Longitudinal Distortions and Transversal Fluctuations of an Actin Filament Sliding on Myosin Molecules

H. HONDA^{1,*}, K. KIKUCHI¹, K. HATORI¹, E. IMAI¹, K. SHIMADA² and K. MATSUNO¹

¹*Department of BioEngineering, Nagaoka University of Technology, Nagaoka, 940-2188 Japan*

²*School of Design and Architecture, Nagoya City University, Nagoya 464, Japan*

(*Author for correspondence, e-mail: hhonda@nagaokaut.ac.jp)

Abstract. An actin filament sliding on myosin molecules demonstrates both longitudinal distortions and transversal fluctuations with the linear dimension far exceeding the diameter of an actin monomer. Local swaying of a single actin filament was identified by reading speckled fluorescent markers attached on the filament. The accuracy of reading each speckled marker was about 10.4 nm (r.m.s.). Longitudinal distortions of an actin filament at a low ATP concentration of 20 μ M were as much as 0.5 μ m for the average filament length of 5.4 μ m. The magnitude of transversal fluctuations was as much as 60 nm, that was independent of the filament length. Both longitudinal distortions and transversal fluctuations are suggested to play a pivotal role for facilitating a smooth sliding movement of an actin filament.

Key words: actin filament, ATP, distortion, fluctuation, myosin, sliding

1. Introduction

An actin filament sliding on a large number of myosin molecules in the presence of ATP to be hydrolyzed is upon two different processes at the least. One is for how a single myosin motor could transform chemical energy stored in an ATP into mechanical one for the sliding movement [1–7]. One more process is for how each myosin motor could generate mechanical forces in a coordinated manner along the filament [8–14]. If adjacent actomyosin complexes along the filament are not coordinated with their timing of ATP hydrolysis and the resulting force generation, there must be a considerable amount of mechanical strains or distortions along the filament [15–17]. The absence of a coordination of molecular motors along the filament could significantly raise both longitudinal distortions and transversal fluctuations, which may turn out to be mechanical loads to the neighboring motors.

In this report, we quantitatively evaluate the longitudinal distortions and transversal fluctuations by reading several fluorescent markers attached on an actin filament sliding on myosin molecules. We then observed that the magnitude of

longitudinal distortions reached up to 10% of the entire filament length while that of transversal fluctuations remained almost constant over the entire length. This may strongly suggest an occurrence of a mechanical coordination upon myosin motors along the sliding filament.

2. Materials and Methods

2.1. PROTEINS AND REAGENTS

ATP and imidazole were purchased from Boehringer Mannheim and Sigma. Rhodamine conjugated phalloidin was purchased from Molecular Probe Inc. Other reagents were from Wako Pure Chemicals (Osaka), of special reagent grade. Rabbit skeletal muscle proteins were used. Actins were purified according to Spudich and Watt [18], and myosins were prepared by the method of Perry [19]. HMM (heavy-meromyosin) was prepared as in Okamoto and Sekine [20]. Purified HMM was dissolved at a concentration of 20 mg/ml in 0.6 M KCl, 10 mM DTT, 10 mM potassium phosphate buffer (pH 7.0), and stored in liquid nitrogen, then thawed and dialyzed before use. Solvent conditions we adopted throughout the experiments were as follows: Unless especially mentioned; 25 mM imidazole-HCl buffer (pH 7.4), 25 mM KCl, 5 mM MgCl₂, 20 μ M ATP, 1 mM CaCl₂, 1 mM DTT.

2.2. MICROSCOPIC OBSERVATION

We then prepared a standardized in vitro motility assay at temperature 25 °C [21]. The slide glass to fix myosin molecules of 50 μ g/ml HMM was hydrophobically treated with nitrocellulose. The solution condition for observing the sliding movement of a speckled actin filament was the one described in the previous section. The inverted microscope (Olympus, IX70) attached with objective lens (Uplan Apo 60x, oil immersion) was used with the aid of the accompanying fluorescent equipment and rhodamine filter.

The observed images of an actin filament were taken and stored in a video recorder equipped with ICCD-camera (Video Scope, ICCD-350F). Each image was taken at every 1/30 s through a video grabber board (Scion, LG-3 PCI) into a computer with use of software NIH-Image 1.6 (Wayne Rasband, NIH). The spacing of neighboring pixels was 61 nm [22].

2.3. ACCURATE POSITIONING

Nine neighboring pixels adjacent to the brightest one were supposed to follow a Gaussian distribution. Our reading of each speckled point was associated with the center of the distribution. The accuracy of this estimation was about 10.4 nm (r.m.s.) on the planar plane of a glass surface fixing the speckled actin filament.

Figure 1. Estimation of the accuracy of measurement The position of a fluorescent mark fixed on the glass slide was traced for 3.3 seconds. Frequency histogram of measured positions was fitted to a Gaussian distribution. The accuracy was about 10.4 nm.

Figure 2. Changes in the skeleton length L (left) and winding index W (right) were displayed. Movement of the filament was observed in the presence of $20 \mu\text{M}$ ATP under the conditions as described in the text.

2.4. FILAMENT WINDING, LONGITUDINAL DISTORTIONS AND TRANSVERSAL FLUCTUATIONS

For a quantitative evaluation for the shape of a speckled actin filament sliding on myosin molecules, we referred to the skeleton length (L) and the winding index (W) [23]. L is the total sum of the lengths of linear segments between two adjacent fluorescent markers. W is equal to 1 when the speckled markers along the filament are arranged in a straight line, and it decreases as the filament is wound.

The deformation of the filament is classified into two part. That is to say, local swaying of each speckled point during the sliding movement was separated into longitudinal distortions and transversal fluctuations. Transversal fluctuations were read from the variations in the moving velocity of the marker over 0.1 sec (3 frames) in the direction perpendicular to the sliding movement. Longitudinal distortions were read from the similar variations in the direction in parallel to the sliding movement.

Figure 3. Longitudinal distortions and transversal fluctuations. Changes in the average displacement along the longitudinal direction during 0.1 sec (a) were estimated from 100 frames (3.3 sec). Horizontal thin line indicates the average and the dashed lines indicate the standard deviation (130 nm) of all points. In (b), changes in the average displacement along the perpendicular direction were estimated from 100 frames (3.3 sec). Horizontal thin line indicates the average and the dashed lines indicate the standard deviation (60 nm) of all points.

3. Results

3.1. FILAMENT LENGTH AT LOW ATP CONCENTRATION

In the presence of 20 μM ATP, the skeleton length L and the winding index W were evaluated for an actin filament sliding at an average velocity of 0.5 $\mu\text{m}/\text{sec}$. Although the value of W was greater than 0.95 throughout the measurement, L varied over the range from 4.9 μm up to 5.4 μm .

3.2. TRANSVERSAL FLUCTUATIONS AND LONGITUDINAL DISTORTIONS

Transversal fluctuations were displayed in Figure 3(b). Neither apparent periodicity nor coordination was detected in the fluctuations. The standard deviation of the

observed fluctuations was about 60 nm, which agreed well with the one observed in independent measurements reported previously [13].

Longitudinal distortions observed for the same filament were displayed in Figure 3(a). Although no apparent correlation with the observed transversal fluctuations was identified, their amplitude was greater than that of transversal fluctuations.

4. Discussion

Considering the fact that the actin filament we examined changed its length over the range from 4.9 μm to 5.5 μm , we concluded that the length of the filament observed in Figure 2 was about 5.5 μm . This suggests that the filament could be longitudinally compressed down to 4.9 μm . The extent of compression was about 10% of the entire length. This may mean a change in the coiled structure of an actin filament during its active sliding along myosin heads [17].

The amplitude of transversal fluctuations in this report (c.a. 60 nm) agreed well with the one measured in independent experiments [13]. The magnitude was almost equal to the one observed in the presence of 2 mM ATP (i.e. saturated ATP). Longitudinal distortions of such a large amplitude (c.a. 100 nm) were identified for the first time in this report. Since the resultant of both transversal fluctuations and longitudinal distortions gives the whole deformation of the filament, the longitudinal distortions may serve as a coordinator of the myosin motors driving the sliding movement.

Our measurement demonstrated that the linear dimension of both transversal fluctuations and longitudinal distortions was greater than the diameter of an actin monomer. This fact may also suggest that the tension generated by single myosin heads would not directly be converted into the sliding movement of an actin filament. Mechanical distortions, both transversal and longitudinal, identified as the deformation of an actin filament may play a significant role to facilitating a smooth movement of an actin filament.

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