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Enhancing the staggered fluctuations of an actin filament sliding on *Chara* myosin

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Abstract

We examined both longitudinal and transversal fluctuations of displacements of an actin filament sliding upon *Chara* myosin molecules. Although the magnitude of transversal fluctuations remained rather independent of ATP concentration, the longitudinal ones were found to increase their magnitude as the concentration increased. In addition, the longitudinal fluctuations gradually increased as the sliding velocity of the filament increased.

Keywords: Actin filament; Fluctuations; *Chara* myosin; ATP-dependence; Sliding movement

1. Introduction

The sliding velocity of an actin filament varies according to the kind of myosin molecules upon which it slides. The fastest motor protein known so far, that is *Chara* myosin molecule, is classified into myosin XI under the myosin superfamily [1,2]. This myosin molecule contributes to generating endoplasmic streaming inside giant plant cells of *Chara* green algae [3,4]. The *Chara* myosin molecules have an ability to enhance the sliding velocity of an actin filament up to approximately 60 μm/s in an in vitro motility assay system that is approximately 10 times greater than that of skeletal muscle ones [5–7]. The mechanism for generating such an extremely fast movement remains to be elucidated because the structural size of the head region and the rate of ATP hydrolysis catalyzed by the head are similar to that of the conventional myosin molecules [5,8,9].

The *Chara* myosin molecule is regarded as non-processive type of motor protein similar to myosin II in skeletal muscle [10]. A myosin XI molecule of *Chara* alone cannot move along an actin filament over a long distance. Because numerous myosin molecules ought to participate in the movement of an actin filament, it is necessary for the myosin molecules to coordinate the sliding forces altogether as with the accompanied ATP hydrolysis.

We have already examined fluctuations of displacements of an actin filament on myosin II molecules derived from skeletal muscle as varying

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ATP concentrations. It was then found that the myosin molecules contacting the filament regulate the filamental fluctuations in a communicative manner along the filament [11–17].

In this article, we report on longitudinal fluctuations of an actin filament sliding on *Chara* myosin molecules, which is quite different from the ones observed with skeletal muscle myosin molecules.

2. Materials and methods

2.1. Preparation of extract containing Chara myosin molecules

An internodal cell (approx. 10-cm length) of green algae, *Chara corallina*, was isolated from its neighbouring cells. The internodal cell was cut at both ends by using precise scissors. Then vacuoles were removed from the cell as absorbing them by a piece of filter paper. Squeezing cyotosol from the cell, approximately 10 μl of extract containing *Chara* myosin molecules was obtained. Immediately after the extraction, 10 μl of extract was added to 40 μl of E-solution (300 mM KCl, 100 mM Tris–acetate (pH 7.5), 20 mM EGTA, 5.6 mM MgATP, 1 mM DTT) containing protease inhibitors (20 μg/ml aprotinin, 20 μg/ml 4-(2-Aminoethyl)-bezenesulfonylfluoride, 50 μg/ml leupeptin) at 4 °C. Then, the mixture was centrifuged at 18 000 rev./min (Kubota, refrigerated centrifuge 6700, angle rotor RA-1M) for 20 min at 4 °C to precipitate chloroplasts. The supernatant was stored on ice and used for assay within 2 h.

2.2. Preparation of a speckled actin filament

Actin was purified from acetone powder of rabbit skeletal muscle according to the method by Spudich and Watt [18]. In order to measure relative displacements occurring in a single actin filament, we prepared a speckled actin filament marked by fluorescent materials, rhodamine-phalloidin [14, 19].

2.3. Motility assay for a speckled actin filament moving on Chara myosin molecules

A flow cell was prepared from a large cover slip (24×50 mm) and a small cover slip (18×5 mm) washed with 0.1 M KOH in ethanol. The *Chara* extract was infused into a gap between cover slips of the flow cell placed on ice. Five minutes after the infusion, the solution in the flow cell was replaced three times by 30 μl of B-solution (20 mM HEPES-KOH (pH 7.5), 25 mM DTT, 2 mM $MgCl_2$, 1 mg/ml bovine serum albumin). Subsequently, those speckled actin filaments (1 μg/ml in 30 μl) were applied into the flow cell. Finally, the solution was replaced four times by 30 μl of Assay-solution (20 mM HEPES-KOH (pH 7.5), 25 mM DTT, 2 mM $MgCl_2$, 0.1 mg/ml glucose oxidase, 0.02 mg/ml catalase, 3 mg/ml glucose and controlled concentrations of ATP) so as to start the sliding movement of actin filaments. The motility assay was performed at 23 °C.

2.4. Analysis of filamental displacements

The microscope (Olympus, IX70) attached with the object lens (Olympus, Uplan Apo 100x, oil) was used with the aid of the fluorescent equipment (Olympus, IX-FLA) and the fluorescent filter (Olympus, rhodamine filter). Images under the microscope were stored in a video recorder equipped with an image-intensified CDD camera (Video Scope International, Ltd., ICCD-350F). Image-processing software we used was NIH Image 1.6 (Wayne Rasband, National Institute of Health, Research Services). Each image was retrieved at every 1/30 s through video grabber board (Scion Co., LG-3 PCI). The displacements of a moving marker on a speckled filament on *Chara* myosin molecules in the presence of ATP at every 0.1 s interval were recorded for both the longitudinal and transversal components. The longitudinal displacement was defined as a component parallel to the direction of the movement of the marker registered at the preceding measurement. The transversal displacement was a component perpendicular to the longitudinal direction. The sliding velocity was estimated as referring to the average of longitudinal displacements measured over 0.1 s for each sample filament examined. Further details of the analysis have been described in our previous report [20].

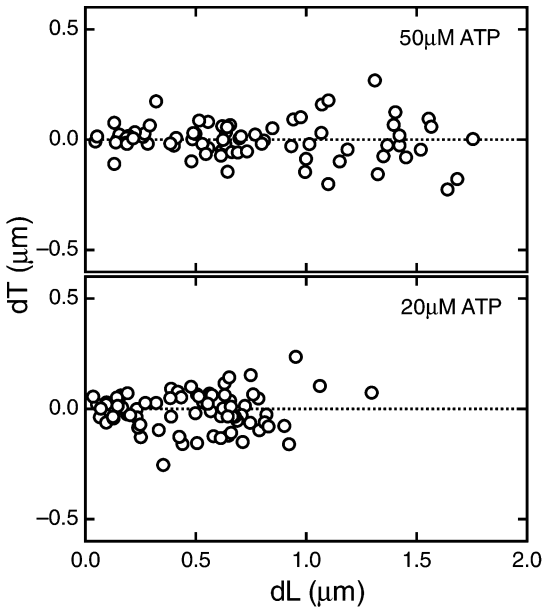

Fig. 1. Two examples of the statistical distribution of displacements of an actin filament on *Chara* myosin molecules, both at 20 μM ATP (under) and at 50 μM ATP (upper). Both the longitudinal and the transversal displacements of a marker of an actin filament moving on a planar plane were measured over the interval of 0.1 s. The precision of measuring the location of each speckled point on the filament was 8 nm [13].

3. Results and discussion

3.1. Fluctuation enhancement of an actin filament on Chara myosin molecules

The filamental fluctuations occurring due to dynamic counterbalancing of the forces generated within the actin filament attached on myosin molecules have already been observed when skeletal muscle myosin molecules were employed [15,17]. In accordance with this context, we examined how the fast movement of the filament on *Chara* myosin could be associated with filamental fluctuations.

Fig. 1 demonstrates a statistical distribution of the observed displacements of the marker attached on an actin filament, both longitudinal and transversal ones, for two different ATP concentrations. The distribution of longitudinal displacements increased its width compared to that of transversal ones, for both ATP concentrations of 20 and 50 μM.

Fig. 2 shows the standard deviations for both the longitudinal and transversal displacements as a quantity indicating the intensity of the fluctuations, in which the ATP concentration is taken as a control parameter. Although the intensity of transversal fluctuations remained rather independent of ATP concentration, the longitudinal ones were found to increase their intensity as the concentration increased.

Fig. 3 shows a relation of the fluctuation intensities of both the longitudinal or transversal displacements to the sliding velocity of actin filaments, measured at various ATP concentrations. In our experiments, the averaged velocity of the filament sliding on *Chara* myosin molecules was approximately 16 μm/s at 0.5 mM ATP. This velocity was roughly five times as much as that on skeletal muscle myosin molecules at the same ATP concentration. The intensity of longitudinal fluctuations increased up to approximately 0.6 μm over the velocity range of 0–15 μm/s. However, the transversal one increased up to only 0.2 μm over this velocity range. Thus, the longitudinal

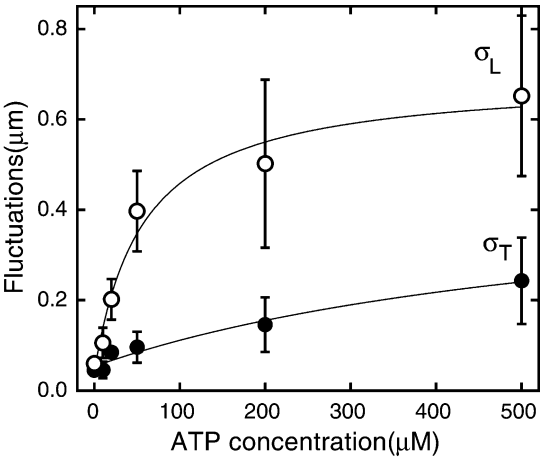

Fig. 2. Standard deviations of both the longitudinal and the transversal displacements. ATP concentration is a control parameter. Fluctuations in terms of standard deviations were evaluated as referring to five independent experiments at each ATP concentration. Open and filled symbols represent the longitudinal fluctuations and the transversal ones, respectively.

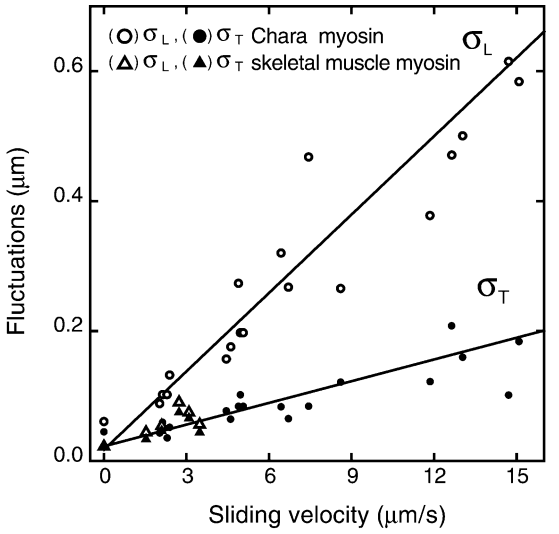

Fig. 3. Relationship between the fluctuation intensity and sliding velocity of actin filaments. The intensities of fluctuations were plotted against the sliding velocities of the filaments obtained in various ATP concentrations. The sliding velocity was estimated as referring to the average of longitudinal displacements measured over 0.1 s for each sample filament examined. Open and filled symbols represent the longitudinal fluctuations and the transversal ones, respectively. The fluctuation intensities on the *Chara* myosin molecules (circle symbols) were compared with that on the skeletal muscle molecules (triangle symbols). The data set presented in the case of skeletal muscle myosin was reproduced from our previous work [17].

fluctuations of the filament were enhanced with an increase of the velocity, compared to the transversal one.

3.2. Chara and skeletal muscle myosin molecules for fluctuations

Fluctuations of the filament attached on *Chara* myosin can be compared with those attached on skeletal muscle myosin, as demonstrated in Fig. 3. In the case of skeletal myosin, the magnitude of longitudinal fluctuations was almost as much as that of transversal ones. The maximum intensities were obtained approximately 100 μM ATP [17]. In contrast, *Chara* myosin molecules enhanced longitudinal fluctuations more prominently compared to transversal ones, and facilitate a faster sliding movement of the filament. Since the intensity of longitudinal fluctuations on *Chara* myosin increased linearly with the sliding velocity, it also increased with the ATP concentration. Even when the sliding velocity of the filament on *Chara* myosin molecules was as low as 4 μm/s, which is almost equal to the sliding velocity expected on skeletal myosin in the presence of sufficient ATP molecules, the longitudinal fluctuations were strongly enhanced. This enhancement of longitudinal fluctuations is quite specific to *Chara* myosin.

3.3. Ionic strength on fluctuations

Ionic strength is a factor influencing the sliding movement of an actin filament on myosin molecules. We then examined the contribution of KCl to filamental fluctuations.

Fig. 4 shows the frequency distribution of the longitudinal displacements of the filament in the presence or in the absence of KCl. At low ionic strength, i.e. in the absence of KCl, the displacements were widely spread and there appeared multiple local maxima in the distribution. When the ionic strength was elevated up to 50 mM KCl, the displacements became rather homogeneous with a single maximum obeying a standard Gaussian distribution. The averaged displacement

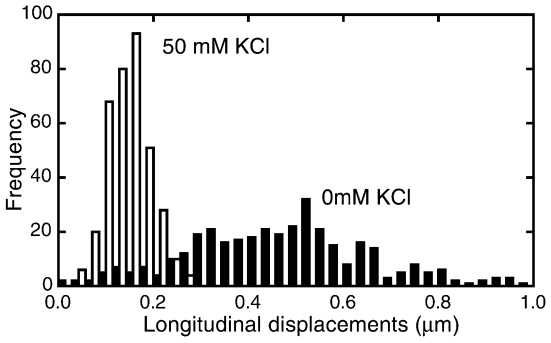

Fig. 4. Frequency distribution of longitudinal displacements of the filament on *Chara* myosin molecules at 20 μM ATP in the absence (filled boxes) or in the presence (open boxes) of KCl. The assay conditions were 20 mM HEPES-KOH (pH 7.5), 25 mM DTT, 2 mM $MgCl_2$, 0.1 mg/ml glucose oxidase, 0.02 mg/ml catalase, 3 mg/ml glucose, 20 μM ATP and either 0 mM or 50 mM KCl. In order to increase the ionic strength, KCl was further added into the Assay-solution.

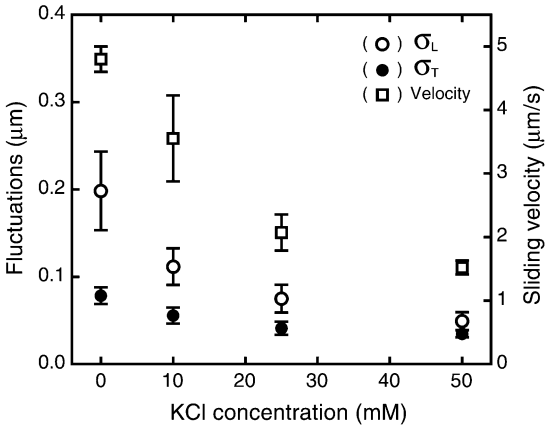

Fig. 5. KCl-dependence of fluctuation intensity and sliding velocity of the filament on *Chara* myosin molecules. ATP concentration was fixed at 20 μM. Open circles, filled circles and open triangles represent the longitudinal fluctuations, the transversal ones and the sliding velocity, respectively.

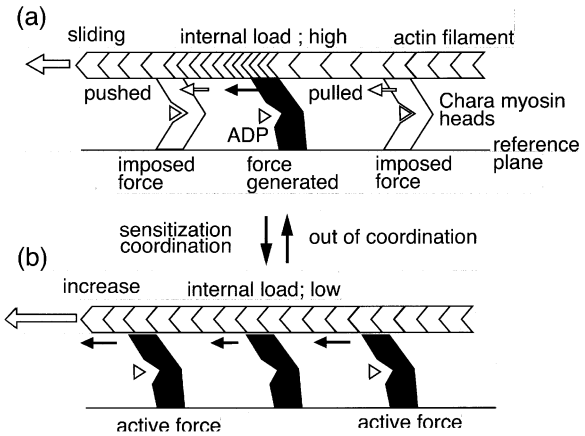

Fig. 6. A schematic representation of a likely mechanism underlying the fluctuation enhancement of an actin filament, induced by *Chara* myosin molecules. (a) Disequilibrating state: A myosin head generating the sliding force influences the kinetics of the neighboring heads along the actin filament, in which either the pushing or the pulling force is generated. (b) Coordinating state: These activated heads develop a coordination among themselves along the filament. Coordination in one place then induces a disequilibrium out of coordination in the neighborhood. Equilibration and desequilibration thus reiterate.

decreased at the same time. Both the intensity of longitudinal fluctuations and the sliding velocity decreased with the increase of KCl concentration, in which ATP concentration was fixed at 20 μM, as shown in Fig. 5. However, the transversal fluctuations remained almost independent of KCl concentrations examined. The sliding velocity of the filament on skeletal myosin increases its magnitude as the ionic strength goes up to 50 mM [21]. In contrast, upon *Chara* myosin molecules, both the longitudinal fluctuations and the sliding velocity decreased with an increase of KCl concentration. The electrostatic interaction between actin monomers in the filament and myosin heads would become overwhelming as the ionic strength decreases. For *Chara* myosin, such a strong interaction between an actin and myosin may be required to facilitate the sliding movement of the filament. In addition, the enhancement of longitudinal fluctuations was coincident with the faster movement.

4. Concluding remarks

The observed enhancement of longitudinal fluctuations could be due to a certain cooperativity resting upon *Chara* myosin molecules aligned along the actin filament. Since the rate of releasing nucleotide molecules such as ADP from *Chara* myosin is influenced by the forces imposed externally on myosin heads [9], allosteric or cooperative behaviors among myosin molecules may come up as sensitizing the neighboring forces. When myosin molecules attaching on an actin filament are activated in response to variable forces occurring within the filament, the staggered movement of the filament [13] would further be enhanced due to the cooperativity, as demonstrated in Fig. 6.

An actin filament can relatively easily be pushed and pulled in the longitudinal direction when touched upon *Chara* myosin molecules. *Chara* myosin molecules may have the capacity of helping an actin filament to transform its longitudinal fluctuations into a unidirectional sliding movement.

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