt, J. Biol. Chem., 246 (1971) 4866–4871.
[20] S.V. Perry, Methods Enzymol., 2 (1955) 582–588.
[21] E.G. Richards, C.S. Chang, D.B. Menzel and H.S. Olcott, Biochemistry, 6 (1967) 528–540.
[22] Y. Harada, K. Sakurada, T. Aoki, D.D. Thomas and T. Yanagida, J. Mol. Biol., 216 (1990) 49–68.
[23] Y. Tanaka, A. Ishijima and S. Ishiwata, Biochim. Biophys. Acta, 1159 (1992) 94–98.
[24] H. Honda, S. Nagao, K. Hatori and K. Matsuno, Biophys. Chem., 54 (1995) 61–66.
[25] K.J. Kinosita, S. Ishiwata, H. Yoshimura, H. Asai and A. Ikegami, Biochemistry, 23 (1984) 5963–5975.
[26] Y.Y. Toyoshima, C. Toyoshima and J.A. Spudich, Nature, 341 (1989) 154–156.
[27] K. Matsuno, BioSystems, 22 (1989) 117–126.
[28] K. Matsuno, Protobiology: Physical Basis of Biology, 1989, CRC Press, Boca Raton, Florida.