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Biophysical Chemistry 70 (1998) 241–245

Biophysical Chemistry

Staggered movement of an actin filament sliding on myosin molecules in the presence of ATP

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Received 14 July 1997; accepted 25 October 1997

Abstract

An actin filament sliding on myosin molecules in the presence of an extremely low concentration of ATP exhibited a staggered movement. Longitudinally sliding movement of the filament was frequently interrupted by its non-sliding, fluctuating movements both in the longitudinal and transversal directions. Intermittent sliding movements of an actin filament indicate establishment of a coordination of ATP-mediated active sites distributed along the filament. © 1998 Elsevier Science B.V.

Keywords: Staggered movement; Actin filament; Myosin molecules

1. Introduction

Physico-chemical elucidation of the interaction between actin and myosin molecules, that underlies muscle contraction, raises a fundamental issue on how the relationship between each microscopic chemical reaction of ATP hydrolysis and an apparently supra-molecular movement of an actin filament sliding on myosin molecules could be established [1–10]. Although each actomyosin complex constitutes a basic functional unit for a muscle contraction, there certainly exists a scheme of functional integration over those units [11–15]. Otherwise, there would be no supra-molecular movement to be coordinated internally.

One particular instance indicating an occurrence of such a functional integration over actomyosin complexes along an actin filament is an observation of ATP-activated transversal fluctuations of the filament propagating along it [16]. The propagating transversal fluctuations come to suggest a likelihood of coordinating ATP hydrolysis over active sites along an actin filament in a communicative manner [16]. Likewise, fluctuations of an actin filament along its longitudinal axis may serve as a marker indicating another type of functional integration.

In this article, we report on the measurement of both transversal and longitudinal fluctuations of an actin filament contacting many myosin molecules in the presence of an extremely low concentration of ATP. Our measurement revealed that a building-up of longitudinal fluctuations is associated with the

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PII S0301-4622(97)00129-4

onset of intermittent sliding movements of an actin filament.

2. Materials and methods

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 [17]. Myosin molecules were obtained from a minced muscle with a Guba–Straub solution and were purified by the method of Perry [18]. Heavy meromyosin (HMM) was prepared by alpha-chymotriptic digestion of myosin [19]. 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. Biotin–NHS (biotin long arm, NHS-water soluble) was from the Vector Laboratories.

2.2. Biotinylated actin

In order to bind avidin-conjugated fluorescent beads to an actin filament, actin monomers in the filament were labeled by biotin. Actin filaments, or F-actin, of 5 mg/ml was suspended in an 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 1/10 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.

2.3. 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 copolymerized actin filaments (33 μg/ml) were labeled with tetramethylrhodamine isothiocyanate (TRITC) phalloidin (Sigma Chemical) at a molar ratio of 1:1 by incubating for 1 day at 4°C in the standard solution. Then, the solution was diluted to 50 folds 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 to be 0.01%. The mixture was incubated for 1 day at 4°C and diluted to two folds with the standard solution just before its usage for further experimentation.

2.4. Observation of a fluorescent bead-labeled actin filament

The observation was made on a fluorescent microscope. The coverslip (Matsunami Glass, cover glass No. 1) were treated with butyltrimethoxysilane (Shinetsu Chem.). The specimen was put between two coverslips with separation 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 (Nikon, DIAPHOT-TMD) carrying a fluorescent illuminating unit (Nikon, TMD-EF2), an objective lens (Nikon, Plan 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).

2.5. Analysis of fluorescent images of a bead-marked actin filament

Fluorescent images were recorded by employing a video cassette recorder (Sony, Hi-8 EVO-9650) with a high sensitive video camera (Hamamatsu Photonics, C2400-08). The video images were fed into a computer (Apple Computer, Power MaCintosh 7600/132) with a video grabber board (Scion, LG-3 PCI) at every interval of 1/30 s. 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 locally maximum while assuming the distribution of detected light intensities to follow a Gaussian, spatially in both the directions. The precision of measuring the spatial position was 8 nm [20].

3. Results and discussion

Fig. 1 demonstrates a typical movement of a fluorescent bead attached to an actin filament contacting heavy meromyosin molecules in the presence of 1 μM ATP. Although the average displacement

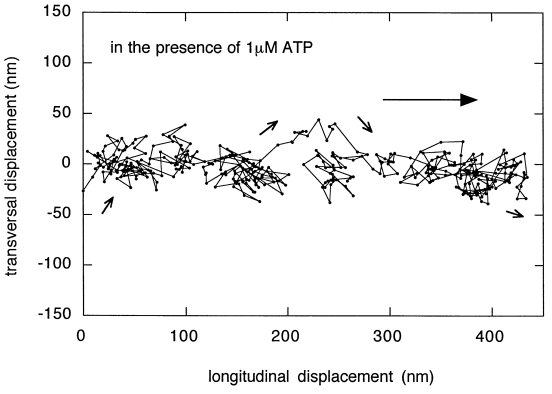

Fig. 1. The development of both longitudinal and transversal displacements of a fluorescent bead attached to an actin filament contacting heavy meromyosin molecules at the ATP concentration of 1 μM. The arrow indicates the direction along which the longitudinal displacements would move. This figure demonstrates the displacements observed over 13 s.

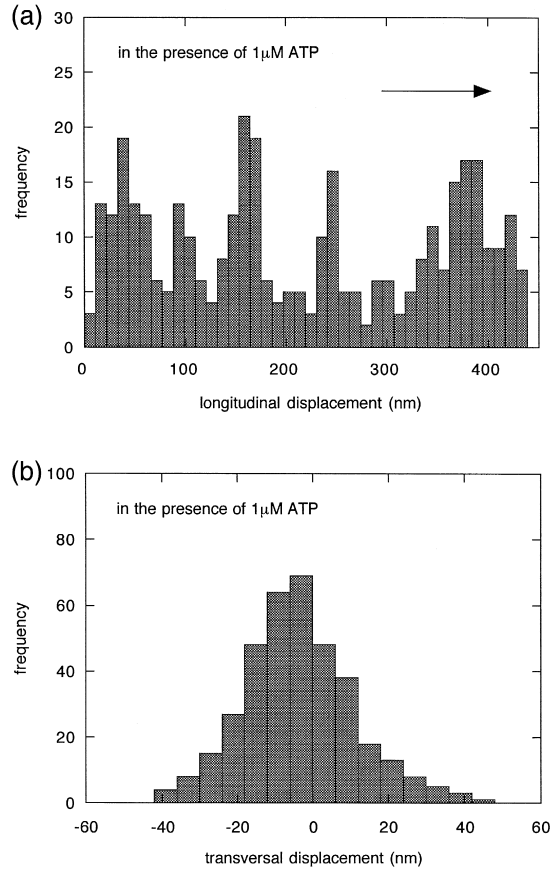

Fig. 2. The frequency distribution of the displacements in the longitudinal direction (a) and in the transversal direction (b). The frequency of finding the florescent marker in a designated window region at every interval of 1/30 s is counted and displayed in the figure. The total time interval set for this observation was 13 s.

velocity along the longitudinal axis of the filament was roughly 35 nm/s, the displacement movement of the filament proceeded in a staggered manner such that the unidirectional movement was frequently interrupted by its non-unidirectional fluctuating movements both in the longitudinal and the transversal directions.

The extent of the staggering was estimated by examining the frequency distribution of each displacement observed in the stationary coordinate space. Fig. 2 displays the frequency distributions of each displacement of the fluorescent bead measured at every interval of 1/30 s for the longitudinal and for the transversal movement. The frequency distri-

bution of the longitudinal displacements exhibited a gradual unidirectional movement while indicating the presence of local maxima of the distribution to be a manifestation of non-unidirectional staggered movements. On the other hand, the transversal displacements observed a Gaussian distribution.

We have examined the motile activity of an actin filament contacting myosin molecules at an extremely low concentration of ATP in order to distinguish between the longitudinally unidirectional movement of the filament and its fluctuations exhibited along the same longitudinal axis and then to relate the two. If the ATP concentration is greater than, say, 10 μM, on the other hand, the unidirectional sliding movement of the filament would far exceed the fluctuating displacements along the direction as examined in our previous study [16]. A rationale for choosing such a low concentration of ATP of order of 1 μM in the present study has been to focus on elucidating the relationship between the unidirectional movement and the fluctuating displacements along the same longitudinal axis.

A distinct characteristic of the filamental movement displayed in Fig. 1 is that non-unidirectional displacements of a bead of 0.04 μm in its diameter attached to an actin filament were followed by intermittent unidirectional displacements along its longitudinal axis. The non-unidirectional displacements were quite similar to those observed in the absence of ATP [20]. This observation is in accord with the presence of ATP in its extremely low concentration. Moreover, the occurrence of those non-unidirectional displacements invites us to ask how ATP would come to be involved in actomyosin complexes distributed along the filament. This will focus what would move along the longitudinal axis of an actin filament.

The average velocity of the unidirectional movement of an actin filament at ATP concentration of 1 μM was found to be roughly 35 nm/s in our present study, while the supposed communication velocity of signaling the onset of ATP hydrolysis along an actin filament at the same ATP concentration was observed to be about 0.6 μm/s [16]. This difference between the sliding velocity of an actin filament and the estimated communication velocity of ATP hydrolysis may suggest a clue to the occurrence of the staggering movement of the filament.

That an actin filament exhibits a uniform unidirectional movement even intermittently while contacting many myosin molecules implies that many active sites for ATP hydrolysis along the filament would be internally coordinated so as to materialize such a supra-molecular movement. In view of the fact that each step size measuring the distance over which an actin filament could slide during one ATP cycle is in the range from 5 to 20 nm depending upon the methods of measurement [21–24], there could be expected many sites that were concurrently ATP-active along the filament. The length of the actin filament we studied was 2.6 μm.

These observations, combined together, may suggest that the staggered movement of an actin filament would correspond to the stage in which a possible coordination of ATP-mediated activation over a supra-molecular length along the filament would not fully be developed yet, while the stage corresponding to occurrence of an intermittent sliding movement in a unidirectional manner would establish such a coordination. This viewpoint would naturally lead to another interpretation such that, as the fractional time over which the coordination of ATP hydrolysis along the actin filament could be established increases, the remaining fractional time allotted to the locally staggered movement would decrease.

4. Concluding remarks

Longitudinal fluctuations of an actin filament contacting many myosin molecules in the presence of ATP and the unidirectional sliding movement of the filament are dynamically inseparable [25]. Nonetheless, the temporal separation between the locally staggered and the relatively uniform movement of the filament in the presence of an extremely low concentration of ATP may indicate the significance of how the signal for the onset of ATP-mediated activation of actomyosin complexes would be communicated along the actin filament. Occurrence of the staggered movement of an actin filament may manifest an importance of communicating signals for establishing a coordinated supra-molecular movement.

Acknowledgements

We appreciate the helpful discussion with Koshin Mihashi of Nagoya University.

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