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Onset of the sliding movement of an actin filament on myosin molecules: from isotropic to anisotropic fluctuations

Kuniyuki Hatori[a], Hajime Honda[a], Katsuhiko Shimada[b], Koichiro Matsuno[a,*]

[a] *Department of BioEngineering, Nagaoka University of Technology, Nagaoka 940-2188, Japan*
[b] *School of Design and Architecture, Nagoya City University, Nagoya 464-0083, Japan*

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Abstract

An actin filament contacting myosin molecules increased the fluctuation intensity of the filamental displacement as the ATP concentration increased. In particular, fluctuations in the filamental displacement in the planar plane in which the sliding movement takes place were isotropic at a low ATP concentration, and became anisotropic as the concentration increased. The build-up of the sliding movement of an actin filament was associated with the transformation from isotropic to anisotropic fluctuations of the filamental displacement.

Keywords: Actin filament; Cell motility; Fluctuations; Myosin; Sliding movement

1. Introduction

Despite the fact that there has been an accumulating amount of evidence on the pivotal role played by myosin molecules as the motor protein for cell motility and muscle contraction [1–8], the microscopic dynamic behaviors of the single molecules have to be supplemented by their collective coordination for actualizing the mesoscopic sliding movement of an actin filament upon myosin molecules [9–12]. If force generation at each actomyosin complex upon an actin filament is completely independent of the similar force generated in its neighborhood, the likelihood of having a uniform sliding movement of the filament would be greatly disturbed. In order to see how such a coordination for the uniform move-

* Corresponding author.

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ment could be established, it would first be required to see how each particular point on the filament moves in relation to the other points on the same filament. For this purpose, we fabricated an actin filament having a fluorescent marker attached on a fixed location on the filament [11], and measured their displacements on the planar plane in which the sliding movement of the filament takes place. We then focused on the extent to which fluctuations associated with the filamental displacement could be isotropic or non-isotropic, and examined how the sliding movement would develop with the increase of the ATP concentration.

2. Material and methods [11]

2.1. Proteins and reagents

Actin and myosin molecules were prepared from rabbit skeletal muscle. Actin monomers were extracted from acetone powder in 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 [13]. Myosin molecules were obtained from a minced muscle with a Guba–Straub solution and were purified by the method of Perry [14]. Heavy meromyosin (HMM) was prepared by alpha-chymotriptic digestion of myosin [15]. In order to visualize the movement of an actin filament, we attached a fluorescent bead to the filament. The fluorescent bead (FluoSpheres, NeuraLite avidin-labeled microspheres with diameter 0.04 μm, yellow–green) was purchased from the Molecular Probes Inc. Biotin-NHS (biotin long arm, NHS-water soluble) was from Vector Laboratories Inc.

Actin monomers in an actin filament were labeled by biotin to bind avidin-conjugated fluorescent beads to the filament. Actin filaments, or F-actin, of 5 mg/ml were suspended in F-solution [100 mM KCl, 50 mM sodium-bicarbonate buffer (pH 9.2), 1 mM ATP, 2 mM $MgCl_2$]. Biotin-NHS was added to the specimen at a final concentration of 0.5 mg/ml. The solution was incubated for 90 min at 25°C. The reaction was terminated by adding one-tenth of the volume of 1 M imidazole-HCl (pH 6.6). The solution was dialyzed against a G-buffer for 2 days at 4°C and then followed by gel chromatography.

2.2. Spot label of an actin filament with a fluorescent bead

Biotinylated actin monomers and unlabeled actin monomers were mixed at a molar ratio of 1:19 in a standard solution [25 mM KCl, 25 mM imidazole–HCl (pH 7.4), 4 mM $MgCl_2$, 0.5 mM DTT]. The co-polymerized actin filaments (33 μg/ml) were labeled with tetramethylrhodamine isothiocyanate (TRITC) phalloidin (Sigma Chemical Co.) at a molar ratio of 1:1 by incubating for 1 day at 4°C in the standard solution. Then, the solution was diluted 50-fold with the standard solution containing 1 mg/ml of bovine serum albumin (BSA). Avidin-conjugated fluorescent beads were added to 500 μl of the labeled actin solution [25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 0.5 mM DTT, 1 μg/ml TRITC-phalloidin labeled actin], and the weight ratio of the beads to the solution was set at 0.01%. The mixture was incubated for 1 day at 4°C and diluted twofold with the standard solution just before its usage for further experimentation.

2.3. Observation of a fluorescent bead-labeled actin filament

The observation was made on a fluorescent microscope [16]. The coverslip (Matsunami Glass Co. Inc., cover glass No. 1) were treated with butyltrimethoxysilane (Shinetsu Chem, Co.). The specimen was put between two coverslips with separation of 0.1 mm, in which a smaller coverslip of 18×18 mm was placed upon a larger one of 24×50 mm. The solution of 50 μl of heavy meromyosin (0.2 mg/ml) was perfused between the two coverslips. Sixty seconds after the perfusion, those heavy meromyosin molecules remaining on the glass surface as being unbounded were removed by further perfusing 100 μl of the standard solution containing 1 mg/ml of BSA. Another 60 s after those bead-labeled actin filaments (0.5 μg/ml) were applied to the 0.1-mm gap

between the two coverslips. After that, the solution was replaced by an assay solution [25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 1 μM ATP, 0.5% 2-mercaptoethanol, 0.1 mg/ml glucose oxidase, 3 mg/ml glucose, 0.018 mg/ml catalase, 1 mM phosphocreatine, 0.1 mg/ml creatine phosphokinase]. The fluorescent images were examined by an optical microscope (Olympus, IX70) carrying a fluorescent illuminating unit (Olympus, IX-FLA), an objective lens (Olympus, UPlan Apo 100×, oil immersion) and a 100-W mercury lamp. Actin filaments were identified by a green excitation, whereas beads by a blue excitation. The experiment was done at room temperature (25°C).

Fluorescent images were recorded by employing a video cassette recorder (Sony, Hi-8 EVO-9650) with a high sensitive video camera (Video Scope International, Ltd., ICCD-350F). The video images were fed into a computer (Apple Computer Inc., Power MaCintosh 7600/132) with a video grabber board (Scion Co., LG-3 PCI) at every interval of 1/30 s. The two-dimensional spatial position of a fluorescent bead, both along the longitudinal axis of the filament, and along the transversal one, was identified by referring to the position at which the measured light intensity would become a local maximum, while assuming the distribution of detected light intensities followed a Gaussian distribution; spatially in both directions. The precision of measuring the spatial position was 8 nm [9].

3. Results and discussion

We measured both the radial and the angular component of the planar displacement that a fluorescent marker attached on an actin filament exhibited over a fixed time interval. The angular component of each planar displacement was measured at every fixed time interval relative to the direction of the movement of the marker registered at the preceding measurement.

Fig. 1 demonstrates the moving velocities of the marker measured over the intervals of 1/30, 2/30 and 30/30 s, respectively, for different ATP concentrations. Each moving velocity was determined by referring to both the initial and the final locations of the marker in the fixed time interval for the measurement. Each value of the moving velocity against the ATP concentration was obtained by averaging over 300 similar samples. For such a low ATP concentration as below 5 μM, the moving velocity of the marker became greater as the time interval for measuring the velocity was shortened. Further details of the planar displacement of the marker were seen in the distribution of the angular component of each planar displacement exhibited.

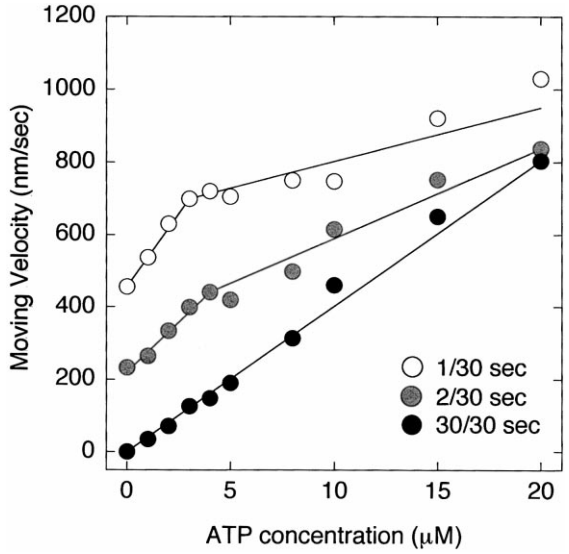

Fig. 1. Relationship between the moving velocity of the fluorescent marker attached on an actin filament contacting myosin molecules and the ATP concentration applied. The moving velocity of the marker in the planar plane was determined by referring to both the initial and the final locations of the marker in the fixed time interval of 1/30, 2/30 or 30/30 s for the measurement.

Fig. 2 shows the distribution of the angular components of planar displacement measured relative to the direction of the movement of the marker exhibited during the preceding interval of 1/30 s, along with their mean magnitude being proportional to its radius. Each displacement was measured at every 1/30 s during a sequence of 10 min, and was parameterized in terms of the applied ATP concentration: 0 μM for (a), 5 μM for (b) and 15 μM for (c). Fig. 3 is a summary diagram demonstrating only the relative fre-

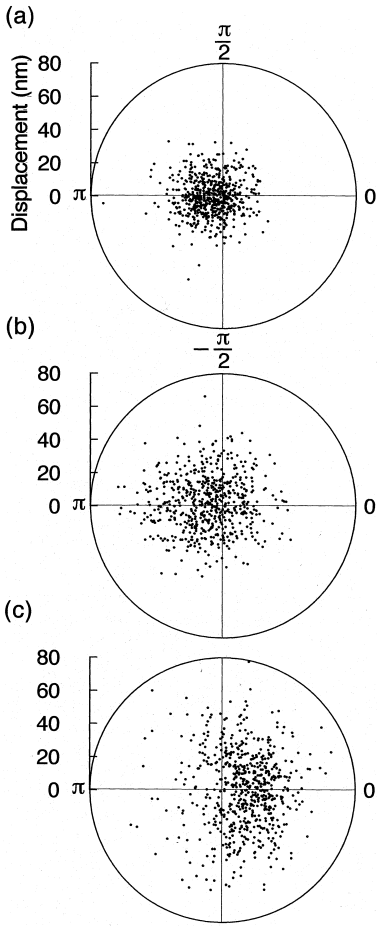

Fig. 2. Angular and radial components of the planar displacement of the fluorescent marker attached on an actin filament parameterized in terms of the ATP concentration: 0 μM for (a); 5 μM for (b); and 15 μM for (c). The angular component of the planar displacement was measured relative to the direction of the movement of the marker exhibited during the preceding interval of 1/30 s, along with their mean magnitude being proportional to its radius. Both the angular and radial components were measured every 1/30 s.

quency of the angular component of planar displacement exhibited over every 1/30 s with the relative frequency (%) being proportional to its radius, also parameterized in terms of the ATP concentration.

Our present measurement revealed two major observations: (1) is that the planar displacement of an actin filament was apparently isotropic for the angular component at a sufficiently low ATP concentration below 5 μM, while the radial component was significant even at that low ATP concentration; and (2) the angular component gradually became anisotropic with the increase of the ATP concentration, while the radial component also increased its magnitude.

The isotropic nature of the planar displacement is in fact implicated in our previous observation of the transversal fluctuations of an actin filament at a low ATP concentration [9]. Although such an isotropic angular displacement can propagate along an actin filament, the propagating velocity of the transversal fluctuations decreases with the increase of the ATP concentration and they become insignificant above the concentration 5 μM [10]. The extent to which the actin filament exhibits its staggered movement is considerably mitigated as the ATP concentration increases [11]. At a sufficiently high ATP concentration (2 mM), the dominant propagating fluctuations turn out to be longitudinal [12]. These longitudinal fluctuations can now come to furnish the actin filament with both contractile and protractile distortions [17].

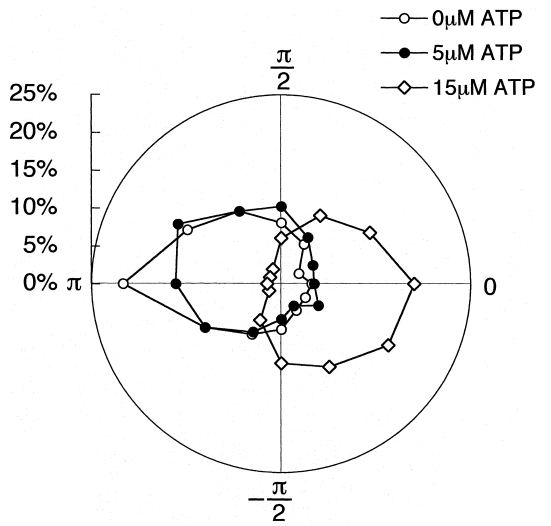

Fig. 3. Relative frequency of the angular component of the planar displacement of the marker measured relative to the direction of the movement exhibited during the preceding interval of 1/30 s with the relative frequency (%) being proportional to its radius, parameterized in terms of the ATP concentration. The angular component was measured every 1/30 s.

Combined with previous observations, we note that the planar displacement of an actin filament sliding on myosin molecules in the presence of ATP exhibits totally different characteristics between the angular and the radial component. Longitudinal coordination of an actin filament sliding on myosin molecules especially with regard to force generation is achieved mainly by mitigating and coordinating the angular component of the planar displacement. Needless to say, this coordination is also accomplished by means of the propagating fluctuations. Fluctuations associated with actomyosin complexes in the presence of ATP are both the cause and effect of coordinating the force generations along the actin filament.

4. Concluding remarks

Fluctuations serving as both the cause and effect of maintaining coordination are ubiquitous. A constant over- and undershoot of these fluctuations survive because of the absence of any means of global coordination on the spot. Fluctuations as a means of establishing a mesoscopic, or even macroscopic, coordination are not globally coordinated by their genesis in advance, while their effect is to maintain global coordination [18]. In other words, fluctuations as a means of global coordination have constantly to be generated to eliminate any mismatching between the preceding fluctuations for coordination and the effected coordination, while this process does not reach a standstill in the course of development.

Since we observed that the sliding movement of an actin filament on myosin molecules in the presence of ATP was a phenomenon upheld and stabilized by intervening fluctuations, the filamental displacement serves as an indicator of the participating fluctuations. The energy source driving the fluctuations is sought in the chemical binding energy stored in ATP. In fact, energy-deficit fluctuations, once they occurr, come to feed on ATP if available, while energy-surplus fluctuations cannot convert the dissociated ADP and inorganic phosphate back into ATP because of the unidirectional nature of energy flow from ATP. This unidirectionality of the fluctuations furnishes the actomyosin complexes with unique characteristics, which cannot be expected in fluctuations occurring in the vicinity of thermodynamic equilibrium. A most conspicuous characteristic must be an onset of the sliding movement when energy-surplus fluctuations would become significant.

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