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Transition from contractile to protractile distortions occurring along an actin filament sliding on myosin molecules

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Abstract

An ATP-activated actin filament sliding on myosin molecules exhibited mechanical distortions or fluctuations both longitudinally and transversally along the filament. Although actin filaments exhibited a uniform sliding movement longitudinally as the ATP concentration increased, the longitudinal fluctuations were found to vary their magnitude with the concentration. The magnitude of longitudinal fluctuations reached its maximum at approximately 100 μM of the ATP concentration. The local enhancement of the longitudinal fluctuations as responding to changes in the ATP concentration is associated with a critical phenomenon bridging the two different kinds of mechanical distortions, either contractile or protractile ones, occurring within a sliding actin filament.

Keywords: Actin filament; Critical phenomena; Fluctuations; Myosin; Sliding movement

1. Introduction

An actin filament sliding on myosin molecules as a basic functional unit of muscle contraction has revealed that the filament is plastic enough to allow its mechanical distortions [1–5]. We have already observed that an actin filament deforms in a contractile manner when the ATP concentration is as low as 20 μM, while it deforms in a protractile manner when the concentration is as high as 2 mM [6]. That an actin filament demonstrates either contractile or protractile distortions in the presence of ATP points up the fact that the filament could maintain a certain extent of plasticity in generating its mechanical distortions. This observation then raises a question of how the relative mechanical motion occurring within an actin filament materializes and transforms itself as responding to changes in the ATP concentration [7–14]. In the present article, we shall examine a dynamic nature of the relative motion internal to an actin filament as focusing on the transition from contractile to protractile distortions, or vice versa, occurring in the filament.

2. Materials and methods

Acitn and myosin were extracted from rabbit skeletal muscle [15,16]. Heavy meromyosin (HMM) was prepared by alpha-chymotriptic

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digestion of myosin [17]. In order to measure relative displacements occurring in a single actin filament, we prepared a speckled actin filament marked by fluorescent materials [18]. Speckled actin filaments were made from a mixture of actin filaments, both unlabeled and labeled by rhodamine–phalloidin. Unlabeled and labeled actin filaments (25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 1 mM DTT) were made and treated with equal molar phalloidin, and their concentrations were maintained at 33 μg/ml. The mixture of the suspensions of labeled and unlabeled actin filaments with their volume ratio 1:4 were subjected to supersonic dissecting (Misonix Corp., Microson model XL2000) at 5 W for 1 s. The mixture of dissected actin filaments, both labeled and unlabeled mixed together, was placed at temperature 4 °C for 1 h for their reformation of the filaments that could be speckled ones. The average distance between two neighboring speckled points in the filament thus prepared was approximately 1 μm.

We also prepared a standardized in vitro motility assay [19]. The slide glass to fix HMM of concentration 0.05 mg/ml was hydrophobically treated with 1% collodion in 3-methylbutyl acetate. The solution condition for observing the sliding movement of an actin filament was 25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 1 mM DTT and controlled concentrations of ATP at temperature 23 °C. 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 precision of measuring the location of each speckled point on the filament was 8 nm by using the method reported previously [20,21]. The measurement was done at every interval of 1/10 s.

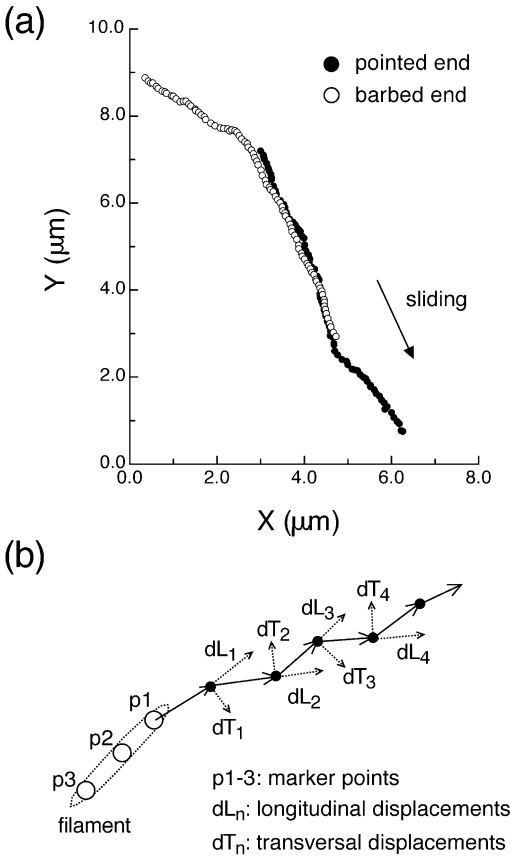

Fig. 1. (a) An example of the traces of moving markers on a speckled actin filament sliding on myosin molecules implanted on a planar plane. One marker is near the pointed end of the filament and the other near the barbed end. (b) Both the longitudinal and the transversal displacements of each marker on the planar plane. Each displacement was the one occurred over the interval of 1/10 s.

3. Results

A typical example of the traces of moving markers on a speckled actin filament sliding on myosin molecules implanted on a planar plane is displayed in Fig. 1a, in which one marker is near the pointed end of the filament and the other near the barbed end. We then focused on both the longitudinal and the transversal displacements of each marker on the planar plane as depicted in Fig. 1b. Each displacement was measured over the interval of 1/10 s.

Fig. 2 demonstrates a statistical distribution of the observed displacements, both longitudinal and transversal ones [22], retrieved from over 1000 sample points, in which the ATP concentration is taken as a control parameter. Frequency distribution of the longitudinal displacements is also presented. Although actin filaments exhibited a uniform sliding movement longitudinally as the ATP concentration increased, fluctuations in the displacement also increased with the concentration until the concentration reaches up to approximately 100 μM. Standard deviations of both the longitudinal and the transversal displacements are shown in Fig. 3, in which the ATP concentration is a controlled parameter.

What has been unique to the observed fluctuations of the displacements was that the fluctuation intensity increased with the ATP concentration up to roughly 100 μM. However, the intensity decreased with the further increase in the concentration.

Fluctuation intensities of the displacements have certainly been subject to the density of myosin molecules or HMM as demonstrated in Fig. 4. However, local enhancement of the intensities near 100 μM ATP has been robust as remaining almost indifferent to the myosin density to a certain extent.

4. Discussions

Our previous studies demonstrated that an actin filament sliding on myosin molecules exhibited contractile distortions along the filament at 20 μM ATP, but did protractile ones at 2 mM ATP [6]. Actin filaments involved in the generation of the sliding force with myosin molecules deformed differently depending upon the magnitude of the concentration of ATP available. Both contractile and protractile distortions are macroscopic or at least mesoscopic over the entire actin filament.

It is likely that below 50 μM ATP, post-stroke heads after releasing nucleotide molecules may be involved in mechanical distortions of an actin filament, while the ATPase activity already reaches its maximum rate at this ATP concentration [23]. Some nucleotide-free heads may give rise to the filamental deformations of actin molecules in the filament. In particular, the force required for detaching a myosin head from an actin filament in the absence of ATP is approximately 9 pN [24], which is roughly twice as large as 5 pN [25] of the maximum force expected at the head through hydrolysis of an ATP molecule. In fact, an actin filament is capable of bending itself down to the curvature of the radius of 0.18 μm when the filament is under the influence of external force as much as 2.4 pN in the lateral direction [4]. Furthermore, it is expected that some interferences between the active and the passive force may occur everywhere along the filament [26]. The disequilibrium between the two forces could be the rule there rather than the exception. If both the affinity of a nucleotide molecule toward a myosin head and the duration time of the binding between the head and an actin filament are sensitive to the external force from those such as kinesin molecules [27], push–pull distortions along the filament may in turn contribute to coordinating the associated myosin heads in action. At higher ATP concentrations, on the other hand, the distortions of the filament could be mitigated with the consequence of more coherent movement over the entire filament [7], since the duration time of the strong binding between each myosin head and the actin filament decreases [28].

An actin filament is in a contractile phase when ATP concentration is low (20 μM), while in a protractile phase at a high concentration as much as 2 mM. When the ATP concentration is raised from below 20 μM, the actin filament would eventually traverse the crossover region between the two phases, the contractile and the protractile ones. Right in the crossover region, a critical enhancement of fluctuations could be expected because of changes in the macroscopic properties as responding to slightest changes in the control parameter that is ATP concentration in our case. Our observation of a critical enhancement of fluctuations at approximately 100 μM ATP suggests that an actomyosin complex may change its dynamic properties macroscopically over the entire actin filament as the ATP concentration approaches 100 μM. Its dynamic background remains to be seen.

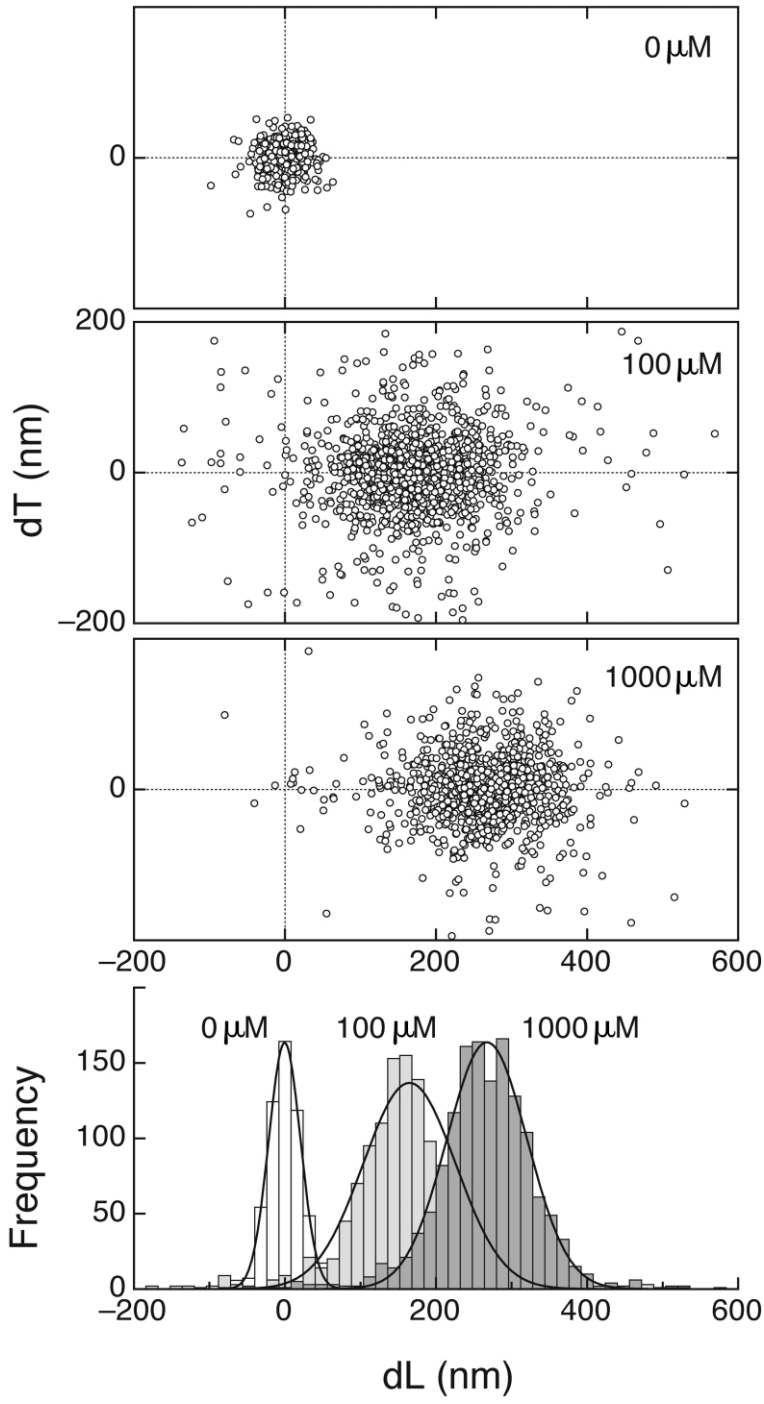

Fig. 2. A statistical distribution of the observed displacements, both longitudinal and transversal ones, retrieved from over 1000 sample points. ATP concentration is taken as a controlled parameter. Frequency distribution of the longitudinal displacements is also presented.

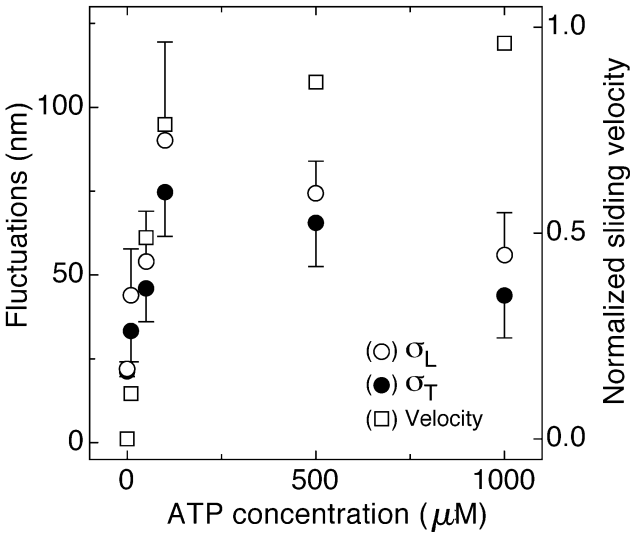

Fig. 3. Standard deviations of both the longitudinal and the transversal displacements. ATP concentration is a control parameter. Fluctuations in terms of standard deviations were evaluated as referring to 10 independent experiments at each ATP concentration. Normalized sliding velocity is also displayed. The velocity increased with the increase of ATP concentration. The half value of the maximum velocity was attained at approximately 50 μM ATP.

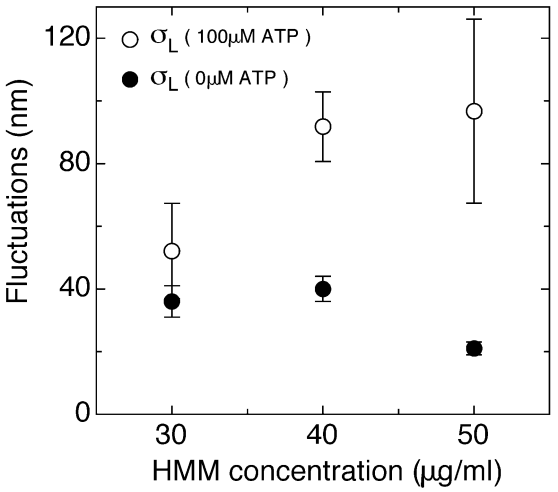

Fig. 4. A relationship between fluctuation intensities of the displacements and the density of myosin molecules or HMM.

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